0022-3565/06/3161-440–447
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
U.S. Government work not protected by U.S. copyright
JPET 316:440–447, 2006

Vol. 316, No. 1
92304/3071860
Printed in U.S.A.

Depressive-Like Effects of the κ-Opioid Receptor Agonist Salvinorin A on Behavior and Neurochemistry in Rats

William A. Carlezon, Jr., Cécile Béguin, Jennifer A. DiNieri, Michael H. Baumann, Michele R. Richards, Mark S. Todtenkopf, Richard B. Rothman, Zhongze Ma, David Y.-W. Lee, and Bruce M. Cohen

Behavioral Genetics Laboratory (W.A.C., J.A.D., M.S.T.), Molecular Pharmacology Laboratory (C.B., M.R.R., B.M.C.), and Bioorganic and Natural Products Laboratory (Z.M., D.Y.-W.L.), Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, Massachusetts; and Clinical Psychopharmacology Section (M.H.B., R.B.R.), Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland

Received July 8, 2005; accepted October 12, 2005

ABSTRACT

Endogenous opioids seem to play a critical role in the regulation of mood states. For example, there is accumulating evidence that stimulation of κ-opioid receptors, upon which the endogenous opioid dynorphin acts, can produce depressive-like behaviors in laboratory animals. Here we examined whether systemic administration of salvinorin A (SalvA), a potent and highly selective κ-opioid agonist, would produce depressive-like effects in the forced swim test (FST) and intracranial self-stimulation (ICSS) test, which are behavioral models often used to study depression in rats. We extracted, isolated, and purified SalvA from *Salvia divinorum* plant leaves and examined its effects on behavior in the FST and ICSS test across a range of doses (0.125–2.0 mg/kg) after systemic (intraperitoneal) administration. SalvA dose dependently increased immobility in the FST, an effect opposite to that of standard antidepressant drugs. Doses of SalvA that produced these effects in the FST did not affect locomotor activity in an open field. Furthermore, SalvA dose dependently elevated ICSS thresholds, an effect similar to that produced by treatments that cause depressive symptoms in humans. At a dose that caused the depressive-like effects in both the FST and ICSS assays, SalvA decreased extracellular concentrations of dopamine (DA) within the nucleus accumbens (NAc), a critical component of brain reward circuitry, without affecting extracellular concentrations of serotonin (5-HT). These data provide additional support for the hypothesis that stimulation of brain κ-opioid receptors triggers depressive-like signs in rats and raise the possibility that decreases in extracellular concentrations of DA within the NAc contribute to these effects.

Although much research on depression has focused on brain norepinephrine and serotonin (5-HT) systems, there is substantial evidence that other systems have important roles in the neurobiology of mood and affective disorders. For example, the mesolimbic dopamine (DA) system—which projects from the ventral tegmental area to the nucleus accumbens (NAc)—contributes importantly to the hedonic (rewarding) effects of food, sexual behavior, and addictive drugs (see Wise, 1998; Nestler and Carlezon, 2005). It has been proposed that disruption of DA function within the NAc causes anhedonia (reduced ability to experience reward) (Wise, 1982), a hallmark sign of clinical depression. The mesolimbic DA system is modulated by noradrenergic and serotonergic inputs (Pasquier et al., 1977), as well as endogenous opioid peptides (Devine et al., 1993; Shippenberg and Rea, 1997; Svingos et al., 1999). Agents that selectively affect the function of κ-opioid receptors cause profound alterations in mood in humans (Pfeiffer et al., 1986; Roth et al., 2002) and motivated behaviors in laboratory animals (Shippenberg and Herz, 1987; Todtenkopf et al., 2004), suggesting that manipulations targeting brain κ-opioid systems might be useful in the study and treatment of depressive disorders.

This work was supported by Grant MH63266 from the National Institute of Mental Health (to W.A.C.) and the Stanley Medical Research Institute (to B.M.C.). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program from the National Center for Research Resources, National Institutes of Health (RR11213).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.105.092304.

ABBREVIATIONS: 5-HT, serotonin; NAc, nucleus accumbens; CREB, cAMP-response element-binding protein; E-2078, *N*-CH_3-Tyr-Gly-Gly-Phe-Leu-Arg-*N*-CH_3-Arg-D-Leu-NHC_2H_5; HPLC, high-performance liquid chromatography; ICSS, intracranial self-stimulation; DMSO, dimethyl sulfoxide; FST, forced swim test; U-50488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methane sulfonate salt; U-69593; (5α,7α,8β)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec8-yl)-benzenacetamide; SalvA, salvinorin A; SSRI, selective serotonin reuptake inhibitor; ANOVA, analysis of variance.

Molecular studies in rodents suggest that complex experience-dependent alterations in the function of κ-opioid systems within the NAc contribute to the development and expression of depressive behaviors. For example, stress elevates the activity of the transcription factor CREB within the NAc (Pliakas et al., 2001). Selective elevation of CREB function within the NAc increases immobility behavior in the forced swim test (FST) (Pliakas et al., 2001), a model often used to study depression. This effect is opposite to that caused by standard antidepressants and thus is a potential sign of depressive-like states (Porsolt et al., 1977; Cryan et al., 2002). Elevated expression of CREB in the NAc also reduces the rewarding effects of cocaine, a putative sign of anhedonia (Carlezon et al., 1998; Pliakas et al., 2001). The depressive-like behavioral effects that accompany elevated CREB function within the NAc seem related, at least in part, to CREB-regulated transcription of dynorphin, an endogenous κ-opioid receptor ligand (Carlezon et al., 1998). The κ-antagonist norbinaltorphimine attenuates the behavioral effects of elevated CREB expression within the NAc (Carlezon et al., 1998; Pliakas et al., 2001), suggesting a role for this receptor subtype in the expression of depressive-like behaviors. κ-Antagonists also have antidepressant-like effects in normal rats (Pliakas et al., 2001; Mague et al., 2003; McLaughlin et al., 2003), even when microinjected directly into the NAc (Newton et al., 2002). In contrast, stimulation of κ-receptors in rats produces complex behaviors that might reflect depressive- or dysphoric-like states. The κ-agonist U-69593 establishes conditioned place aversions (Shippenberg and Herz, 1987), increases immobility in the FST (Mague et al., 2003), and elevates ICSS thresholds (Todtenkopf et al., 2004). These findings are consistent with the observation that κ-agonists produce depressive or dysphoric states in humans (Pfeiffer et al., 1986). The mechanism of these effects likely involves reduced DA function. κ-Agonists decrease extracellular concentrations of DA within the NAc (DiChiara and Imperato, 1988; Spanagel et al., 1992; Devine et al., 1993) through stimulation of κ-receptors that regulate DA release from mesolimbic neurons (Donzanti et al., 1992; Shippenberg and Rea, 1997; Svingos et al., 1999). Considered together, these findings raise the possibility that elevated CREB-mediated transcription of dynorphin within the NAc leads to increased κ-receptor activity, which decreases local DA function and triggers certain signs of depression.

Salvinorin A (SalvA) is a psychoactive compound found in the leaves of *Salvia divinorum*, a plant from the mint family (Roth et al., 2002). Binding and function studies indicate that SalvA is a potent and highly selective κ-agonist, with greater efficacy than that of the synthetic κ-agonists U-50488H and U-69593 (Roth et al., 2002; Chavkin et al., 2004; Munro et al., 2005). SalvA is non-nitrogenous and has no structural similarity with other opioid agonists, making it an ideal agent with which to further examine relationships among dynorphin, κ-opioid receptors, and depressive behavior. The present studies were designed to examine the effects of SalvA in tests (FST, ICSS) that have previously identified the depressive-like effects of U-69593 (Mague et al., 2003; Todtenkopf et al., 2004) and other treatment regimens (e.g., drug withdrawal) that cause depression in humans (Cryan et al., 2002). We also examined the effects of SalvA on locomotor activity to determine whether it causes nonspecific behavioral suppression that might complicate data interpretation. Finally, we examined the effects of SalvA on extracellular concentrations of DA and serotonin within the NAc using in vivo microdialysis in freely moving rats.

Materials and Methods

Rats. A total of 129 male Sprague-Dawley rats (Charles River Laboratories, Boston MA) were used in these studies. Rats used for forced swim testing or locomotor activity testing were housed in groups of four and weighed 325 to 375 g at the time of testing, whereas those used for ICSS testing or brain microdialysis were housed singly and weighed 350 to 400 g at the time of stereotaxic surgery. All rats were maintained on a 12-h light (7:00 AM to 7:00 PM)/12-h dark cycle with free access to food and water, except during testing. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, 1996) as well as McLean Hospital and National Institute on Drug Abuse-Intramural Research Program policies.

Salvinorin A. Dried *S. divinorum* leaves were purchased from Salvia Space (Lawrence, KS). SalvA (Fig. 1) was extracted, isolated, and purified using published methods with minor modifications (see Lee et al., 2005 and references therein). In brief, the leaves were treated sequentially with hexane and acetone. The acetone extract was purified by chromatography on activated carbon, and SalvA was crystallized from acetone and methanol to yield a white crystalline solid. Spectroscopic analyses confirmed that the SalvA obtained with these methods is chemically identical to that described in other reports (Roth et al., 2002). The samples used for testing were determined by HPLC to be >99% pure. SalvA was dissolved in a vehicle of 75% dimethyl sulfoxide (DMSO), 25% distilled water and was administered by i.p. injection in a volume of 1 ml/kg.

Forced Swim Test. Forty rats were used to study the effects of SalvA in the FST. The FST is a two-day procedure in which rats swim under conditions where escape is not possible. On the first day, the rats are forced to swim for 15 min. The rats initially struggle to escape from the water, but eventually they adopt a posture of immobility in which they make only the movements necessary to keep their heads above water. When the rats are retested 24 h later, immobility is increased. Treatment with standard antidepressant drugs within the 24-h period between the first exposure to forced swimming and retesting can block facilitated immobility, an effect correlated with antidepressant efficacy in humans (Porsolt et al., 1977; Detke et al., 1995).

On the first day of the FST, rats were placed in clear 65-cm tall, 25-cm diameter Plexiglas cylinders filled to 48 cm with 25°C water.

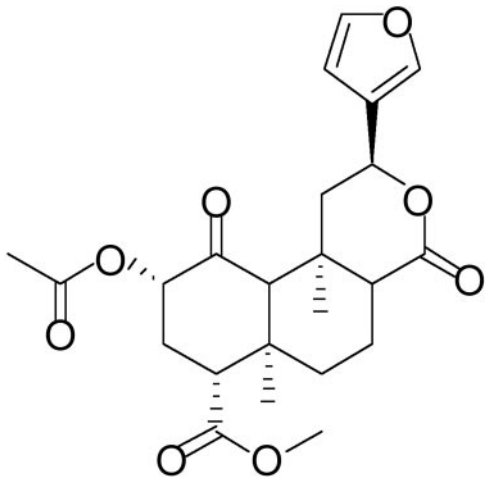

Fig. 1. Chemical structure of salvinorin A, a potent, efficacious, and highly selective non-nitrogenous κ-opioid receptor agonist.

After 15 min of forced swimming, the rats were removed from the water, dried with towels, and placed in a warmed enclosure for 30 min. The cylinders were emptied and cleaned between rats. Rats (6–8 per treatment condition) received three separate injections of SalvA (0.125–2.0 mg/kg, i.p.) at 1, 19, and 23 h after the first exposure to forced swimming. This is a commonly used treatment regimen (Porsolt et al., 1977; Detke et al., 1995) that we followed to enable qualitative comparisons with previous studies of agents with antidepressant-like and prodepressant-like effects (Mague et al., 2003). At 24 h after the forced swim, rats were retested for 5 min (300 s) under identical swim conditions. Retest sessions were videotaped from the side of the cylinders and scored using a behavioral sampling method (Detke et al., 1995; Mague et al., 2003) by raters unaware of the treatment condition. Rats were rated at 5-s intervals throughout the duration of the retest session; at each 5-s interval, the predominant behavior was assigned to one of four categories: immobility, swimming, climbing, or diving. A rat was judged to be immobile if it was making only movements necessary to keep its head above water, climbing if it was making forceful thrashing movements with its forelimbs directed against the walls of the cylinder, swimming if it was actively making swimming movements that caused it to move within the center of the cylinder, and diving if it swam below the water toward the bottom of the cylinder. (Data quantifying diving behavior is not shown in the present report because it rarely occurred, and it was not affected by any of the treatments tested.) This behavioral sampling method differentiates certain classes of antidepressant drugs. For example, tricyclic antidepressants decrease immobility and increase climbing without affecting swimming, whereas selective serotonin reuptake inhibitors (SSRIs) decrease immobility and increase swimming without affecting climbing (Detke et al., 1995). It is also sensitive to treatments that cause depressive effects in humans, including antimanic agents and drug withdrawal (Carlezon et al., 2002; Cryan et al., 2002).

The number of occurrences (to a maximum of 60) of each category of behavior was analyzed using separate one-way (treatment) analyses of variance (ANOVAs). Significant effects were analyzed using post hoc Newman-Keuls tests.

Locomotor Activity. Fifty-eight rats were used to determine whether the doses of SalvA examined in the FST studies alter locomotor activity. These studies were conducted exactly as the FST studies had been conducted until the point of re-testing; i.e., all rats (8–11/group) underwent the first day of the FST and were treated with SalvA at the normal pretreatment times (1, 19, and 23 h after swimming). At 24 h after the first exposure to forced swimming, the rats were placed for 1 h in automated 43.2 × 43.2 × 30.5 cm (L × W × H) activity chambers (MED Associates, St. Albans, VT) instead of being retested in the FST. The total number of activity counts (photocell beam breaks) during the 30-min test session was quantified in 5-min bins, and differences among the treatment groups were analyzed using a one-way ANOVA (for total counts) and a two-way (treatment × time) ANOVA with repeated measures.

ICSS. Each of seven rats was anesthetized with a mixture of ketamine plus xylazine (80 mg/kg plus 12 mg/kg i.p.; Sigma-Aldrich, St. Louis, MO), and given subcutaneous atropine sulfate (0.25 mg/kg) to reduce bronchial secretions. Each rat was then implanted with a monopolar stainless steel electrode (0.250-mm diameter; Plastics One, Roanoke, VA) aimed at the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from the midsaggital suture, and 7.8 mm below dura; Paxinos and Watson, 1986). The electrodes were coated with polyamide insulation, except at the flattened tip. Skull screws (one of which served as the ground) and the electrode were secured to the skull with dental acrylic.

After at least one week of recovery, the rats were trained on a continuous reinforcement schedule (FR1) to respond for brain stimulation using procedures described previously (Todtenkopf et al., 2004). Each lever-press earned a 0.5-s train of square-wave cathodal pulses (0.1-ms pulse duration) at a set frequency of 141 Hz. The delivery of the stimulation was accompanied by the illumination of a 2-watt house light. Responses during the 0.5-s stimulation period did not earn additional stimulation. The stimulation current (100–300 μA) was adjusted gradually to the lowest value that would sustain a reliable rate of responding (at least 40 rewards/min). Once the minimal effective current was found for each rat, it was held constant for all subsequent phases of training and testing.

Each rat was then adapted to brief tests at its minimal effective current with each of the descending series of 15 stimulation frequencies. Each series comprised 1-min test trials at each frequency. For each frequency tested, there was an initial 5-s "priming" phase during which noncontingent stimulation was given followed by a 50-s test phase during which the number of responses was counted. Following the test phase, there was a 5-s time-out period during which no stimulation was available. The stimulation frequency was then lowered by approximately 10% (0.05 $\log_{10}$ units), and another trial was started. After responding had been evaluated at each of the 15 frequencies, the procedure was repeated such that each rat was given six such series per day (90 min of training). During the training procedure, the range of frequencies was adjusted for each rat so that only the highest seven to eight frequencies would sustain responding. To characterize the functions relating response strength to reward magnitude, a least-squares line of best fit was plotted across the frequencies that sustained responding at 20, 30, 40, 50 and 60% of the maximum rate using customized analysis software. ICSS threshold was defined as the frequency at which the line intersected the x-axis (θ-0; Miliaressis et al., 1986). Drug testing started when mean ICSS thresholds varied by less than 10% over three consecutive sessions.

For drug testing, three rate-frequency functions ("curves") were determined for each rat immediately before drug treatment. The first curve served as a warm-up period and was discarded because it tended to be unreliable. The second and third curves were averaged to obtain the baseline (threshold and maximal response rates) parameters. Each rat then received an i.p. injection of drug or vehicle, and four more 15-min rate-frequency curves were obtained (1 h of testing). All rats received the same daily treatments in a standardized order: saline, vehicle (75% DMSO), and SalvA at 0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg i.p. The doses were given in ascending and then descending order, such that each rat received vehicle and each dose of the drug twice. In addition, on alternate days, rats were tested after injections of saline to ensure that they had recovered from prior treatment and to minimize the possibility of conditioned drug effects.

To determine whether there were differences between the first and second tests with each treatment, the effects of saline, vehicle (75% DMSO), and SalvA on ICSS thresholds and maximal response rates over the test period were evaluated in separate two-way analyses of variance (ANOVAs) (drug dose × test number) with repeated measures. Data from the first and last tests with saline were used. The first and second tests at each dose were then combined into single means, and the drug effects on thresholds and maximum rates were evaluated with separate one-way ANOVAs. Significant effects were analyzed further using post hoc Newman-Keuls tests.

In Vivo Microdialysis. Each of the 24 rats was anesthetized as described above, and an indwelling jugular catheter was implanted (Baumann et al., 2001) to enable administration of anesthetic during insertion of the microdialysis probes (see below). The rat was then placed in a stereotaxic instrument. A plastic intracerebral guide cannula (CMA 12; CMA/Microdialysis, Solna, Sweden) was implanted above the NAc (1.6 mm anterior to bregma, 1.6 mm lateral from the midsaggital suture, and 6.2 mm below dura; Paxinos and Watson, 1986) according to published methods (Baumann et al., 2001). The guide cannula was fixed to the skull using stainless steel screws and dental acrylic. Animals were singly housed postoperatively and were allowed 7 to 10 days to recover. On the evening before an experiment, rats were moved to the testing room and lightly anesthetized with an intravenous injection of 10 mg/kg methohexital, an ultra-rapid short-acting anesthesia. A microdialysis probe

with a 2 × 0.5-mm exchange surface (CMA/12) was lowered into the guide cannula, and an extension tube (PE-50; Becton Dickinson, Sparks, MD) was attached to the jugular catheter. Each rat was placed into its own plastic container and was connected to a tethering system that allowed unrestricted movement within the container. The microdialysis inflow and outflow tubing, as well as the catheter extension tubing, were connected to a fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA). Artificial Ringers' solution containing 147.0 mM NaCl, 4.0 mM KCl, and 1.8 mM $CaCl_2$ was pumped through the probe overnight at 0.5 μl/min. On the next morning, 10-μl dialysate samples were collected at 20-min intervals. Samples were immediately assayed for DA and 5-HT by HPLC with electrochemical detection as described below. When three stable baseline samples were obtained, drug treatments were administered; rats received an i.p. injection of 1.0 mg/kg SalvA, 0.125 mg/kg SalvA, or vehicle (75% DMSO). Sampling continued for 2 h after treatment.

Aliquots of the dialysate were injected into a microbore HPLC column (5 μm, C18, 100 × 1 mm, Unijet; BAS Bioanalytical Systems, West Lafayette, IN) that was coupled to an amperometric detector (Model LC-4C; BAS Bioanalytical Systems). A glassy carbon electrode was set at a potential of +650 mV relative to an Ag/AgCl reference. Mobile phase consisting of 150 mM monochloroacetic acid, 150 mM NaOH, 2.5 mM sodium octanesulfonic acid, and 250 μM disodium EDTA with 1 ml of triethylamine, 6% MeOH, and 6% CH_3CN per liter of water (final pH = 5) was pumped (model 260D; ISCO, Lincoln, NE) at a rate of 60 μl/min. Chromatographic data were acquired on-line and exported to a Millennium software system (Waters, Milford, MA) for peak amplification, integration, and analysis. Standards of DA and 5-HT were run daily before dialysate samples, and standard curves were linear over a wide range of concentrations (0.1–100 pg). A monoamine standard mixture containing DA, 5-HT, and their respective acid metabolites was injected before and after the experiment to ensure validity of the constituent retention times. Peak heights of unknowns were compared with peak heights of standards and the lower limit of assay sensitivity (3× baseline noise) was 50 fg/5-μl sample.

Extracellular concentrations of DA and 5-HT were expressed as percentage of baseline, and the data for each neurotransmitter were analyzed separately. Differences among the treatment groups were analyzed using two-way (treatment × time) ANOVA with repeated measures on the time factor. Significant effects were analyzed further using post hoc Newman-Keuls tests.

Histology. Rats that had undergone stereotaxic surgery to implant ICSS electrodes or microdialysis probes were overdosed with pentobarbital (130 mg/kg i.p.) and perfused with 4% paraformaldehyde. The fixed brains were sliced in 40-μm sections for cresyl violet staining to confirm placements.

Results

In the FST, SalvA produced effects on behavior (Fig. 2a) that were opposite to those typically seen after administration of selective serotonin reuptake inhibitors (see Detke et al., 1995; Mague et al., 2003). Specifically, SalvA dose dependently increased occurrences of immobility ($F_{5,34} = 5.98$, $P < 0.01$) and decreased occurrences of swimming behavior ($F_{5,34} = 6.07$, $P < 0.01$). There was no effect on climbing or diving behaviors (data not shown). Post hoc analyses revealed that SalvA significantly increased immobility at 0.25 mg/kg ($P < 0.05$, Newman-Keuls test), 0.5 mg/kg ($P < 0.05$), 1.0 mg/kg ($P < 0.01$), and 2.0 mg/kg ($P < 0.01$). Similarly, SalvA significantly decreased swimming behavior at all doses from 0.25 to 2.0 mg/kg ($P < 0.01$). In contrast, SalvA caused no treatment-related differences in locomotor activity in an open field at any of the doses tested, regardless of whether the data were analyzed as 5-min bins (Fig. 2b) or as total activity over the 30-min test period (data not shown). As would be expected, activity levels decreased significantly in all groups over the course of the 30-min test period ($F_{5,260} = 350.5$, $P < 0.01$).

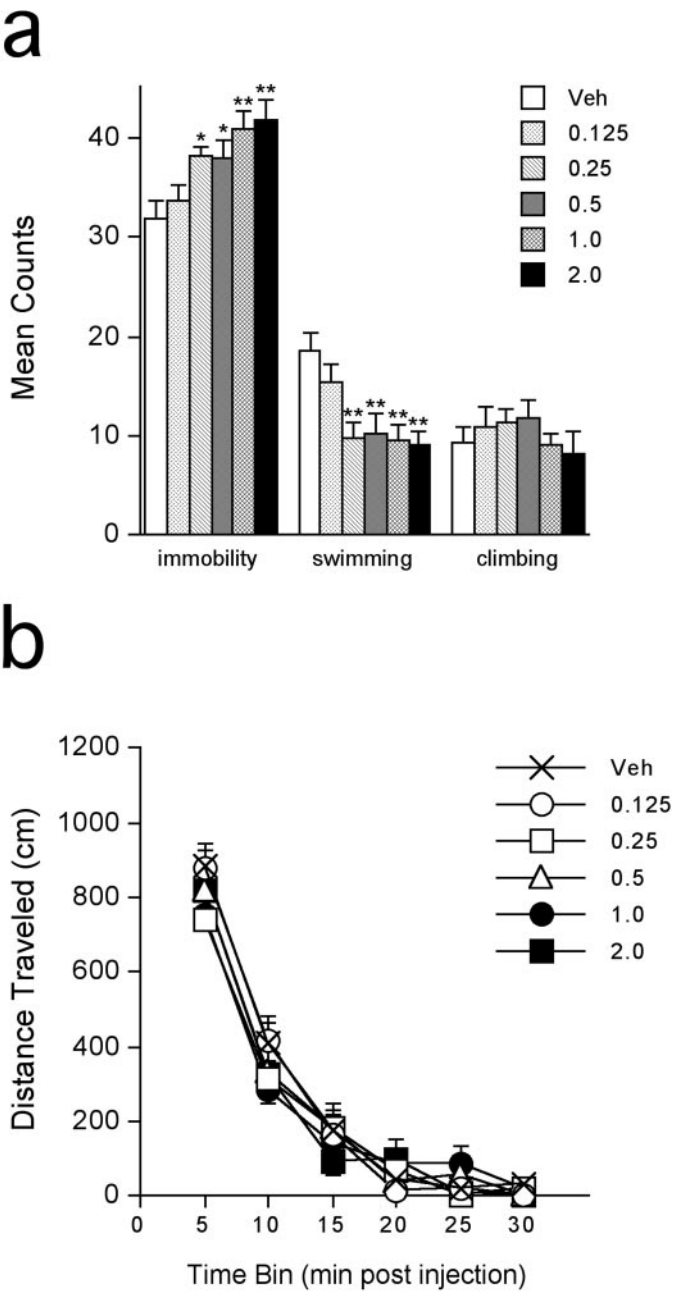

Fig. 2. Effects of SalvA on behavior in the FST and in an open field. Doses are expressed as milligram/kilogram. a, in the FST, SalvA increased occurrences of immobility and decreased occurrences of swimming, without affecting climbing (means ± S.E.M.). *, $P < 0.05$; **, $P < 0.01$ compared with vehicle, Newman-Keuls tests, six to eight rats/group. b, SalvA did not affect locomotor activity (means ± S.E.M.) at doses with prodepressant-like effects in the FST (8–11 rats per group).

In the ICSS assay, there was no effect of repeated testing with SalvA on thresholds or maximal rates at any of the doses tested (data not shown). Because there were no effects of repeated testing, data from the first and second test sessions were combined into single means for each condition. The effects of SalvA on ICSS thresholds depended upon treat-

ment ($F_{6,42} = 19.0$, $P < 0.01$); the drug produced effects on behavior (Fig. 3a) that were opposite to those typically seen after administration of psychomotor stimulant drugs such as cocaine (see Mague et al., 2003). Treatment with vehicle tended to cause small nonsignificant increases in ICSS thresholds and decreases in maximum response rates in comparison with saline, suggesting nonspecific effects of DMSO. In comparison with vehicle, SalvA significantly increased ICSS thresholds within the same range of doses that had behavioral effects in the FST: 0.5 mg/kg ($P < 0.05$), 1.0 mg/kg ($P < 0.01$), and 2.0 mg/kg ($P < 0.01$). SalvA also appeared to affect maximal response rates ($F_{6,42} = 3.72$, $P < 0.01$). However, post hoc analyses revealed that this effect was due to differences between saline treatment and SalvA at 1.0 and 2.0 mg/kg. There were no differences between maximum rates after vehicle and any dose of SalvA, again suggesting nonspecific effects of DMSO on behavior in this assay.

In the in vivo microdialysis studies, systemic administration of SalvA produced effects on extracellular concentrations of DA in the NAc that depended upon an interaction between dose and time ($F_{16,168} = 9.47$, $P < 0.01$) (Fig. 4a). Injection of 1.0 mg/kg SalvA produced rapid decreases in DA that were detectable for the entire 2-h test period ($P < 0.01$ for 0–100 min; $P < 0.05$ for 100–120 min), whereas DMSO or 0.125 mg/kg SalvA had no effect at any time point. In contrast, none of the treatments affected extracellular concentrations of 5-HT at any of the time points tested (Fig. 4b).

All ICSS electrodes were located within the medial forebrain bundle at the level of the lateral hypothalamus. The placements were indistinguishable from those depicted previously (Todtenkopf et al., 2004). Similarly, all microdialysis probes were located within the NAc (Fig. 5). As such, data from all rats were included in the analyses.

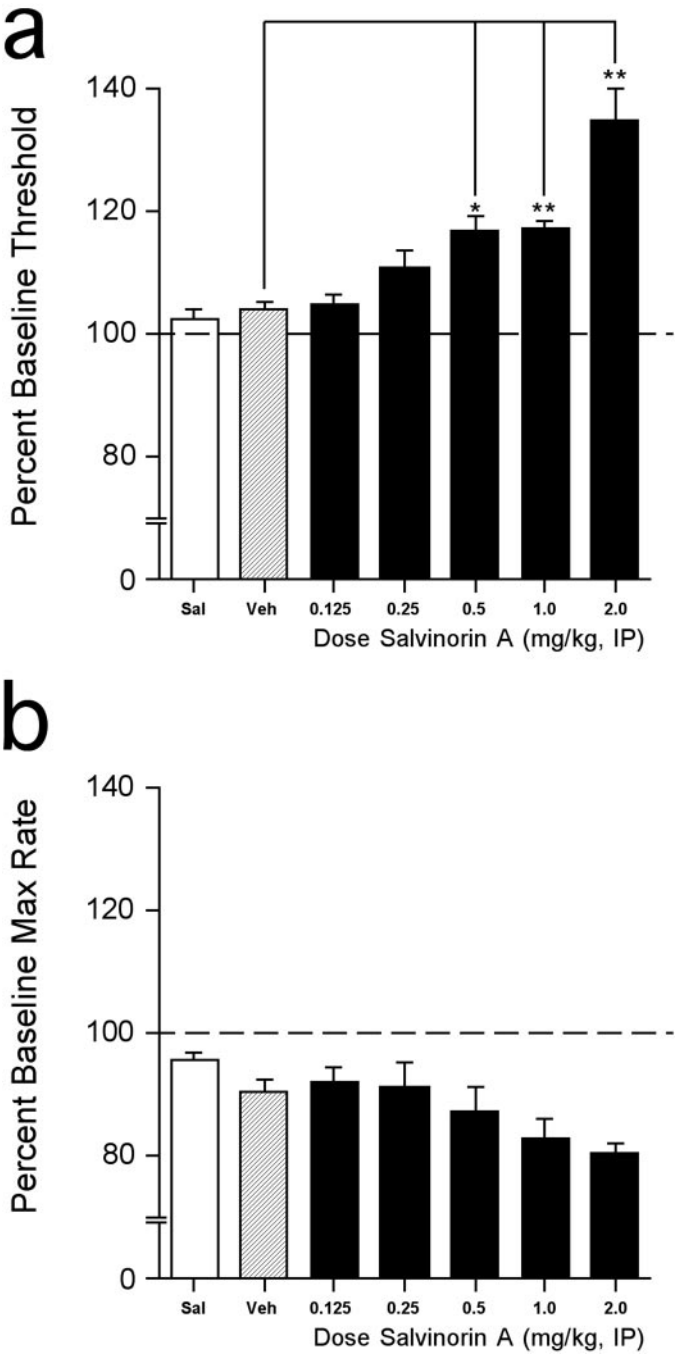

Fig. 3. Effect of SalvA on ICSS. a, treatment with vehicle (75% DMSO) tended to cause small nonsignificant increases in ICSS thresholds (mean ± S.E.M.) over the course of the 1-h test sessions. SalvA significantly increased ICSS thresholds at the doses that produced prodepressant-like effects in the FST. *, $P < 0.05$; **, $P < 0.01$ compared with vehicle, Newman-Keuls tests, seven rats per group. b, in comparison with vehicle, none of the treatments affected response capabilities (maximal rates).

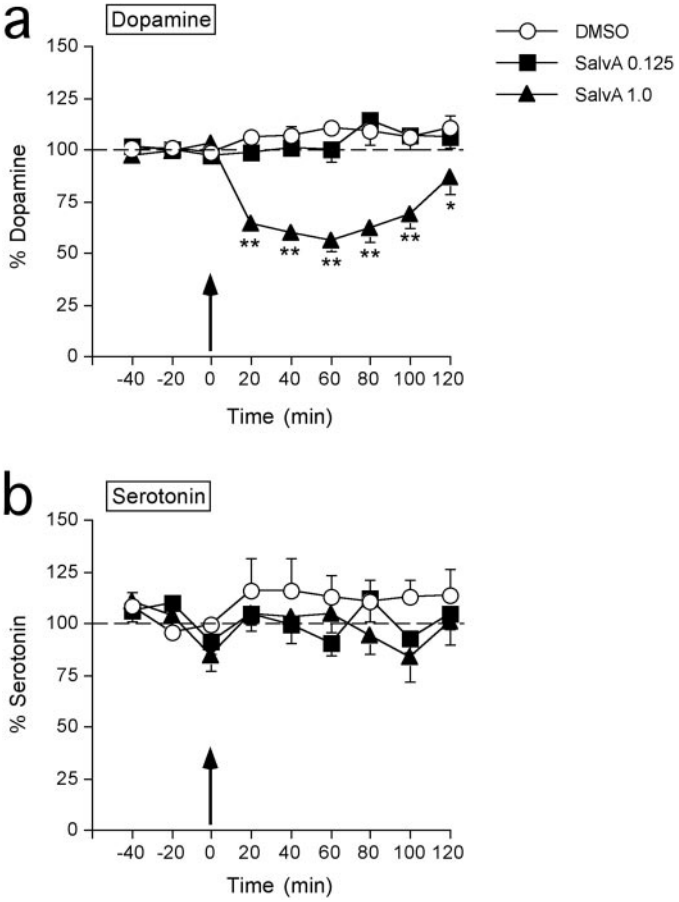

Fig. 4. Effect of SalvA on extracellular concentrations of DA and 5-HT in the NAc, as measured by in vivo microdialysis and HPLC. a, SalvA caused rapid and long-lasting decreases in extracellular concentrations of DA. *, $P < 0.05$; **, $P < 0.01$ compared with vehicle, Neuman-Keuls tests, eight rats per group. b, in contrast, the drug did not affect extracellular concentrations of 5-HT.

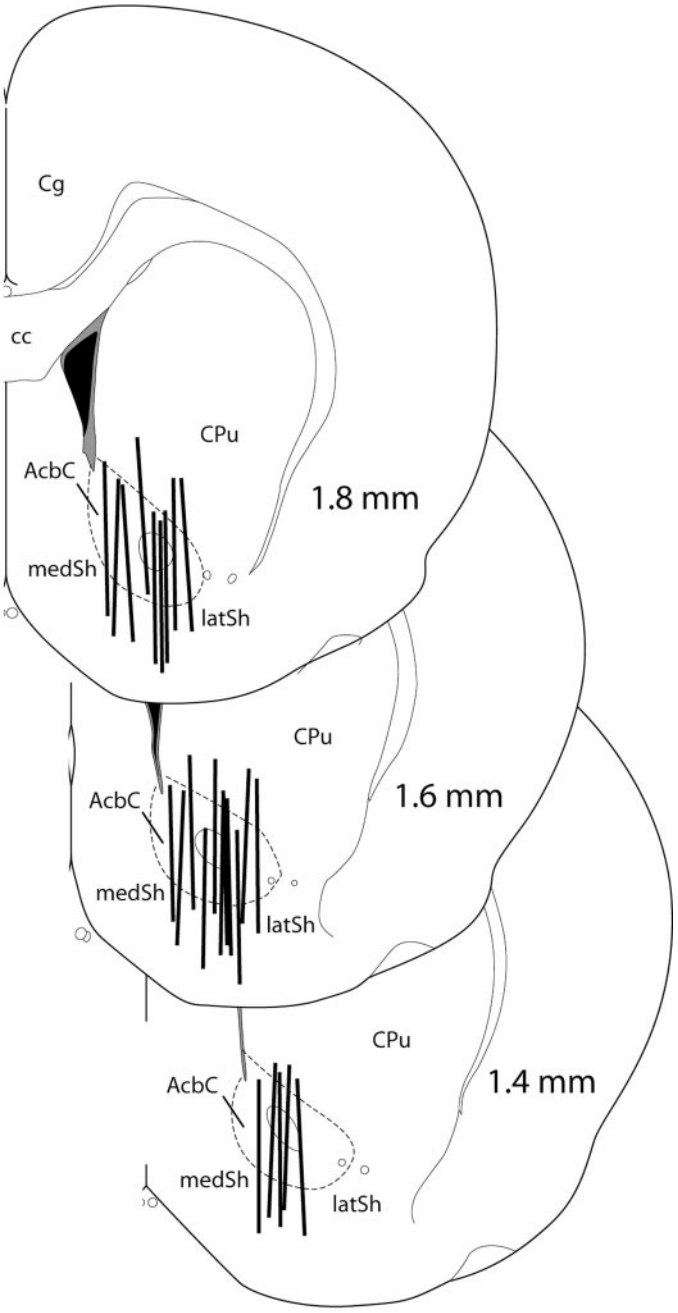

Fig. 5. Histological reconstruction of the areas sampled in the microdialysis studies. All probes were located in the NAc; the active portions of the membranes are depicted by the black lines.

Discussion

Systemic administration of SalvA—a potent, efficacious, and highly selective κ-opioid receptor agonist (Roth et al., 2002; Chavkin et al., 2004)—has profound behavioral effects in rats. SalvA increased the occurrences of immobility behavior in the FST, an assay often used in the study of depression (Cryan et al., 2002). This general effect is opposite to that seen with virtually all types of antidepressant treatments used in humans, including noradrenergic reuptake inhibitors and SSRIs (Porsolt et al., 1977; Detke et al., 1995; Mague et al., 2003). This finding suggests that SalvA produces prodepressant-like effects in the FST. The increase in immobility behavior was accompanied by a decrease in swimming behaviors. This specific effect is opposite to that seen with SSRIs, which decrease immobility and increase swimming behaviors (Detke et al., 1995). Although an increase in swimming behaviors is associated with the serotonergic actions of SSRIs, the significance of a decrease in swimming behaviors is unknown. This pattern of results might be an early indicator that the effects of SalvA on behavior in the FST involve 5-HT systems in critical brain regions. Moreover, SalvA increased ICSS thresholds, suggesting that it reduced the rewarding impact of medial forebrain bundle stimulation. Considering that reduced sensitivity to rewarding stimuli in rodents reflects anhedonia—a hallmark sign of clinical depression—these ICSS data provide additional evidence that SalvA produces depressive-like effects. We have reported previously that U-69593 produces the same pattern of results in these tests (Mague et al., 2003; Todtenkopf et al., 2004), suggesting a general effect of κ-agonists rather than a specific effect of SalvA. The potency and efficacy of SalvA in these tests are approximately equivalent to those seen with U-69593, although the present studies were designed to enable qualitative rather than quantitative comparisons between these agents. Considering that SalvA binds to few (if any) other types of receptors in the brain (Roth et al., 2002), these data suggest that selective stimulation of κ-receptors can produce depressive signs, at least in rats.

One concern when using the FST is that nonspecific treatment effects on activity levels could complicate data interpretation. We conducted locomotion studies in parallel with the FST studies to identify potentially confounding effects. To facilitate comparisons between the assays, the locomotion studies were conducted under conditions identical to those used in the FST studies, except during the second day of testing the rats were placed in activity chambers rather than being re-exposed to forced swimming. SalvA did not alter activity at any of the doses tested. Additionally, the methods we use to conduct the ICSS test also enable analyses of whether SalvA affects the performance capabilities of the rats. Although there were nominal decreases in response rates over the 1-h ICSS test session, this was a general effect of the 75% DMSO vehicle rather than a specific effect of SalvA. Thus it seems unlikely that nonspecific effects of SalvA on performance capabilities can explain the prodepressant-like effects of the drug in the FST or ICSS tests at the doses tested here.

The mechanisms that mediate these putative prodepressant actions of SalvA are not known. One possibility is that SalvA, through selective actions at κ-receptors, affects the function of DA systems. Indeed, previous reports in rodents indicate that systemic or intracerebroventricular administration of κ-agonists (e.g., U-50488H or the dynorphin analog E-2078) decreases extracellular concentrations of DA within the NAc (DiChiara and Imperato, 1988; Spanagel et al., 1992; Devine et al., 1993; Maisonneuve et al., 1994). These effects may be mediated, at least in part, within the NAc itself (Donzanti et al., 1992). Within the NAc, κ-receptors are located on the terminals of DA neurons (Shippenberg and Rea, 1997; Svingos et al., 1999), where they are in a position to regulate DA release. In addition, inhibitory actions within the midbrain may also contribute to decreased extracellular concentrations of DA within the NAc (Margolis et al., 2003). Regardless, our microdialysis data confirm previous observations that κ-agonists reduce activation of the mesolimbic DA

system and extend them to SalvA, which is more selective and efficacious than the other agents. In contrast to the effects of SalvA on extracellular DA in the NAc, the drug had no effects on 5-HT concentrations in this region. These data provide early evidence that the decreases in swimming activity in the FST do not reflect reductions in 5-HT activity within the NAc, although different effects in other brain regions (e.g., raphe nucleus) cannot be ruled out. Considered together, our data raise the possibility that decreases in DA in the NAc contribute to the depressive-like behaviors that were observed in the present studies. Indeed, it has been proposed previously (Wise, 1982) that decreased DA function in the NAc produces anhedonia.

Interestingly, a recent report in which in vivo microdialysis was performed in mice suggests that SalvA reduces extracellular concentrations of DA within the caudate putamen, but not within the NAc (Zhang et al., 2005). It is not clear why stimulation of κ-receptors in mice would produce a different pattern of results than those seen here with SalvA, or in previous studies with other κ-agonists (DiChiara and Imperato, 1988; Spanagel et al., 1992; Donzanti et al., 1992; Devine et al., 1993; Maisonneuve et al., 1994). This discrepancy raises the possibility that rats and mice have different κ-receptor distributions or different sensitivities to κ-opioid modulation of mesolimbic DA function, or that the small size of the striatum in mice makes it difficult to resolve the caudate putamen from the NAc in microdialysis studies.

Alterations in the function of κ-opioid systems may be part of a complex process that leads to the development and expression of depression or other mood disorders. Factors that can trigger depression in humans and depressive-like behaviors in laboratory animals—including stress and drug withdrawal—increase the activity of CREB in the NAc (Pliakas et al., 2001; Barrot et al., 2002; Shaw-Lutchman et al., 2002; Chartoff et al., 2003). Increased CREB activity in the NAc, in turn, leads to elevated expression of the gene for dynorphin (Carlezon et al., 1998). The possibility that increased stimulation of κ-receptors causes depressive-like behaviors is supported by the finding that selective κ-antagonists produce antidepressant-like effects in models used to study depression (Pliakas et al., 2001; Newton et al., 2002; Mague et al., 2003; McLaughlin et al., 2003). These findings, together with other evidence, have raised the possibility that selective κ-antagonists might have clinical utility as antidepressants (Pliakas et al., 2001; Mague et al., 2003) or treatments for drug addiction (Rothman et al., 2000). Under some circumstances, the ability of κ-agonists to decrease DA function in the basal forebrain might also have clinical utility. For example, antipsychotic drugs—which are frequently used to treat mania—activate dynorphinergic neurons (Ma et al., 2003). Although SalvA produces hallucinogenic effects under some conditions (e.g., when smoked) (Roth et al., 2002; Chavkin et al., 2004; Bucheler et al., 2005), it is conceivable that administration of a derivative or related compound under more carefully controlled conditions might be useful for the treatment of disorders characterized by hyperfunction of dopamine systems (e.g., mania). Less selective κ-agonists (e.g., enadoline, spiradoline) can produce measures of sedation and decrease the frequencies of tics in individuals with Tourette's Syndrome (Chappell et al., 1993; Walsh et al., 2001). Regardless, the present studies confirm and extend the idea that κ-opioid receptors may play a key role in regulating complex mood states.

References

Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, Impey S, Storm DR, Neve RL, Yin JC, et al. (2002) CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc Natl Acad Sci USA* **99:**11435–11440.

Baumann MH, Ayestas MA, Dersch CM, and Rothman RB (2001) 1-(m-Chlorophenyl)piperazine (mCPP) dissociates in vivo serotonin release from long-term serotonin depletion in rat brain. *Neuropsychopharmacology* **24:**492–501.

Bucheler R, Gleiter CH, Schwoerer P, and Gaertner I (2005) Use of nonprohibited hallucinogenic plants: increasing relevance for public health? A case report and literature review on the consumption of *Salvia divinorum* (Diviner's Sage). *Pharmacopsychiatry* **38:**1–5.

Carlezon WA Jr, Pliakas AM, Parrow AM, Detke MJ, Cohen BM, and Renshaw PF (2002) Antidepressant-like effects of cytidine in the forced swim test in rats. *Biol Psychiatry* **51:**882–889.

Carlezon WA Jr, Thome J, Olson V, Lane-Ladd SB, Brodkin ES, Hiroi N, Duman RS, Neve RL, and Nestler EJ (1998) Regulation of cocaine reward by CREB. *Science (Wash DC)* **282:**2272–2275.

Chappell PB, Leckman JF, Scahill LD, Hardin MT, Anderson G, and Cohen DJ (1993) Neuroendocrine and behavioral effects of the selective kappa agonist spiradoline in Tourette's syndrome: a pilot study. *Psychiatry Res* **47:**267–280.

Chartoff EH, Papadopoulou M, Konradi C, and Carlezon WA Jr (2003) Dopamine-dependent increases in CREB phosphorylation during precipitated morphine withdrawal in primary cultures of rat striatum. *J Neurochem* **87:**107–118.

Chavkin C, Sud S, Jin W, Stewart J, Zjawiony JK, Siebert DJ, Toth BA, Hufeisen SJ, and Roth BL (2004) Salvinorin A, an active component of the hallucinogenic sage *Salvia divinorum* is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther* **308:**1197–1203.

Cryan JF, Markou A, and Lucki I (2002) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **23:**238–245.

Detke MJ, Rickels M, and Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology* **121:**66–72.

Devine DP, Leone P, Pocock D, and Wise RA (1993) Differential involvement of ventral tegmental mu, delta, and kappa opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. *J Pharmacol Exp Ther* **266:**1236–1246.

DiChiara G and Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85:**5274–5278.

Donzanti BA, Althaus JS, Payson MM, and Von Voigtlander PF (1992) Kappa agonist-induced reduction in dopamine release: site of action and tolerance. *Res Commun Chem Pathol Pharmacol* **78:**193–210.

Lee DY-W, Ma Z, Liu-Chen L-Y, Wang Y, Chen Y, Carlezon WA Jr, and Cohen BM (2005) Three new neoclerodane diterpenoids from the leaves of *Salvia divinorum*. *Bioorg Med Chem* **13:**5635–5639.

Ma J, Ye N, Lange N, and Cohen BM (2003) Dynorphinergic GABA neurons are a target of both typical and atypical antipsychotic drugs in the nucleus accumbens shell, central amygdaloid nucleus and thalamic central medial nucleus. *Neurosci* **121:**991–998.

Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC Jr, Jones RM, Portoghese PS, and Carlezon WA Jr (2003) Antidepressant-like effects of κ-opioid receptor antagonists in the forced swim test in rats. *J Pharmacol Exp Ther* **305:**323–330.

Maisonneuve IM, Archer S, and Glick SD (1994) U50,488, a κ-opioid receptor agonist, attenuates cocaine-induced increases in extracellular dopamine in the nucleus accumbens of rats. *Neurosci Lett* **181:**57–60.

Margolis EB, Hjelmstad GO, Bonci A, and Fields HL (2003) Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. *J Neurosci* **23:**9981–9986.

McLaughlin JP, Marton-Popovici M, and Chavkin C (2003) Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J Neurosci* **23:**5674–5683.

Miliaressis E, Rompre PP, Laviolette P, Philippe L, and Coulombe D (1986) The curve-shift paradigm in self-stimulation. *Physiol Behav* **37:**85–91.

Munro TA, Rizzacasa MA, Roth BL, Toth BA, and Yan F (2005) Studies toward the pharmacophore of salvinorin A, a potent kappa opioid receptor agonist. *J Med Chem* **48:**345–348.

Nestler EJ and Carlezon WA Jr (2005) The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry*, in press.

Newton SS, Thome J, Wallace TL, Shirayama Y, Schlesinger L, Sakai N, Chen J, Neve R, Nestler EJ, and Duman RS (2002) Inhibition of cAMP response element-binding protein or dynorphin in the nucleus accumbens produces an antidepressant-like effect. *J Neurosci* **22:**10883–10890.

Pasquier DA, Kemper TL, Forbes WB, and Morgane PJ (1977) Dorsal raphe, substantia nigra and locus coeruleus: interconnections with each other and the neostriatum. *Brain Res Bull* **2:**323–339.

Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd ed., Academic Press, San Diego, CA.

Pfeiffer A, Brantl V, Herz A, and Emrich HM (1986) Psychotomimesis mediated by kappa opiate receptors. *Science (Wash DC)* **233:**774–776.

Pliakas AM, Carlson R, Neve RL, Konradi C, Nestler EJ, and Carlezon WA Jr (2001) Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element binding protein expression in nucleus accumbens. *J Neurosci* **21:**7397–7403.

Porsolt RD, Le Pichon M, and Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature (Lond)* **266:**730–732.

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

Rothman RB, Gorelick DA, Heishman SJ, Eichmiller PR, Hill BH, Norbeck J, and Liberto JG (2000) An open-label study of a functional opioid kappa antagonist in the treatment of opioid dependence. *J Subst Abuse Treat* **18:**277–281.

Shaw-Lutchman TZ, Barrot M, Wallace T, Gilden L, Zachariou V, Impey S, Duman RS, Storm D, and Nestler EJ (2002) Regional and cellular mapping of cAMP response element-mediated transcription during naltrexone-precipitated morphine withdrawal. *J Neurosci* **22:**3663–3672.

Shippenberg TS and Herz A (1987) Place preference conditioning reveals the involvement of D1-dopamine receptors in the motivational properties of mu- and kappa-opioid agonists. *Brain Res* **436:**169–172.

Shippenberg TS and Rea W (1997) Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* **57:**449–455.

Spanagel R, Herz A, and Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci USA* **89:**2046–2050.

Svingos AL, Colago EE, and Pickel VM (1999) Cellular sites for dynorphin activation of kappa-opioid receptors in the rat nucleus accumbens shell. *J Neurosci* **19:**1804–1813.

Todtenkopf MS, Marcus JF, Portoghese PS, and Carlezon WA Jr (2004) Effects of kappa opioid ligands on intracranial self-stimulation in rats. *Psychopharmacology* **172:**463–470.

Walsh SL, Strain EC, Abreu ME, and Bigelow GE (2001) Enadoline, a selective kappa opioid agonist: comparison with butorphanol and hydromorphone in humans. *Psychopharmacology* **157:**151–162.

Wise RA (1982) Neuroleptics and operant behavior: the anhedonia hypothesis. *Behav Brain Sci* **5:**39–87.

Wise RA (1998) Drug-activation of brain reward pathways. *Drug Alcohol Depend* **51:**13–22.

Zhang Y, Butelman ER, Schlussman SD, Ho A, and 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.

Address correspondence to: Dr. Bill Carlezon, Department of Psychiatry, McLean Hospital, MRC 217, 115 Mill Street, Belmont, MA 02478. E-mail: bcarlezon@mclean.harvard.edu