Pharmacology Biochemistry and Behavior, Vol. 57, Nos. 1/2, pp. 89–100, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00117-7

Persistent Increases in Basal Cerebral Metabolic Activity Induced by Morphine Sensitization

MARK A. KRAUS, JEAN M. PIPER AND CONAN KORNETSKY[1]

Departments of Pharmacology and Psychiatry, Boston University School of Medicine, Boston, MA 02118

Received 15 December 1995; Revised 5 March 1996; Accepted 20 March 1996

KRAUS, M. A., J. M. PIPER AND C. KORNETSKY. *Persistent increases in basal cerebral metabolic activity induced by morphine sensitization.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 89–100, 1997.—To characterize the underlying neuroanatomic substrate of morphine (MS) sensitization, changes in the local cerebral metabolic rate for glucose ($LCMR_{glu}$) were examined in 95 brain regions of male F-344 rats using the 2-deoxy-D-[1-^{14}C]glucose method. The results of these experiments demonstrate that MS-induced sensitization is manifested by increases in basal metabolic activity that last for at least 6 days. Although changes in basal metabolic rate were found to be more extensive in the presence of conditioned cues, the increases in $LCMR_{glu}$ in nonconditioned sensitized rats indicate a basic underlying pharmacologic effect of MS sensitization on basal brain activity. Regions in which MS sensitization had a lasting pharmacologic effect include the shell of the nucleus accumbens, the prelimbic area of the prefrontal cortex, and the dorsolateral prefrontal cortex. Interestingly, the core of the nucleus accumbens and regions of the caudate were found to have an increased $LCMR_{glu}$ only in the presence of conditioned cues, indicating conditioned brain activity without observable changes in behavior. The previous administration of an MS-sensitizing treatment was also found to alter the cerebral metabolic response to a subsequent acute MS challenge (0.5 mg/kg, subcutaneously), most notably in forebrain systems. The more widespread activation of brain structures in the basal state in the presence of conditioned cues suggests that these MS-sensitized rats may model an altered brain state related to craving in the abstinent opiate addict. © 1997 Elsevier Science Inc.

Morphine Oral stereotypy Conditioning Glucose utilization 2-Deoxyglucose Nucleus accumbens Craving Limbic system Extrapyramidal system

HIGH doses of morphine sulfate (MS), repeatedly administered to rats, result in a progressive increase in the expression of oral stereotypy characterized by intense gnawing and biting behavior (15,46). The reexpression of biting behavior in response to a subsequent low dose MS challenge by pretreated rats, but not MS-naive rats (43,56,57), demonstrates the presence of MS-induced behavioral sensitization. However, in the absence of an MS challenge, oral stereotypy is not expressed and sensitized rats appear to be qualitatively similar to nonsensitized rats. Because sensitization has been observed to endure for months after the initial treatment (3,57), it appears that long-lasting changes in basal brain function have occurred and are responsible for maintaining the increased susceptibility to the oral stereotypic effects of MS.

The persistence of an enhanced responsiveness to subsequent MS challenges indicates that some aspect of basal brain physiology has been altered as a result of MS sensitization and is involved in maintaining the sensitization process in the absence of additional drug administration. Although an altered mesolimbic dopamine transmission is involved in the initiation and expression of sensitization (33), it is not indicated in the long-term endurance of sensitization (29–32,72). In fact, the mechanisms responsible for preserving sensitization for long periods remain unknown.

Using the quantitative 2-deoxy-D[1-^{14}C]glucose (2-DG) technique, the present study was designed to test the hypothesis that MS-sensitized rats have an altered basal metabolic activity in distinct brain regions 6 days after the completion of the sensitizing treatment. Because these sensitized rats are examined in the absence of an MS challenge, changes in local

[1]Requests for reprints should be addressed to C. Kornetsky, Boston University School of Medicine, 80 East Concord Street, L-602, Boston, MA 02118.

TABLE 1

TREATMENT REGIMEN ADMINISTERED TO EACH GROUP OF THE TWO EXPERIMENTS

	Experiment 1 (Conditioned Cues Present)				Experiment 2 (Conditioned Cues Absent)	
Injection Time	Non-Sz-Sal*	Non-Sz-MS	Sz-Sal	Sz-MS	Non-Sz-nc*	Sz-nc
0 h	Saline	Saline	Morphine (10 mg/kg)	Morphine (10 mg/kg)	Saline	Morphine (10 mg/kg)
12 h	Saline	Saline	Morphine (10 mg/kg)	Morphine (10 mg/kg)	Saline	Morphine (10 mg/kg)
24 h	Saline	Saline	Morphine (10 mg/kg)	Morphine (10 mg/kg)	Saline	Morphine (10 mg/kg)
36 h	Saline	Saline	Morphine (10 mg/kg)	Morphine (10 mg/kg)	Saline	Morphine (10 mg/kg)
7 days	Saline	Morphine (0.5 mg/kg)	Saline	Morphine (0.5 mg/kg)	None	None

*Because the saline controls of each experiment did not differ significantly from each other, they were combined. Sal indicates the administration of a saline injection (1 ml/lg, SC). All injections were administered subcutaneously at the indicated dose.

cerebral metabolic rate for glucose ($LCMR_{glu}$) will indicate brain regions in which an altered basal synaptic activity is involved in the persistence of MS sensitization. In addition, because classical conditioning has been reported to be involved in sensitization to the locomotor-activating effects of both MS and psychostimulants (14,47,58,59,73), the basal $LCMR_{glu}$ was analyzed in sensitized rats in both the presence and absence of environmental cues that could act as conditioned stimuli. Also, we sought to determine whether the MS-sensitizing treatment altered MS-evoked changes in cerebral metabolism, by comparing $LCMR_{glu}$ in brain regions of sensitized and nonsensitized rats challenged with a low, behaviorally ineffective dose of MS. We were interested not only in which brain structures had an altered metabolic response to MS, but also in testing the hypothesis that MS-induced sensitization involves a hyperactivity of the mesolimbic system during a subsequent drug challenge.

METHODS

Subjects and Drug Treatment

Forty-three male Fischer-344 rats (300–350 g) were used in this study. Rats were maintained on a 12 L:12 D cycle (lights on at 0700 h), housed individually in stainless-steel cages and received food and water ad lib. All animals were handled extensively before testing.

Experiment 1: Basal and MS-Evoked Effects

Rats were first divided into two groups (Table 1) and given one of two pretreatment regimens: (a) the MS-sensitizing treatment (Sz), which consisted of four injections of morphine sulfate (MS) [10 mg/kg, subcutaneously (sc)] administered at 12-h intervals; or (b) a nonsensitizing treatment (non-Sz), which consisted of four saline injections (1 ml/kg, SC) administered at similar intervals. Following each treatment injection, rats were placed in the 2-DG test chamber for 1 h. Six days after the completion of the pretreatment, half the rats from each treatment group received an injection of 0.5 mg/kg (SC) MS, while the other half received an injection of saline. The challenge injection was administered 10 min before the 2-DG infusion. Four groups were studied in this experiment ($n = 8$ each): non-Sz-Sal, non-Sz-MS, Sz-Sal, and Sz-MS.

The MS-sensitizing treatment was based on previous investigations in our laboratory demonstrating the enhancement of oral stereotypy following later MS challenges (43,56,57). Although sensitization persists for months after the initial treatment, we used a relatively short time period between the establishment of MS sensitization and testing to maximize the possibility of measurable effects using the 2-DG procedure, while avoiding the presence of MS withdrawal (25,46,78) and the effects it would have on $LCMR_{glu}$. The challenge dose of MS (0.5 mg/kg, SC) was chosen because of its inability to evoke oral stereotypy in MS-sensitized rats (unpublished observations). The expression of MS-induced oral stereotypy was avoided, due to concerns that the cerebral metabolic effects of this behavior would confound the characterization of the underlying neuroanatomic substrate of MS-induced sensitization. Finally, the 10-min delay between MS administration and 2-DG infusion was derived from previous investigations from our laboratory that demonstrated significant MS effects on $LCMR_{glu}$ (20,42).

Experiment 2: Nonconditioned Effects

After analyzing the results of Experiment 1, a second experiment (Table 1) was designed to test for the concurrent expression of conditioned responding with pharmacologic effect in the MS-sensitized group (Sz-Sal). To determine which changes in basal $LCMR_{glu}$ are due to the pharmacologic treatment completed 6 days earlier and not to conditioned responding elicited by drug-associated stimuli, the treatment regimen was altered to exclude any potential association to drug effect. There were two potential drug associated stimuli in the pretreatment regimen described in Experiment 1: the injection experience 10 min before the 2-DG infusion and the test environment. The first potential conditioned stimulus was avoided by not administering an acute injection of saline on the test day. The second conditioned stimulus, the test chamber, was avoided by first acclimating rats to the test apparatus before the sensitizing treatment and then placing the rat in a neutral environment rather than the test chamber (as in Experiment 1) immediately after each treatment injection.

Experiment 2 consisted of two groups (Table 1): (a) a nonconditioned MS-sensitized group (Sz-nc) and (b) a nonconditioned nonsensitized group (non-Sz-nc), $n = 8$ and 3, respectively.

Although we did not expect the non-Sz-nc rats to differ from the non-Sz-Sal group (Experiment 1), three additional control subjects were added because Experiment 2 was carried out after the completion of Experiment 1. Because the two control groups do not differ significantly from each other, they were combined and served as the control for both experiments. To keep the number of subjects in the control group close to the number in experimental groups, controls with the earliest calendar dates of experimentation (non-Sz-Sal group) were dropped from the analysis. In analyses the treatment groups will be compared with this combined (non-Sz-control) group.

Measurement of Local Cerebral Metabolic Rate for Glucose

$LCMR_{glu}$ was measured according to the procedure described by Sokoloff and colleagues (67) and adapted for freely moving animals by Crane and Porrino (11). On the day of the experiment, each rat was anesthetized with a mixture of halothane and surgical-grade air while polyethylene catheters (PE50) were inserted into an ipsilateral femoral vein and artery. The catheters were threaded subcutaneously and exited via an incision at the nape of the neck. This approach allowed the animal to be unrestrained during the experimental procedure. Following postoperative recovery of at least 3 h, the rat was placed in the experimental chamber. Ten minutes after placement into the chamber, blood pressure was measured using a mercury manometer and the hematocrit was recorded. At this time, plasma glucose and background plasma radioactivity were also determined. Next, MS (0.5 mg/kg, SC) or saline was administered followed, 10 min later, by an infusion of 2-DG at a dose of 125 μCi/kg (sp. act. 50–55 mCi/mmol; New England Nuclear, Boston, MA) via the femoral venous catheter. Timed arterial blood samples were drawn during the 45-min experimental period. Samples were collected at the following times: continuous sampling from 0 to 30 s, 45 s, and 1, 2, 3, 5, 7.5, 10, 15, 25, 35, and 45 min. Blood samples were immediately centrifuged and plasma concentrations of 2-DG were determined by liquid scintillation counting (Beckman Instruments, Fullerton, CA). Plasma glucose concentrations were assayed by means of a glucose analyzer (Beckman Instruments). Rats were killed at the end of the 45-min experimental period by an intravenous injection of 50 mg sodium pentobarbital. Brains were rapidly removed, frozen in isopentane (−35°C), and stored in a freezer at −86°C until sectioning. Coronal brain sections were cut at 20 μm in a cryostat maintained at −24°C to −29°C. The sections were thaw-mounted onto glass cover slips, dried on a hotplate (70°C), and apposed to X-ray film (Kodak EMC-1; Rochester, NY) with [^{14}C]methyl methacrylate standards (Amersham, Arlington Heights, IL) in stainless-steel cassettes for 12 days.

Autoradiograms were analyzed by quantitative densitometry with a computerized-image processing system (MCID; Imaging Research, St. Catherine's, Ontario, Canada). Optical density measurements for each structure were made in a minimum of four of six consecutive brain sections per animal. Anatomic structures were identified according to the rat brain atlases of Paxinos and Watson (54) and Swanson (69). When necessary, autoradiograms were compared with adjacent thionin-stained sections. The optical densities were converted to ^{14}C concentrations on the basis of a calibration curve determined by densitometric analysis of the autoradiograms of the calibrated ^{14}C standards. The lumped constant used for each rat in the operational equation was based on its average arterial plasma glucose concentration and the published lumped constant equivalents (66).

Data Analysis

Student *t*-tests for independent samples were performed to determine the effects of drug treatment compared with saline. Our analysis was restricted to differences that yielded a $p < 0.01$ confidence level to reduce the probability of a type I statistical error. Two other levels of significance ($p < 0.02$ and $p < 0.05$) are also listed in Tables 2–4 so that relative probability levels may be assessed by the reader.

RESULTS

Tables 2–4 present the mean $LCMR_{glu}$ values obtained from the treatment groups of Experiments 1 and 2 in each of the 95 regions analyzed, along with the results of the statistical analysis. As discussed in the Methods section, the control group consists of the last five subjects in the non-Sz-Sal plus the three subjects in the non-Sz-nc group. These two control groups were not significantly different from each other ($p > 0.05$).

Experiment 1: Effects of MS Sensitization on Basal $LCMR_{glu}$ (Sz-Sal vs. Non-Sz-Controls)

To determine whether basal metabolic activity was altered following the sensitizing treatment, glucose utilization following a saline challenge administered to sensitized (Sz-Sal) was compared with nonsensitized controls (non-Sz-control). Significant increases ($p < 0.01$) in $LCMR_{glu}$ were found in 54 of the 95 brain regions analyzed in the Sz-Sal rats 6 days following the completion of the MS-sensitizing treatment (Tables 2–4). Within the limbic system (Table 2), significant increases ($p < 0.01$) were present in 22 of the 40 regions analyzed. Significant elevations in $LCMR_{glu}$ were also found in 12 of the 17 areas of the basal ganglia and seven of the nine cortical areas analyzed (Table 3). Also, 10 of the 11 thalamic nuclei and three of the 18 hindbrain regions analyzed were found to have a significantly elevated metabolic rate in Sz-Sal rats compared with non-Sz-controls (Table 4). These results indicate that in the absence of drug challenge, MS-sensitized rats have an abnormally high glucose utilization throughout the brain.

Effects of MS Challenge on $LCMR_{glu}$ in Nonsensitized Rats (Non-Sz-MS vs. Non-Sz-Control). To characterize the acute effect of a low-dose MS challenge in nonsensitized rats, the $LCMR_{glu}$ following the non-Sz-MS treatment was compared with the saline controls. The MS challenge (0.5 mg/kg, SC) produced a generalized reduction in rates of glucose use throughout the brain compared with saline controls. This dose of MS significantly ($p < 0.01$) reduced $LCMR_{glu}$ in 12 of the 95 areas analyzed. Significant decreases occurred in three of the 40 limbic regions (Table 2), six of the 11 thalamic nuclei, and three of the 18 hindbrain (Table 4) structures analyzed.

Effects of MS Challenge on $LCMR_{glu}$ in Sensitized Rats (Sz-MS vs. Sz-Sal). To determine the acute effect of an MS challenge on brain metabolism in sensitized rats, the $LCMR_{glu}$ following the Sz-MS treatment was compared with the Sz-Sal treatment. The results of this comparison are interesting because sensitized behaviors are only expressed in the presence of an MS challenge. However, as stated in the Methods section, a subthreshold dose of MS was used to avoid the confound of expressed oral stereotypy on the effects of MS on the brain

TABLE 2

MEAN ± SEM LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE (μMOL/100 G/PER MIN) IN LIMBIC REGIONS OF NONSENSITIZED AND SENSITIZED RATS AFTER ADMINISTRATION OF A SALINE OR MS CHALLENGE

Brain Regions Analyzed (Limbic system)	Experiments 1 and 2 Saline Controls[a]	Experiment 1 (Conditioned Cues Present)			Experiment 2 (Conditioned Cues Absent)
		Non-Sz-MS	Sz-Sal	Sz-MS	Sz-nc
Nucleus accumbens, core—rostral	71.2 ± 2.6 (8)	68.8 ± 1.4 (8)	83.2 ± 0.7 (7)***	74.6 ± 2.0 (7)‡‡	76.4 ± 1.8 (8)
Nucleus accumbens, core—caudal	57.5 ± 1.9 (8)	56.5 ± 0.8 (8)	66.3 ± 1.6 (8)***	58.0 ± 1.6 (8)‡‡	55.3 ± 1.4 (8)
Nucleus accumbens, shell—rostral	65.1 ± 3.0 (8)	66.2 ± 1.6 (8)	80.5 ± 1.0 (7)***	69.7 ± 2.1 (7)‡‡	77.4 ± 1.4 (8)***
Nucleus accumbens, shell—caudal	48.2 ± 1.9 (8)	48.1 ± 1.0 (8)	54.8 ± 1.5 (8)**	50.7 ± 1.1 (8)	49.3 ± 1.5 (8)
Nucleus accumbens, whole—rostral	68.1 ± 2.5 (8)	67.2 ± 1.8 (8)	81.4 ± 0.9 (7)***	72.4 ± 2.2 (7)‡‡	75.3 ± 1.9 (8)*
Nucleus accumbens, whole—caudal	51.5 ± 1.9 (7)	52.1 ± 0.7 (5)	59.3 ± 0.4 (5)***	53.7 ± 1.2 (6)‡‡	51.4 ± 1.2 (7)
Olfactory tubercle, anteroventral	89.3 ± 5.4 (6)	82.8 ± 2.7 (6)	95.7 ± 3.0 (8)	94.4 ± 2.1 (6)	96.6 ± 4.4 (6)
Olfactory tubercle, anterodorsal	86.6 ± 4.0 (6)	82.5 ± 2.3 (6)	95.1 ± 3.3 (8)	89.9 ± 4.1 (6)	81.5 ± 4.3 (7)
Olfactory tubercle, posteroventral	74.4 ± 3.4 (7)	69.6 ± 2.0 (7)	78.2 ± 1.9 (5)	75.6 ± 3.6 (6)	82.3 ± 2.2 (3)
Olfactory tubercle, posterodorsal	65.8 ± 2.5 (7)	63.1 ± 2.2 (7)	72.9 ± 1.4 (5)*	71.3 ± 4.1 (6)	67.2 ± 2.7 (4)
Ventral pallidum	46.4 ± 0.9 (8)	45.3 ± 1.0 (8)	53.3 ± 0.8 (8)***	52.2 ± 0.6 (8)	52.4 ± 1.0 (7)***
Ventral tegmental area—rostral	54.3 ± 1.6 (7)	51.5 ± 1.7 (7)	61.0 ± 2.0 (8)*	57.8 ± 2.1 (8)	58.3 ± 2.1 (8)
Ventral tegmental area—caudal	54.3 ± 1.7 (7)	51.4 ± 3.0 (6)	60.6 ± 2.1 (7)*	56.9 ± 1.9 (8)	57.0 ± 2.6 (7)
Major Isle of Calleja	64.0 ± 1.8 (8)	65.6 ± 2.3 (8)	74.0 ± 1.2 (8)***	67.4 ± 2.0 (8)‡	68.1 ± 1.8 (8)
Septohippocampal nucleus	64.5 ± 2.1 (8)	64.9 ± 2.0 (8)	76.5 ± 1.3 (8)***	66.4 ± 2.0 (8)‡‡	68.0 ± 1.8 (7)
Medial septal nucleus	54.9 ± 1.7 (8)	54.0 ± 3.4 (8)	62.1 ± 1.3 (8)***	57.2 ± 2.1 (7)	55.0 ± 2.2 (7)
Lateral septal nucleus	46.2 ± 1.8 (8)	43.4 ± 1.9 (8)	51.4 ± 1.3 (8)*	46.9 ± 1.7 (8)	46.1 ± 0.9 (8)
Diagonal band, vertical limb	63.1 ± 1.7 (7)	60.1 ± 2.1 (7)	73.0 ± 1.5 (8)***	66.5 ± 1.3 (8)‡‡	69.7 ± 1.4 (7)***
Diagonal band, horizontal limb	71.1 ± 1.8 (8)	70.7 ± 1.4 (8)	80.6 ± 1.0 (8)***	78.3 ± 1.1 (8)	82.6 ± 2.2 (6)***
Amygdala, basolateral nucleus	69.7 ± 2.2 (8)	64.7 ± 1.3 (8)	83.5 ± 1.5 (7)***	71.6 ± 1.0 (8)‡‡	78.1 ± 2.2 (8)**
Amygdala, lateral nucleus	55.2 ± 1.9 (8)	50.5 ± 1.2 (8)	63.3 ± 1.6 (7)***	55.2 ± 1.5 (8)‡‡	57.5 ± 1.7 (8)
Amygdala, central nucleus	42.7 ± 1.7 (8)	43.8 ± 1.1 (8)	52.2 ± 1.4 (7)***	48.7 ± 0.7 (8)†	48.5 ± 1.0 (8)***
Amygdala, medial nucleus	44.7 ± 2.4 (8)	43.6 ± 1.4 (8)	51.0 ± 1.9 (5)	50.3 ± 2.0 (6)	45.0 ± 1.5 (7)
Amygdala, posteromedial cortical nucleus	61.5 ± 3.5 (7)	51.3 ± 2.8 (8)	74.2 ± 4.9 (5)*	67.3 ± 5.9 (6)	81.4 ± 1.4 (7)***
Amygdala, posterolateral cortical nucleus	70.3 ± 3.0 (6)	74.4 ± 1.5 (8)	82.6 ± 1.4 (5)***	82.1 ± 3.2 (6)	82.8 ± 1.6 (7)***
Bed nucleus of the stria terminalis	39.0 ± 1.0 (8)	40.8 ± 1.3 (8)	45.8 ± 0.7 (8)***	43.2 ± 1.3 (8)	42.5 ± 0.6 (7)***
Medial habenular nucleus	65.1 ± 2.1 (8)	58.3 ± 1.4 (8)*	75.8 ± 2.5 (6)***	68.3 ± 1.6 (8)†	68.2 ± 2.1 (7)
Lateral habenular nucleus, medial	73.3 ± 1.9 (8)	64.5 ± 1.8 (8)**	85.4 ± 3.5 (6)***	73.9 ± 2.2 (8)‡	79.2 ± 2.4 (7)
Lateral habenular nucleus, lateral	93.7 ± 3.2 (8)	78.6 ± 2.0 (8)**	106.9 ± 4.1 (6)*	90.1 ± 2.4 (8)‡‡	98.0 ± 4.8 (7)
Dentate gyrus	52.2 ± 2.0 (8)	47.9 ± 1.3 (8)	58.8 ± 1.8 (7)*	56.6 ± 1.7 (8)	53.0 ± 1.5 (8)
Dorsal hippocampus, CA 1	48.9 ± 2.0 (8)	45.2 ± 1.6 (8)	57.6 ± 1.1 (7)***	55.7 ± 2.1 (8)	48.4 ± 1.3 (8)
Mid hippocampus, CA 3	62.0 ± 2.8 (7)	57.3 ± 2.8 (6)	65.6 ± 5.1 (5)	70.7 ± 3.6 (8)	69.2 ± 1.7 (8)*
Subiculum	72.6 ± 2.1 (8)	63.4 ± 1.6 (8)*	84.7 ± 3.0 (7)***	71.3 ± 1.6 (8)‡‡	77.7 ± 1.5 (5)
Mammilary body	98.1 ± 3.0 (7)	82.8 ± 1.4 (5)***	114.7 ± 4.8 (8)**	99.2 ± 3.3 (8)‡	105.7 ± 3.9 (7)
Anterior pretectal area	85.9 ± 1.8 (8)	69.7 ± 1.4 (7)***	97.6 ± 1.7 (8)***	85.7 ± 1.4 (8)‡‡	85.8 ± 3.9 (8)
Lateral hypothalamus	51.6 ± 1.7 (8)	48.8 ± 1.1 (8)	60.7 ± 1.6 (7)***	54.1 ± 1.3 (8)‡‡	54.7 ± 1.2 (8)
Medial preoptic area	42.3 ± 1.6 (7)	41.1 ± 1.3 (8)	45.1 ± 1.6 (8)	45.5 ± 2.0 (8)	42.0 ± 1.2 (7)
Lateral preoptic area	59.0 ± 2.8 (7)	55.4 ± 1.2 (8)	62.1 ± 1.1 (8)	61.0 ± 1.6 (8)	62.8 ± 1.9 (7)
Periventricular hypothalamic nucleus	46.0 ± 1.3 (6)	42.1 ± 1.9 (5)	51.8 ± 1.9 (5)*	46.8 ± 1.4 (7)	43.4 ± 2.5 (5)
Suprachiasmatic nucleus	78.0 ± 1.5 (6)	64.6 ± 3.1 (5)**	78.8 ± 4.3 (5)	69.5 ± 1.7 (8)	84.2 ± 4.0 (5)

[a] The saline control group is composed of controls from both experiments 1 and 2. Refer to Table 1 and Methods for further descriptions of these groups.

*,**,*** Differences from the saline control (non-Sz-Sal) group $p < 0.05$, $p < 0.02$, $p < 0.01$; two tailed *t*-test.

†, ‡, ‡‡ indicate differences between Sz-Sal and Sz-MS $p < 0.05$, $p < 0.02$, $p < 0.01$; two tailed *t*-test.

TABLE 3

MEAN ± SEM LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE (μMOL/100 G/PER MIN) IN REGIONS OF THE BASAL GANGLIA AND CEREBRAL CORTEX OF NONSENSITIZED AND SENSITIZED RATS AFTER ADMINISTRATION OF A SALINE OR MS CHALLENGE

Brain Regions Analyzed	Experiments 1 and 2 Saline Controls[a]	Experiment 1 (Conditioned Cues Present)			Experiment 2 (Conditioned Cues Absent)
		Non-Sz-MS	Sz-Sal	Sz-MS	Sz-nc
Basal Ganglia					
Dorsomedial caudate, rostral	78.7 ± 1.3 (8)	70.8 ± 2.0 (8)*	89.0 ± 2.0 (8)***	78.3 ± 1.6 (8)‡‡	81.7 ± 2.0 (8)
Dorsolateral caudate, rostral	89.2 ± 1.6 (8)	81.5 ± 1.7 (8)*	99.6 ± 2.0 (8)***	88.9 ± 1.0 (8)‡‡	89.3 ± 2.5 (8)
Dorsal caudate, mid 1	77.4 ± 1.9 (8)	71.2 ± 1.9 (8)*	84.9 ± 1.3 (8)***	78.8 ± 0.9 (8)‡‡	77.2 ± 2.5 (7)
Dorsal caudate, mid 2	68.7 ± 1.1 (8)	64.9 ± 1.6 (8)	78.9 ± 2.2 (7)***	72.9 ± 1.9 (8)	70.8 ± 2.6 (8)
Dorsal caudate, caudal	66.5 ± 1.3 (7)	61.7 ± 2.0 (7)	75.9 ± 1.6 (6)***	69.7 ± 1.2 (8)‡‡	71.5 ± 2.1 (4)
Ventromedial caudate, rostral	77.3 ± 2.2 (8)	73.5 ± 2.1 (8)	90.8 ± 2.2 (8)***	81.1 ± 2.0 (7)‡‡	85.1 ± 2.2 (8)*
Ventrolateral caudate, rostral	76.3 ± 2.3 (8)	75.0 ± 1.6 (8)	87.3 ± 2.1 (8)***	81.1 ± 1.2 (7)†	83.6 ± 2.2 (8)*
Ventral caudate, mid 1	77.4 ± 1.9 (8)	76.4 ± 2.1 (8)	86.6 ± 1.3 (8)***	82.2 ± 1.3 (8)†	82.0 ± 1.6 (7)
Ventral caudate, mid 2	68.7 ± 1.1 (8)	71.8 ± 1.3 (8)*	85.3 ± 2.0 (7)***	79.7 ± 1.4 (7)†	81.1 ± 2.5 (8)
Ventral caudate, caudal	76.6 ± 2.0 (7)	67.6 ± 2.7 (7)*	85.3 ± 1.8 (6)***	76.4 ± 1.2 (8)‡‡	81.4 ± 2.2 (4)
Globus pallidus	44.1 ± 1.0 (8)	42.3 ± 1.2 (8)	51.7 ± 1.0 (8)***	47.9 ± 1.1 (8)†	50.5 ± 1.3 (8)***
Entopedunucular nucleus	45.7 ± 1.5 (8)	42.2 ± 1.0 (8)	53.9 ± 0.8 (7)***	47.3 ± 0.8 (8)‡‡	52.5 ± 0.9 (8)***
Subthalamic nucleus	80.0 ± 2.7 (8)	73.2 ± 1.4 (8)	87.2 ± 1.9 (8)*	84.1 ± 1.3 (7)	83.7 ± 1.8 (8)
Substantia nigra, pars compacta, rostral	69.9 ± 1.9 (7)	63.6 ± 1.3 (7)	73.2 ± 1.6 (8)	73.0 ± 2.2 (8)	70.9 ± 2.4 (8)
Substantia nigra, pars compacta, caudal	65.4 ± 2.0 (7)	56.7 ± 1.9 (6)*	71.3 ± 1.7 (7)†	68.0 ± 1.5 (8)	63.7 ± 2.7 (7)
Substantia nigra, pars reticulata rostral	45.0 ± 1.2 (7)	41.4 ± 0.9 (7)	48.0 ± 2.1 (8)	49.0 ± 2.0 (8)	46.4 ± 1.5 (8)
Substantia nigra, pars reticulata caudal	45.9 ± 1.7 (7)	41.7 ± 1.4 (6)	50.2 ± 2.3 (7)	48.8 ± 1.8 (8)	49.5 ± 2.1 (7)
Cortex					
Medial prefrontal cortex, rostral	75.6 ± 2.3 (6)	72.5 ± 2.4 (8)	86.2 ± 1.5 (8)***	76.8 ± 0.9 (6)‡‡	82.8 ± 2.2 (7)*
Medial prefrontal cortex, prelimbic	79.1 ± 2.2 (8)	74.2 ± 2.4 (8)	92.8 ± 1.7 (8)***	85.6 ± 4.5 (7)	88.6 ± 1.6 (8)***
Medial prefrontal cortex, infralimbic	75.0 ± 2.3 (7)	73.8 ± 2.8 (7)	82.8 ± 2.3 (8)*	79.9 ± 1.8(6)	81.6 ± 2.9 (7)
Dorsolateral prefrontal cortex	70.0 ± 2.5 (8)	68.2 ± 2.9 (8)	84.7 ± 1.8 (8)***	76.5 ± 2.8 (6)†	79.7 ± 1.8 (7)***
Frontal cortex	71.8 ± 1.6 (8)	70.2 ± 2.7 (7)	83.4 ± 1.6 (8)***	79.9 ± 2.6 (7)	77.9 ± 1.2 (8)***
Ventrolateral orbital cortex	106.7 ± 1.4 (8)	97.7 ± 3.2 (8)*	125.9 ± 3.2 (8)***	109.7 ± 2.5 (7)‡‡	118.7 ± 2.9 (8)***
Anterior cingulate cortex	84.4 ± 2.8 (8)	76.7 ± 2.4 (8)	95.5 ± 2.4(8)***	85.9 ± 1.7 (8)‡‡	83.6 ± 2.2 (8)
Somatosensory cortex	81.5 ± 2.1 (8)	74.3 ± 2.1 (8)	92.9 ± 2.0 (8)***	85.6 ± 1.3 (8)‡‡	87.5 ± 2.3 (8)
Entorhinal cortex	58.1 ± 2.1 (7)	55.3 ± 1.1 (7)	67.5 ± 5.2 (7)	65.3 ± 4.5 (4)	70.5 ± 2.2 (8)***

[a] The saline control group is composed of controls from both Experiments 1 and 2. Refer to Table 1 and Methods for further descriptions of these groups.

*, **, *** Differences from the saline control (non-Sz-Sal) group $p < 0.05$, $p < 0.02$, $p < 0.01$; two-tailed *t*-test.

†, ‡, ‡‡ Differences between Sz-Sal and Sz-MS $p < 0.05$, $p < 0.02$, $p < 0.01$; two-tailed *t*-test.

metabolism of sensitized rats. Therefore, differences in the profiles of $LCMR_{glu}$ following MS challenge in Sz-MS and non-Sz-MS rats would indicate brain regions contributing to the altered response to acute MS in sensitized rats. Similar to the overall effects of MS in nonsensitized rats, decreases in glucose metabolism, compared with saline-treated sensitized rats, were observed in Sz-MS rats. The MS challenge administered to sensitized rats significantly reduced $LCMR_{glu}$ in 33 of the 95 areas analyzed. Moreover, except for several thalamic nuclei, sensitized rats challenged with MS had significantly ($p < 0.01$) reduced $LCMR_{glu}$ in areas unaffected in non-Sz-MS rats (Tables 2–4). These areas include the rostral prefrontal and ventrolateral orbital cortices, regions of the caudate, the rostral core and shell of the nucleus accumbens, and the mediodorsal thalamic nucleus. Overall, significant decreases ($p < 0.01$) were present in 13 of the 40 limbic areas, seven of the 17 basal ganglia

TABLE 4

MEAN ± SEM LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE (μMOL/100 G/PER MIN) IN THALAMIC NUCLEI AND HINDBRAIN REGIONS OF NONSENSITIZED AND SENSITIZED RATS AFTER ADMINISTRATION OF A SALINE OR MS CHALLENGE

Brain Regions Analyzed (Thalamic nuclei)	Experiments 1 and 2 Saline Controls[a]	Experiment 1 (Conditioned Cues Present)			Experiment 2 (Conditioned Cues Absent)
		Non-Sz-MS	Sz-Sal	Sz-MS	Sz-nc
Thalamic nuclei					
Paraventricular	66.7 ± 1.6 (8)	59.5 ± 1.1 (8)***	77.3 ± 2.5 (7)***	69.3 ± 1.6 (8)‡	68.7 ± 2.0 (7)
Paratenial	82.4 ± 1.3 (8)	74.2 ± 1.2 (8)***	97.2 ± 3.2 (7)***	85.9 ± 3.0 (8)†	89.4 ± 2.0 (7)**
Anteroventral	86.3 ± 1.9 (8)	80.8 ± 1.8 (8)	97.9 ± 2.5 (8)***	89.6 ± 1.4 (8)‡	88.0 ± 2.1 (7)
Ventrolateral	92.3 ± 1.2 (7)	75.4 ± 1.9 (6)***	107.2 ± 3.0 (6)***	93.8 ± 1.3 (8)‡‡	100.5 ± 3.9 (4)
Ventral posterolateral	83.4 ± 1.6 (7)	71.6 ± 2.7 (6)***	98.3 ± 1.8 (6)***	87.7 ± 1.1 (8)‡‡	93.6 ± 5.3 (4)
Gelatinosus	102.3 ± 2.9 (8)	87.3 ± 1.3 (7)***	122.2 ± 4.4 (7)***	104.4 ± 2.1 (8)‡‡	109.9 ± 2.5 (8)
Ventromedial	93.9 ± 2.4 (8)	79.9 ± 1.6 (8)***	109.9 ± 1.9 (7)***	93.9 ± 1.7 (8)‡‡	100.9 ± 2.2 (8)*
Mediodorsal	79.7 ± 1.4 (6)	74.4 ± 1.8 (8)	102.9 ± 2.0 (7)***	92.0 ± 1.3 (7)‡‡	93.9 ± 2.2 (8)***
Central medial	67.9 ± 1.7 (7)	61.0 ± 2.0 (7)*	80.7 ± 3.5 (7)***	71.3 ± 1.4 (7)†	67.0 ± 1.6 (8)
Ventral posteromedial	79.3 ± 3.1 (8)	70.8 ± 1.5 (8)*	92.5 ± 1.8 (7)***	83.6 ± 1.5 (8)‡‡	81.3 ± 2.8 (8)
Parafasicular	78.5 ± 3.3 (6)	64.2 ± 2.0 (6)	85.6 ± 2.6 (5)	79.1 ± 2.4 (6)	82.1 ± 2.9 (6)
Midbrain/hindbrain					
Red nucleus	68.2 ± 2.1 (8)	62.2 ± 0.8 (8)	76.6 ± 2.4 (8)**	73.2 ± 2.0 (8)	67.7 ± 2.8 (7)
Dorsal periaqueductal grey	55.3 ± 1.6 (8)	51.4 ± 1.4 (8)	64.9 ± 1.8 (8)***	61.3 ± 2.4 (8)	60.8 ± 1.5 (7)
Ventral periaqueductal grey	67.9 ± 2.3 (8)	57.5 ± 2.1 (8)*	79.4 ± 3.2 (8)***	72.1 ± 3.3 (8)	74.2 ± 2.4 (7)
Medial reticular formation	63.4 ± 2.0 (7)	56.0 ± 1.2 (8)	69.8 ± 2.0 (8)*	67.8 ± 2.4 (8)	62.8 ± 2.7 (7)
Interpeduncular nucleus	91.2 ± 3.6 (7)	84.4 ± 2.4 (8)	99.7 ± 1.7 (8)	89.7 ± 2.7 (8)‡‡	97.6 ± 4.3 (7)
Pedunculopontine tegmental nucleus	52.4 ± 1.8 (6)	50.3 ± 2.6 (8)	59.3 ± 2.1 (8)*	52.4 ± 1.1 (3)‡	54.6 ± 0.8 (7)
Dorsal raphe	63.6 ± 1.8 (8)	58.6 ± 2.3 (7)	70.1 ± 2.3 (8)*	63.7 ± 1.6 (7)†	69.3 ± 3.4 (7)
Medial raphe	76.8 ± 2.2 (8)	69.3 ± 1.6 (7)	88.5 ± 3.3 (8)***	78.9 ± 1.6 (8)†	80.5 ± 3.0 (7)
Raphe magnus	50.1 ± 1.4 (6)	44.4 ± 0.8 (3)	51.7 ± 1.9 (7)	50.2 ± 1.8 (7)	48.7 ± 1.5 (6)
Posterodorsal tegmental nucleus	78.4 ± 2.4 (7)	65.6 ± 2.1 (7)***	83.6 ± 4.2 (8)	82.1 ± 1.9 (7)	81.9 ± 3.0 (8)
Locus coeruleous	54.5 ± 0.7 (7)	46.9 ± 2.3 (7)*	58.6 ± 2.7 (7)	55.3 ± 1.9 (7)	52.7 ± 1.8 (8)
Lateral parabrachial area	45.5 ± 1.0 (5)	41.4 ± 1.6 (6)	48.1 ± 1.2 (8)	47.7 ± 1.4 (6)	45.7 ± 1.6 (8)
Olive	96.6 ± 2.3 (6)	81.0 ± 3.4 (7)*	110.1 ± 5.2 (8)*	100.6 ± 4.2 (7)	91.5 ± 2.9 (8)
Parvocellular reticular nucleus	58.1 ± 1.5 (7)	48.1 ± 1.3 (4)*	62.0 ± 1.3 (8)	56.5 ± 1.0 (8)‡‡	55.1 ± 1.2 (8)
Gigantocellular reticular nucleus	57.6 ± 1.7 (6)	48.5 ± 1.1 (4)*	59.3 ± 1.3 (8)	55.8 ± 1.7 (8)	53.5 ± 1.1 (8)
Dorsal paragigantocellular nucleus	54.6 ± 1.7 (7)	46.6 ± 1.3 (4)***	58.4 ± 1.7 (8)	54.5 ± 1.2 (7)	52.2 ± 1.0 (8)
Prepositus hypoglossal nucleus	68.5 ± 1.7 (7)	54.3 ± 1.6 (3)***	75.1 ± 1.4 (8)**	65.3 ± 1.5 (7)‡‡	69.3 ± 2.1 (8)
Facial nucleus	52.0 ± 2.4 (4)	46.2 ± 2.2 (4)	54.2 ± 0.6 (7)	52.0 ± 2.2 (6)	50.8 ± 1.0 (3)

[a] The saline control group is composed of controls from both Experiments 1 and 2. Refer to Table 1 and Methods for further descriptions of these groups.

*, **, *** Differences from the saline control (non-Sz-Sal) group $p < 0.05$, $p < 0.02$, $p < 0.01$; two-tailed *t*-test.

†, ‡, ‡‡ Differences between Sz-Sal and Sz-MS $p < 0.05$, $p < 0.02$, $p < 0.01$; two-tailed *t*-test.

regions, four of the nine cortical areas, six of the 11 thalamic nuclei, and three of the 18 hindbrain regions analyzed.

Experiment 2: Effects of MS Sensitization on Basal $LCMR_{glu}$ in the Absence of Conditioned Cues (Sz-nc vs. Non-Sz-Control)

To characterize changes in basal $LCMR_{glu}$ induced by the MS-sensitizing treatment and not concurrent conditioned responding, glucose use in the Sz-nc group was compared with nonsensitized controls. The results of this comparison identify brain regions in which the sensitizing MS injections result in long-lasting changes in synaptic activity that are dependent on pharmacologic and not conditioning effects. Significant increases ($p < 0.01$) in $LCMR_{glu}$ were found in 16 of the 95 brain regions analyzed in Sz-nc rats compared with saline controls (Tables 2–4). Within the limbic system (Table 2), increases ($p < 0.01$) were found in eight of the 40 analyzed regions. These included the rostral shell of the nucleus accumbens, the ventral pallidum, the vertical and horizontal limb of the diagonal band, several amygdaloid nuclei, and the bed nucleus of the stria terminalis. In the basal ganglia, increases were found in two of 17 areas. These were the globus pallidus and the entopeduncular nucleus (Table 3). Of the nine cortical areas analyzed, five showed significant increases, including the medial and dorsolateral prefrontal, frontal, ventrolateral orbital, and entorhinal cortices (Table 3). Only one thalamic nucleus was found to be increased: the mediodorsal thalamic nucleus (Ta-

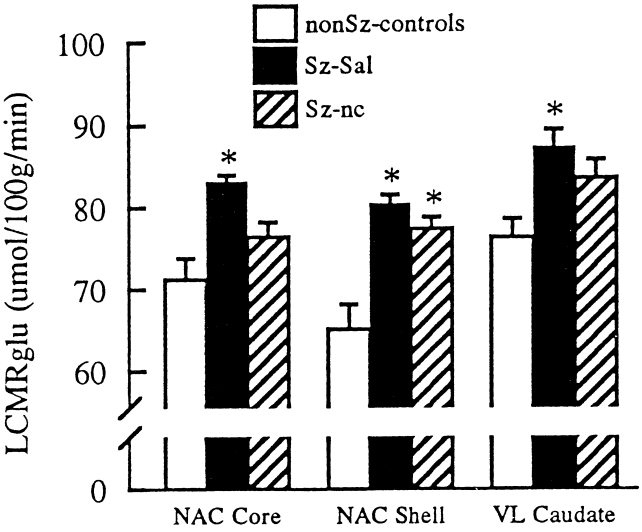

FIG. 1. Mean ± SEM $LCMR_{glu}$ in three forebrain areas of nonsensitized control rats (nonSz-controls), sensitized rats in the presence of conditioned cues (Sz-Sal), and sensitized rats in the absence of conditioned cues (Sz-nc). NAC core, nucleus accumbens core; NAC shell, nucleus accumbens shell; VL caudate, ventrolateral caudate. *Significance ($p < 0.01$) vs. non-Sz-controls (see also Tables 2 and 3).

ble 4). No significant changes ($p < 0.01$) were found in the 18 hindbrain structures of Sz-nc rats compared with saline controls.

A comparison of several relevant forebrain areas in MS-sensitized rats in the presence (Sz-Sal) and absence (Sz-nc) of conditioned cues is further depicted in Figs. 1 and 2. Figure 1 illustrates the differential effect of each experimental condition on the nucleus accumbens core and shell and the ventrolateral caudate. Figure 2 presents the changes in $LCMR_{glu}$ in each condition in the prelimbic and infralimbic areas of the medial prefrontal cortex and the dorsolateral prefrontal cortex.

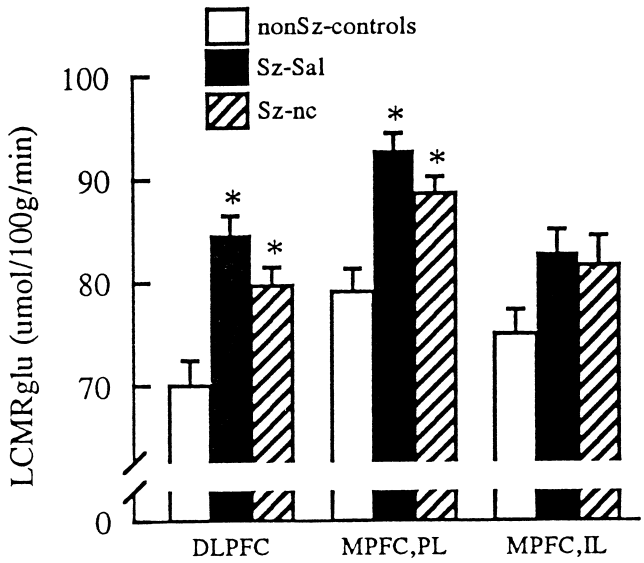

FIG. 2. Mean ± SEM $LCMR_{glu}$ in three cortical areas of nonsensitized control rats (nonSz-controls), sensitized rats in the presence of conditioned cues (Sz-Sal), and sensitized rats in the absence of conditioned cues (Sz-nc). DLPFC, dorsolateral prefrontal cortex; MPFC, PL, medial prefrontal cortex, prelimbic areas; MPFC, IL, medial prefrontal cortex, infralimbic area. *Significance ($p < 0.0l$) vs. non-Sz-controls (see also Table 3).

Physiologic Parameters

The blood pressure and hematocrit recorded 10 min before and 25 min following the intravenous infusion of 2-DG are presented in Table 5. These values did not vary significantly during the course of the experiment.

Although arterial plasma glucose concentrations for each of the groups did not remain constant over the course of metabolic testing (Table 5), they were not outside the normoglycemic range, nor did they vary more than the acceptable amount from initial plasma glucose concentrations (65). Furthermore, even at our highest average arterial plasma glucose concentration, the experimental groups remained well below the glucose concentrations that have been reported to cause changes in $LCMR_{glu}$ (53). As described in Methods, the lumped constant applied in the analysis of each animal varied according to the average arterial plasma glucose concentration for the respective animal. The mean lumped constant and its respective standard deviation for each of the treatment conditions is as follows: non-Sz-Sal, 0.46 ± 0.01; non-Sz-MS, 0.47 ± 0.03; Sz-Sal, 0.45 ± 0.01; Sz-MS, 0.45 ± 0.01; and Sz-nc, 0.45 ± 0.01. These values are comparable to the lumped constant originally reported by Sokoloff and colleagues (67) in conscious rats.

DISCUSSION

Effects of Morphine Sensitization on Basal $LCMR_{glu}$

The major finding of this investigation was the increased basal metabolic activity present 6 days after the sensitizing treatment in both the presence and absence of conditioned environmental cues. In the presence of conditioned cues (Sz-Sal), Experiment 1, significant increases in basal $LCMR_{glu}$ ($p < 0.01$) were found in 54 of the 95 brain sites analyzed in MS-sensitized rats acutely challenged with saline compared with saline controls (non-Sz-control). These changes were widely distributed in forebrain systems including the limbic system, basal ganglia, cerebral cortex, and thalamic nuclei (Tables 2–4). Effects were also found in midbrain and hindbrain areas (Table 4) which have been reported to be involved in sensitization (71) as well as oral stereotypy (28). In the absence of conditioned cues (Sz-nc), Experiment 2, significant increases in metabolic rate were found in 16 areas of the 95 brain sites analyzed, all of which were distributed in the forebrain (Tables 2–4). Therefore, although conditioning is involved in the basal metabolic changes found in sensitized rats exposed to drug-associated stimuli (Sz-Sal), MS sensitization has a basic underlying pharmacologic effect on basal $LCMR_{glu}$ which is independent of conditioning.

Within the limbic telencephalon, basal $LCMR_{glu}$ was significantly increased as a consequence of MS sensitization. In sensitized rats in which conditioned cues were present (Sz-Sal), basal metabolic rate is significantly elevated from control levels (non-Sz-control) in 22 of the 40 limbic areas analyzed. However, the majority of these effects appear to be associated with a response to conditioned cues, because, in the absence of conditioning, only eight limbic structures were significantly

TABLE 5

MEAN ± SEM OF THE PHYSIOLOGIC PARAMETERS MEASURED IN THE FIVE EXPERIMENTAL GROUPS BOTH PRIOR TO AND AT VARIOUS TIMES AFTER ADMINISTRATION OF 2-DG

Physiologic Parameters	Experiments 1 and 2 Non-Sz Controls*	Experiment 1			Experiment 2 Sz-nc
		Non-Sz-MS	Sz-Sal	Sz-MS	
Blood pressure					
Pre	143 ± 2 (8)	142 ± 2 (8)	141 ± 2 (8)	142 ± 1 (8)	142 ± 2 (8)
25 min	141 ± 2 (8)	146 ± 2 (8)	141 ± 2 (8)	143 ± 2 (8)	137 ± 3 (7)
Hematocrit					
Pre	0.46 ± 0.02 (8)	0.46 ± 0.02 (8)	0.46 ± 0.01 (8)	0.44 ± 0.02 (8)	0.45 ± 0.01 (8)
25 min	0.39 ± 0.03 (7)	0.41 ± 0.02 (7)	0.40 ± 0.02 (8)	0.39 ± 0.02 (8)	0.44 ± 0.01 (8)
Plasma glucose					
Pre	139 ± 8 (8)	119 ± 8 (8)	139 ± 9 (8)	114 ± 8 (8)	141 ± 6 (8)
0 min	127 ± 13 (8)	105 ± 9 (8)	127 ± 7 (8)	118 ± 11 (8)	142 ± 9 (8)
*	120 ± 9 (8)	107 ± 9 (8)	131 ± 7 (8)	115 ± 8 (8)	140 ± 6 (8)
45 min	146 ± 9 (8)	134 ± 12 (8)	146 ± 7 (8)	132 ± 11 (8)	166 ± 7 (8)
†	128 ± 9 (8)	115 ± 11 (8)	136 ± 6 (8)	122 ± 8 (8)	144 ± 7 (8)

All three physiologic parameters were measured 10 min prior to the infusion of 2-DG (Pre). The blood pressure and hematocrit were also measured 25 min following the 2-DG infusion. Plasma glucose values are given for various times: 0 min, at the time of 2-DG infusion; * the mean of the first 20 min; 45 min, 45 min after the 2-DG infusion; † the mean of the entire experiment. There were no significant differences between relevant groups.

increased compared with controls (Sz-nc vs. non-Sz-controls). Conditioning has been reported to influence locomotor sensitization induced by both opiates and psychostimulants (14,47,58,59,73). In these earlier studies, conditioning was reported to influence only the reexpression of sensitization after a later drug challenge. The results of the present experiment, however, indicate that in the absence of a drug challenge, drug-associated stimuli (acting as the CS) increase glucose utilization (the CR) throughout the brain 6 days after the last MS dose without causing any observable change in behavior.

Because the extensive increase in brain metabolic activity 6 days after the last MS dose is in part conditioned by environmental cues, the results suggest that MS-induced sensitization may be a model for the conditioned craving seen in opiate addicts (6,7,50,52,79). The generalization of our findings as a model of conditioned craving is supported by the report of Volkow and colleagues (75) in which 1 wk after the last cocaine experience, cocaine users had increased glucose metabolism in areas of the cortex, including the ventrolateral orbital and prefrontal cortices, and the basal ganglia during self-reported periods of drug craving.

In the absence of conditioning, the increased basal metabolic activity in select limbic structures of Sz-nc rats indicates that the MS-sensitizing treatment had pharmacologic effects which persist for at least 6 days. Of the areas affected, the shell of the nucleus accumbens (NAC shell) is of special interest (Fig. 1). The NAC shell and core act as convergence sites for mesolimbic dopamine neurons and for projections from other limbic and cortical areas. These NAC regions have also been shown to be involved in the expression of oral stereotypy (44,60) and locomotor activity (33,34,55). An increased basal metabolic rate in the NAC shell 6 days after the last MS dose, suggests that in addition to a role in the expression of these behaviors, the shell is involved in maintaining the increased susceptibility of sensitized rats to express these behaviors. Further, because Kalivas and Duffy (29–32) and Vezina and colleagues (72) demonstrated that basal dopamine release is unchanged in the NAC of rats sensitized to the locomotor effects of MS, the change in basal $LCMR_{glu}$ suggests an increased neurotransmission from nondopaminergic terminals. The presence of an increased glucose utilization in the NAC shell and other limbic structures of both the conditioned and nonconditioned MS-sensitized rats, supports the hypothesis that MS sensitization relies on an altered neurotransmission in the limbic system, and extends it by suggesting that an increased basal metabolic rate in select limbic structures may be responsible for the persistence of MS sensitization.

The core of the NAC, unlike the shell, was not affected by the sensitized nonconditioned (Sz-nc) treatment, indicating a selectivity of pharmacologic effects for the shell. In the presence of conditioning, however, glucose metabolism in both the core and shell were significantly elevated in sensitized rats (Table 2 and Fig. 1). Thus, while the effect of MS sensitization in the shell is a pharmacologic phenomenon, the effect in the core is a conditioning phenomenon. The importance of the distinction of shell from core in the present results is underscored by neuroanatomic (24,76,82,85) and pharmacologic (12,45) studies which suggest that each subterritory mediates different functions. These investigators hypothesized that the NAC shell mediates limbic functions, whereas the core is associated with motor processes. Although these proposed functions were based on differences in the neuroanatomic organization of each subterritory, the present results provide functional evidence that the shell and core differ in their involvement in MS-induced sensitization. Therefore, the persistence of MS sensitization relies on a reorganization of basal synaptic activity in the "limbic" shell and not in the "motor" core, while both subterritories are involved in the conditioned response to drug-associated stimuli.

The increased basal $LCMR_{glu}$ in the NAC shell is also important because of interconnections with other similarly affected forebrain structures in the Sz-nc group. These structures include the amygdala (16,37), the prelimbic area of the medial prefrontal cortex (4,8,16), and the entorhinal cortex (16). However, in reference to the prelimbic area of the prefrontal cortex, Zahm and Brog (84) stated in a review article that projections from the prelimbic area are mainly to the NAC core. These regions are relevant to MS sensitization

because each contributes to the glutamatergic afferents of the NAC. It has been suggested that the behavioral activating effects of MS may be due to an enhancement of glutamatergic influences on dopamine transmission (5,23). Previous work in our laboratory has indicated that the neurotransmitter glutamate, acting at the NMDA receptor, is required for the development and expression of MS sensitization of oral stereotypy (43). Similarly, a role for glutamatergic transmission has been reported in psychomotor stimulant-induced sensitization (13,35,36,61,68,80).

The primary projection field of both the NAC shell and core, the ventral pallidum (VP) (24), was also found to have a significantly increased basal activity in Sz-nc rats (Table 2). The VP may play a special role in the sensitized brain by virtue of its presumed role in limbic-mediated locomotor activity (1,2,27,70) and conditioned place preference (26,48). Afferents projecting from the NAC (10,24,49) and ventral tegmental area (VTA) (40) have been shown to be influenced by opiates (9,51) and to act through the VP to affect locomotor behavior (1,2,41,64). Therefore, the VP may be functioning as a coupler between the limbic and extrapyramidal motor systems, allowing motivational signals to gain access to motor behavior. Such a circuit involving the accumbens-pallidal region has been proposed by Austin and Kalivas (1,2), Koob (41), and Robinson and Berridge (64). VP neurons innervated by the NAC shell have been shown to project to the more limbic targets of the VP, while the NAC core innervates VP neurons which project to motor areas (24,85). Among the sites innervated by the VP that are involved in the control of locomotor activity and were significantly increased in Sz-nc rats are the mediodorsal thalamic nucleus and entopeduncular nucleus (22,74,77,83).

Several structures innervated by the mediodorsal thalamic nucleus were also affected by MS sensitization, including the medial and dorsolateral prefrontal cortices (Fig. 2) and entorhinal cortex (21,62) (Table 3). Thus, the corticostriatal–pallidothalamic–thalamocortical circuit of the NAC shell appears to have been reset to a hyperactive and presumably hyperresponsive state by the pharmacologic actions of the MS-sensitizing treatment. Furthermore, because the prelimbic area of the medial prefrontal cortex and entorhinal cortex project back onto the NAC shell, the change in synaptic activity induced by MS sensitization in this circuit may be self-sustaining and therefore may be responsible for maintaining the sensitization process for long periods of time in the absence of additional drug.

Effects in the dorsolateral prefrontal cortex of sensitized rats (Table 3 and Fig. 2) are notable not only because of the innervation it receives from the mediodorsal thalamic nucleus, but also because it projects preferentially to the NAC core and regions of the basal ganglia (84). These regions were unaffected in MS-sensitized nonconditioned (Sz-nc) rats, but were significantly activated in the presence of conditioned cues. Thus, the conditioned response characterized in Sz-Sal rats may involve an increased outflow from the corticostriatal–pallidothalamic–thalamocortical circuit of the NAC shell via efferent fibers projecting from the dorsolateral prefrontal cortex. Projection fields of this area of the cortex which were significantly increased in the presence of conditioning (Sz-Sal vs. non-Sz-control) include the NAC core and regions of the striatum.

Surprisingly, no significant effect of MS sensitization was seen in the VTA. The lack of metabolic effects in the VTA is of particular interest because this region has been implicated in the development and expression of MS- and psychostimulant-induced sensitization of locomotor activity (33,63,71). Our failure to find effects in the VTA indicates that basal synaptic activity within this structure does not have a role in the maintenance of MS sensitization, which is in agreement with Kalivas and Stewart (33). Alternatively, the 2-DG technique may lack the sensitivity to detect changes relevant to MS sensitization in the VTA. However, this possibility is unlikely, because even a trend toward an altered $LCMR_{glu}$ was not present in the VTA.

Although abstinence-induced MS withdrawal may have been present soon after the MS-sensitizing treatment, it was unlikely to have occurred during the 2-DG experiment, for several reasons. First, MS withdrawal has been characterized to develop fully within 1 day and to last for approximately 4 days in the human and the rat (25,46,78). The present results were collected 6 days after the last MS injection. Second, none of the commonly reported opiate withdrawal signs, including weight loss, diarrhea, hyperactivity, and wet-dog shakes, were observed in any of the sensitized groups at the time of metabolic testing. Third, although a comparison of the current results seen in Sz-Sal rats with earlier 2-DG studies investigating MS withdrawal (17–19,38,39,81) reveal similar metabolic effects in many regions, the gross differences between these earlier 2-DG studies and the sensitized nonconditioned (Sz-nc) group of the current study clearly indicate that MS withdrawal was not present in MS-sensitized rats at the time of metabolic testing. Given these facts, it is unlikely that physical dependence was present 6 days after the last MS dose. It is important to note that a conditioned withdrawal response is not present in the current investigation, because rats were not exposed to the test apparatus during periods of MS withdrawal, and therefore, no association could have developed.

Effects of the MS Challenge on $LCMR_{glu}$

The MS challenge (0.5 mg/kg), administered to both nonsensitized and sensitized rats, significantly reduced metabolic activity from basal levels in a number of distinct structures compared with the respective control. However, there were clear differences in the MS effect that are dependent on the treatment history. In nonsensitized rats, MS (Sz-MS) significantly ($p < 0.01$) reduced $LCMR_{glu}$ in 12 brain sites (Tables 2–4). In agreement with an earlier 2-DG experiment examining the metabolic effects of MS (20), there were significant decreases in a number of midline thalamic nuclei. In sensitized rats, however, MS significantly ($p < 0.01$) lowered metabolic activity from basal levels in 23 structures (Tables 2–4), many of which were not affected by the non-Sz-MS treatment. Also, in sensitized rats challenged with 0.05 mg/kg of MS (Sz-MS), metabolic decreases were absent in a number of brain areas effected by 0.05 mg/kg MS administered to nonsensitized rats (non-Sz-MS). These results indicate that the MS-sensitizing treatment altered the responsiveness of several brain structures to the acute metabolic effects of MS.

The differential effect of the MS challenge in nonsensitized and sensitized rats was most evident in the forebrain (Tables 2–4). An altered response in the NAC shell and core is of special interest in the study of MS sensitization, for several reasons. First, the NAC is the primary structure hypothesized to be responsible for the expression of both MS and psychostimulant sensitization. Second, because the MS challenge significantly reduced glucose metabolism in the NAC of sensitized rats only, it indicates that the role of MS in determining the activity of the NAC shell and core is more important in rats sensitized to MS. Also, if the increase in basal metabolic

rate 6 days after the last MS injection is related to the phenomenon of craving, then the decreases observed in MS-sensitized rats following an MS challenge (Sz-MS) may be interpreted as a partial normalization of the induced excitatory state.

SUMMARY AND CONCLUSIONS

The results of these experiments demonstrate that MS-induced sensitization is manifested by increases in basal metabolic activity that last for at least 6 days. Although a majority of the brain structures analyzed were found to be significantly altered in MS-sensitized rats exposed to drug-associated stimuli, a basic underlying pharmacologic effect of MS sensitization on basal brain activity was characterized in the corticostriatal–pallidothalamic–thalamocortical circuit of the NAC shell. Therefore, the "limbic" shell of the NAC and associated structures appear to have been reset to a hyperactive and presumably hyperresponsive state by the nonconditioning MS-sensitizing treatment, whereas the "motor" core of the NAC remained unaffected by the same regimen.

Upon challenge with morphine, sensitized rats were found to have significant reductions in metabolic activity in many structures in which MS had no affect in nonsensitized rats. The differential effect of MS is evident in the forebrain, most notably the limbic and extrapyramidal systems. In sensitized rats, MS acutely reduced $LCMR_{glu}$ in the core and shell of the nucleus accumbens and regions of the basal ganglia, but had no effect in these structures in nonsensitized rats.

The presence of altered basal and MS-evoked metabolic activity 6 days after the completion of the MS-sensitizing treatment indicates a deviation from normal brain physiology resulting in an altered functional state in forebrain systems. Finally, the activation of brain structures in the presence of conditioned cues suggests that these rats (Sz-Sal) may model an altered brain state related to craving in the abstinent opiate addict.

ACKNOWLEDGEMENTS

This work was supported by NIDA Grant DA02326 and NIDA Research Scientist Award DA00099 to C.K.

REFERENCES

1. Austin, M. C.; Kalivas, P. W. Enkephalinergic and GABAergic modulation of motor activity in the ventral pallidum. J. Pharmacol. Exp. Ther. 252:1370–1377; 1990.
2. Austin, M. C.; Kalivas, P. W. Dopaminergic involvement in locomotion elicited from the ventral pallidum/substantia innominata. Brain Res. 542:123–131; 1991.
3. Bartoletti, M.; Gaiardi, M.; Gubellini, G.; Bacchi, A.; Babbini, M. Long-term sensitization to the excitatory effects of morphine. A motility study in post-dependent rats. Neuropharmacology 22:1193–1196; 1983.
4. Berendse, H. W.; Galis-de Graaf, Y.; Gronewegen, H. J. Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J. Comp. Neurol. 316:314–347; 1992.
5. Calenco-Choukroun, G.; Dauge, V.; Gacel, G.; Roques, B. P.; Lesion of dopamine mesolimbic neurons blocks behavioral effects induced by the endogenous enkephalins but not by a μ-opioid receptor agonist. Eur. J. Pharmacol. 209:267–271; 1991.
6. Childress, A. R.; McLellan, A. T.; O'Brien, C. P. Abstinent opiate abusers exhibit conditioned craving, conditioned withdrawal and reductions in both through extinction. Br. J. Addict. 81:655–660; 1986.
7. Childress, A. R.; McLellan, A. T.; Ehrman, R.; O'Brien, C. P. Classically conditioned responses in opioid and cocaine dependence: A role in relapse? In: Ray, B. A., ed. Learning factors in substance abuse. NIDA Research Monograph vol. 84; 1988:25–43.
8. Christie, M. J.; James, L. B.; Beart, P. M. An excitatory amino-acid projection from the medial prefrontal cortex to the anterior part of nucleus accumbens in the rat. J. Neurochem. 45:477–482; 1985.
9. Chrobak, J. J.; Napier, T. C. Opioid and GABA modulation of accumbens-evoked ventral pallidal activity. J. Neural Transm Gen Sect 93:123–143; 1993.
10. Churchill, L.; Dilts, R. P.; Kalivas, P. W. Changes in γ-aminobutyric acid, μ-opioid and neurotensin receptors in the accumbens-pallidal projection after discrete quinolinic acid lesions in the nucleus accumbens. Brain Res. 511:41–54; 1990.
11. Crane, A. M.; Porrino, L. J. Adaptation of the quantitative 2-[^{14}C] deoxyglucose method for use in freely moving rats. Brain Res. 499:87–92; 1989.
12. Deutch, A. Y.; Cameron, D. S. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46:49–56, 1992.
13. Druhan, J. P.; Jakob, A.; Stewart, J. The development of behavioral sensitization to apomorphine is blocked by MK-801. Eur. J. Pharmacol. 243:73–77; 1993.
14. Ellinwood, E. H.; Kilby, M. M. Amphetamine stereotypy: The influence of environmental factors and prepotent behavioral patterns on its topography and development. Biol. Psychiatry 10:3–16; 1975.
15. Fog, R. Behavioural effects in rats of morphine and amphetamine and of a combination of the two drugs. Psychopharmacologia 16:305–312; 1970.
16. Fuller, T. A.; Russchen, F. T.; Price, J. L. Sources of presumptive glutamatergic/aspartegenic afferents to the rat ventral striatopallidal region. J. Comp Neurol. 258:317–338; 1987.
17. Geary, W. A. II; Wooten, G. F. A behavioral and 2-deoxyglucose autoradiographic study of the effects of cumulative morphine dose on naloxone precipitated withdrawal in the rat. Brain Res. 275:117–126, 1983.
18. Geary, W. A. II; Wooten, G. F. Similar functional anatomy of spontaneous and precipitated morphine withdrawal. Brain Res. 334:183–186; 1985.
19. Geary, W. A. II; Wooten, G. F. Time action profiles of regional cerebral glucose utilization during naloxone-precipitated morphine withdrawal. Brain Res. 399:181–184; 1986.
20. Gescuk, B.; Lang, S.; Porrino, L. J.; Kornetsky, C. The local cerebral metabolic effects of morphine in rats exposed to escapable foot shock. Brain Res. 663:303–311; 1994.
21. Groenewegen, H. J. Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to mediodorsal-prefrontal topography. Neuroscience 24:379–431; 1988.
22. Groenewegen, H. J.; Berendse, H. W.; Haber, S. N. Organization of the output of the ventral striatopallidal system in the rat: Ventral pallidal efferents. Neuroscience 57:113–142; 1993.
23. Hakan, R. L.; Henriksen, S. J. Opiate influences on nucleus accumbens neuronal electrophysiology: Dopamine and nondopamine mechanisms. J. Neurosci. 9:3538–3546; 1989.
24. Heimer, L.; Zahm, D. S.; Churchill, L.; Kalivas, P. W.; Wohltmann, C. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89–125; 1991.
25. Himmelsbach, C. K. Clinical studies on drug addiction. Physical dependence, withdrawal and recovery. Arch. Intern Med. 69:766–772, 1942.
26. Hiroi, N.; White, N. M. The ventral pallidum area is involved in the acquisition but not expression of the amphetamine conditioned place preference. Neurosci. Lett. 156:9–12; 1993.
27. Hooks, M. S.; Kalivas, P. W. Involvement of dopamine and excit-

atory amino acid transmission in novelty-induced motor activity. J. Pharmacol. Exp. Ther. 269:976–988; 1994.
28. Iwamoto, E. T.; Way, E. L. Circling behavior and stereotypy induced by intranigral opiate microinjections. J. Pharmacol. Exp. Ther. 203:347–359; 1977.
29. Kalivas, P. W.; Duffy, P. Sensitization to repeated morphine injection in the rat: Possible involvement of A10 dopamine neurons. J. Pharmacol. Exp. Ther. 241:204–212; 1987.
30. Kalivas, P. W.; Duffy, P. Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. J. Neurochem. 50:1498–1504; 1988.
31. Kalivas, P. W.; Duffy, P. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J. Neurosci. 13:266–275; 1993.
32. Kalivas, P. W.; Duffy, P. Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. J. Neurosci. 13:276–284; 1993.
33. Kalivas, P. W.; Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223–244; 1991.
34. Kalivas, P. W.; Widerlov, E.; Stanley, D.; Breese, G.; Prange, A. J. Jr. Enkephalin action on the mesolimbic system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. 227:229–237; 1983.
35. Karler, R.; Calder, L. D.; Turkanis, S. A. DNQX blockade of amphetamine behavioral sensitization. Brain Res. 552:295–300; 1991.
36. Karler, R.; Calder, L. D.; Chaudhry, I. A.; Turkanis, S. A. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci. 45:599–606; 1989.
37. Kelley, A. E.; Domesick, V. B.; Nauta, W. J. The amygdalostriatal projection in the rat. An anatomical study by anterograde and retrograde tracing methods. Neuroscience 7:615–630; 1982.
38. Kimes, A. S.; Bell, J. A.; London, E. D. Clonidine attenuates increased brain glucose metabolism during naloxone-precipitated morphine withdrawal. Neuroscience 34:633–644, 1990.
39. Kimes, A. S.; London, E. D. Glucose utilization the rat brain during chronic morphine treatment and naloxone-precipitated morphine withdrawal. J. Pharmacol. Exp. Ther. 248:538–545; 1989.
40. Klitenick, M. A.; Deutch, A. Y.; Churchill, L.; Kalivas, P. W. Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50:371–386; 1992.
41. Koob, G. F. Dopamine, addiction and reward. Semin. Neurosci. 4:139–148; 1992.
42. Kornetsky, C.; Huston-Lyons, D.; Porrino, L. J. The role of the olfactory tubercle in the effects of cocaine, morphine and brain-stimulation reward. Brain Res. 541:75–81; 1991.
43. Livezey, R. T.; Pearce, L. B.; Kornetsky, C. The effect of MK-801 and SCH23390 on the expression and sensitization of morphine-induced oral stereotypy. Brain Res. 692:93–98, 1995.
44. Longoni, R.; Spina, L.; Mulas, A.; Carboni, E.; Garau, L.; Melchiorri, P.; Di Chiara, G. (D-Ala_2)Deltorphin II: D1-dependent stereotypies and stimulation of dopamine release in the nucleus accumbens. J. Neurosci. 11:1565–1576; 1991.
45. Maldonado-Irizarry, C. S,; Kelly, A. E. Differential behavioral effects following microinjection of an NMDA antagonist into nucleus accumbens subregions. Psychopharmacology 116:65–72; 1994.
46. Martin, W. R.; Wikler, A.; Eades, C. G.; Pescor, F. T. Tolerance to and physical dependence on morphine in rats. Psychopharmacologia 4:247–260; 1963.
47. Mattingly, B. A,; Gotsick, J. E. Conditioning and experiential factors affecting the development of sensitization to apomorphine. Behav. Neurosci. 103:1311–1317; 1989.
48. McAlonan, G. M.; Robbins, T. W.; Everitt, B. J. Effects of medial dorsal thalamic and ventral pallidal lesions on the acquisition of a conditioned place preference: Further evidence for the involvement of the ventral striatopallidal system in reward-related processes. Neuroscience 52:605–620; 1993.
49. Mogenson, G. J.; Nielsen, M. A. Evidence that an accumbens to subpallidal GABAergic projection contributes to locomotor activity. Brain Res. Bull. 11:309–314; 1983.
50. Nader, M. A.; Tatham, T. A.; Barrett, J. E. Behavioral and pharmacological determinants of drug abuse. Ann. NY Acad. Sci. 654:368–385; 1992.
51. Napier, T. C.; Chrobak, J. J.; Yew, J. Systemic and microiontophoretic administration of morphine differentially effect ventral pallidum/substantia innominata neural activity. Synapse 12:214–219; 1992.
52. Newlin, D. B.; Pretorius, M. B.; Jaffe, J. H. Pavlovian conditioning to morphine in opiate abusers. In: Harris, L. S., ed. Problems of drug dependence. NIDA Research Monograph vol. 95; 1989: 390–391.
53. Orzi, F.; Lucignani, G.; Dow-Edwards, D.; Namba, H.; Nehlig, A.; Patlak, C. S.; Pettigrew, K.; Schuier, F.; Sokoloff, L. Local cerebral glucose utilization in controlled graded levels of hyperglycemia in the conscious rat. J. Cereb. Blood Flow Metab. 8:346–356; 1988.
54. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. New York: Academic Press; 1986.
55. Pert, A.; Sivit, C. Neuroanatomical focus for morphine and enkephalin-induced hypermotility. Nature 265:645–647; 1977.
56. Pollock, J.; Kornetsky, C. Evidence for the role of dopamine D1 receptors in morphine induced stereotypic behavior. Neurosci. Lett. 102:291–296; 1989.
57. Pollock, J.; Kornetsky, C. Reexpression of morphine-induced oral stereotypy six months after last morphine sensitizing dose. Pharmacol. Biochem. Behav. 53:67–71; 1996.
58. Post, R. M.; Lockfeld, A.; Squillace, K. H.; Contel, N. R. Drug-environment interaction: Context dependency of cocaine-induced behavioral sensitization. Life Sci. 28:755–760; 1981.
59. Post, R. M.; Weiss, S. R.; Fontana, D.; Pert, A. Conditioned sensitization to the psychomotor stimulant cocaine. Ann. NY Acad. Sci. 654:386–399; 1992.
60. Prinssen, E. P.; Balestra, W.; Bemelmans, F. F.; Cools, A. R. Evidence for a role of the shell of the nucleus accumbens in oral behavior of freely moving rats. J. Neurosci. 14:1555–1562; 1994.
61. Pudiak, C. M.; Bozarth, M. A. L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. Life Sci. 53:1517–1524; 1993.
62. Ray, J. P.; Price, J. L. The organization of thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. J. Comp. Neurol. 323:167–197; 1992.
63. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. 396:157–198, 1986.
64. Robinson, T. E.; Berridge, K. C. The neural basis of drug craving: An incentive-sensitization theory of addiction. Brain Res. Rev. 18:247–291; 1993.
65. Savaki, H. E.; Davidsen, L.; Smith, C.; Sokoloff, L. Measurement of free glucose turnover in brain. J. Neurochem. 35:495–502; 1980.
66. Schuier, F.; Orzi, F.; Suda, S.; Lucignani, G.; Kennedy, C.; Sokoloff, L. Influence of plasma glucose concentration on lumped constant of the deoxyglucose method: Effects of hyperglycemia in the rat. J. Cereb. Blood Flow Metab. 10:765–773; 1990.
67. Sokoloff, L.; Reivich, M.; Kennedy, C.; Des Rosiers, M. H.; Patlak, C. S.; Pettigrew, K. D.; Sakurada, O.; Shinohara, M. The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28:897–916; 1977.
68. Stewart, J.; Druhan, J. P. Development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the noncompetitive NMDA receptor antagonist MK-801. Psychopharmacology 110:125–132; 1993.
69. Swanson, L. W. Brain maps: Structure of the rat brain. New York: Elsevier; 1992.
70. Swerdlow, N. R.; Koob, G. F. Lesions of the dorsomedial nucleus of the thalamus, medial prefrontal cortex and pedunculopontine

nucleus: Effects on locomotor activity mediated by nucleus accumbens-ventral pallidal circuitry. Brain Res. 412:233–243; 1987.
71. Vezina, P.; Kalivas, P. W.; Stewart, J. Sensitization occurs to the locomotor effects of morphine and the specific opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but not to the nucleus accumbens. Brain Res. 417:51–58; 1987.
72. Vezina, P.; Blanc, G.; Glowinski, J.; Tassin, J. P. Nicotine and morphine differentially activate brain dopamine in prefrontocortical and subcortical terminal fields: Effects of acute and repeated injections. J. Pharmacol. Exp. Ther. 261:484–490; 1992.
73. Vezina, P.; Stewart, J. Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacol. Biochem. Behav. 20:925–934; 1984.
74. Vives, F.; Mogenson, G. J. Electrophysiological evidence that the mediodorsal nucleus of the thalamus is a relay between the ventral pallidum and the medial prefrontal cortex in the rat. Brain Res. 344:329–337; 1985.
75. Volkow, N. D.; Fowler, J. S.; Wolf, A. P.; Hitzemann, R.; Dewey, S.; Bendriem, B.; Alpert, R.; Hoff, A. Changes in brain glucose metabolism in cocaine dependence and withdrawal. Am. J. Psychiatry 148:621–626; 1991.
76. Voorn, P.; Gerfen, C. R.; Groenewegen, H. J. Compartmental organization of the ventral striatum of the rat: Immunohistochemical distribution of enkephalin, substance P, dopamine, and calcium-binding protein. J. Comp. Neurol. 289:189–201, 1989.
77. Walaas, I.; Fonnum, F. The distribution and origin of glutamate decarboxylase and choline acetyltransferase in ventral pallidum and other basal forebrain regions. Brain Res. 177:325–336, 1979.
78. Wikler, A. Recent progress in research on the neurophysiological basis of morphine addiction. Am. J. Psychiatry 105:329–338, 1948.
79. Wikler, A. Opioid dependence mechanisms and treatment. New York: Plenum Press; 1980.
80. Wolf, M. E.; Jeziorski, M. Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res. 613:291–294; 1993.
81. Wooten, G. F.; DiStefano, P.; Collins, R. C. Regional cerebral glucose utilization during morphine withdrawal in the rat. Proc. Natl. Acad. Sci. USA 79:3360–3364, 1982.
82. Zaborsky, L.; Alheid, G. F.; Beinfeld, M. C.; Eiden, L. E.; Heimer, L.; Palkovits, M. Cholecystokinin innervation of the ventral striatum: A morphological and radioimmunological study. Neuroscience 14:427–453; 1985.
83. Zahm, D. S. The ventral striatopallidal parts of the basal ganglia in the rat—II. Compartmentation of ventral pallidal efferents. Neuroscience 30:33–50; 1989.
84. Zahm, D. S.; Brog, J. S. On the significance of subterritories in the "accumbens" part of the rat ventral striatum. Neuroscience 50:751–767; 1992.
85. Zahm, D. S.; Heimer, L. Two transpallidal pathways originating in the rat nucleus accumbens. J. Comp. Neurol. 302:437–446; 1990.