Pergamon

0091-3057(95)00086-0

Rats Self-Inject a Dopamine Antagonist in the Lateral Hypothalamus Where It Acts to Increase Extracellular Dopamine in the Nucleus Accumbens

MARCO A. PARADA,*† MARINA PUIG DE PARADA*† AND BARTLEY G. HOEBEL*[1]

*Princeton University, Department of Psychology, Princeton, NJ
†Los Andes University, Department of Physiology, School of Medicine, Mérida, Venezuela

PARADA, M. A., M. PUIG DE PARADA AND B. G. HOEBEL. *Rats self-inject a dopamine antagonist in the lateral hypothalamus where it acts to increase extracellular dopamine in the nucleus accumbens.* PHARMACOL BIOCHEM BEHAV 52(1) 179–187, 1995. — Local injection of sulpiride to block dopamine (primarily D_2-type) receptors in the perifornical lateral hypothalamus (pf-LH) can induce locomotion, feeding, and drinking, and in the present study, local sulpiride induced reward and dopamine (DA) release in the nucleus accumbens. Sulpiride injected bilaterally (4, 8, and 16 µg/0.3 µl), ipsilaterally, or contralaterally (8 µg) in the pf-LH increased extracellular levels of DA and its metabolites in the accumbens. Bilateral sulpiride injected posterior and medial to the pf-LH controlled for diffusion to the ventricle or ventral midbrain. Rats self-injected sulpiride (210 ng/21 nl/2 s) in the pf-LH (111 resp/2 h on drug lever vs. 20 resp on a blank lever). Thus, cells in the pf-LH establish connections with mesolimbic DA neurons involved in the behavior reinforcement process. Evidently hypothalamic cells with DA receptors normally inhibit aspects of behavior reinforcement. Disinhibition with hypothalamic sulpiride is reward for self-injection and cause of overeating that can lead to obesity.

| Neuroleptic | Sulpiride | Intracranial self-administration | | Self-injection | Feeding | Food intake | Obesity |
| Microdialysis | Dopamine | Reinforcement | Reward | | | | |

THE PERIFORNICAL area of the lateral hypothalamus (pf-LH) is a dopaminergic terminal field, as evidenced by fluorescence microscopy (20,31), biochemical analysis (40), and microdialysis (45). These terminals could belong to projections arising from DA cell bodies within the hypothalamus or the midbrain. The hypothalamic source could be the incerto-hypothalamic cell group originally demonstrated by fluorescence histochemistry (1) and further characterized through the visualization of tyrosine-hydroxylase-immunoreactive neurons (4,17), whereas the mesencephalic origin might be represented by DA neurons belonging to the A10 group of the ventral tegmental area (VTA) (6,26,31). DA may exert its functions through D_2 receptors that have been identified in this area (50).

Earlier work implicated hypothalamic DA in the inhibitory control of food and water intake (30,32,33,42) and the inhibi-

tion of locomotion related to procurement of food and water (43). Localized pf-LH administration of sulpiride, a relatively selective D_2-type receptor blocker (22,25), induced feeding, drinking, and a state of psychomotor activation characterized by hyperlocomotion, rearing, and sniffing, which are typical components of exploratory behavior (42,43,44).

Increased levels of forward locomotion seem to reflect the activation of biological mechanisms involved in positive reinforcement (10,60), and many reports have established an association between the meso-accumbens DA pathway, locomotor activity (19,23,29,58), and reinforcement (8,9,15,16,18,19, 53,60,61). Feeding, drinking, mating, stress, and associated stimuli have also been correlated with DA in the nucleus accumbens (NAc) (9,13,14,37,55). For example, microdialysis studies have shown an increase in DA metabolism in the NAc in relation with drinking (64,65) and feeding induced by food

deprivation (11,54,64) or by electrical stimulation of the lateral hypothalamus (11).

Taken together, these data suggest two predictions that were experimentally addressed in the present report. First, hypothalamic administration of sulpiride at a dose that can induce feeding, drinking, and locomotion, might also be able to release DA in the NAc. Second, release of DA produced by intrahypothalamic sulpiride might induce a state similar to that produced during activation of reinforcement mechanisms and, in consequence, the animals would self-administer sulpiride in the pf-LH.

METHOD

Animals and Surgery

For Experiments 1, 2, and 3, rats had guide shafts implanted in the hypothalamus for microinjections of sulpiride plus a guide shaft in the NAc for microdialysis. For Experiment 4, there was just a pf-LH guide shaft for sulpiride self-injections.

Thirty-four adult male Sprague-Dawley rats, weighing between 380 and 420 g at the time of the surgery, were individually housed at 21–23°C on a 15 L : 9 D cycle (lights on at 0700 h), with Purina chow pellets and tap water ad lib. Under ketamine (80 mg/kg) and pentobarbital (12 mg/kg) anesthesia, 29 rats were implanted with chronic bilateral 27 ga stainless steel guide cannulas (47) aimed 2 mm above the pf-LH for later microinjections. Of these rats, 24 had an additional guide shaft for microdialysis in the right NAc. The stereotaxic coordinates for the pf-LH were: 6.2 mm anterior to the interaural line, 1.6 mm lateral to the midsagittal sinus, and 6.6 mm perpendicularly below the surface of the skull (A 6.2, L 1.6, V 6.6). Coordinates for the posterior medial accumbens were: 1.2 mm anterior to the bregma, L 1.2, and V 3.5. In five rats used in Experiment 3 as control for drug diffusion to the VTA the guide shafts for microinjection were placed at A 5, L 0.8, V 6.6. The incisor bar was always placed 3.5 mm below the interaural line. All the experiments were carried out after at least 1 week of postsurgical recovery.

Accumbens Microdialysis During Hypothalamic Microinjections

At least 16 h prior to the collection of dialysates, obturators were removed and a dialysis probe was inserted into the right NAc and attached to a flexible spring connecting the rat to a water-tight swivel (46). Long (200 mm) pieces of PE 20 tubing extended from the hypothalamic guide shafts up the spring and served as guides for the remote insertion of fused silica microinjectors during the experiment the next day (47). The animal was then placed in a dialysis cage for the night. The next day food and water were removed just prior to the collection of dialysates starting at 0800 h. Samples were taken every 20 min for immediate analysis of DA, dihydroxyphenylacetic acid (DOPAC), and homovanilic acid (HVA). After a stable baseline of at least three consecutive DA samples within 10% of each other, the animals received a microinjection of sulpiride or vehicle in the hypothalamus followed by four to five additional NAc samples to assess the injection effects on the meso-accumbens DA system.

The injections were performed following a remote procedure (47). In brief, immediately after the collection of the last baseline sample and without handling the animals, a long injector was inserted through the PE 20 guide and into the intracerebral guide shaft. The injector was made of glass capillary tubing (145 µm o.d. × 21.2 cm, Polymicro Technologies

Inc.) and protruded exactly 2 mm beyond the tip of the intracerebral guide shaft to reach the intended target in the hypothalamus. Polyethylene tubing connected the injector to a 10-µl syringe driven by a syringe pump. The injection volume (0.3 µl) was delivered during 1 min while the rat was freely moving, and microdialysis perfusion in the NAc went on unabated. The hypothalamic injectors were withdrawn 30 s after the injection.

Microdialysis probes were made of fused silica capillary inside of 26 ga stainless steel tubing ending in a tip of 200 µm × 3 mm cellulose dialysis fiber with a 6,000 molecular weight cutoff. The probes were perfused with a Ringer's solution (146 mM NaCl, 4 mM KCl and 1.2 mM CaCl₂) at a flow rate of 1 µl/min, with the capillary tubing as the outlet. Dialysate samples were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-EC). The 20 µl samples were injected into a 50 µl valve loop leading to a 10 cm long, 3.2 mm bore, 3 µm ODS phase II column (Brownlee, Co.). The mobile phase contained 60 mM sodium phosphate monobasic, 100 µm EDTA, 1 mM heptanesulfonic acid and 6% v/v methanol with pH adjusted to 3.6. The mobile phase flow rate was set at 1 ml/min provided by pressure generated by a single-piston pump (SSI Inc., Model 220 B). Neurochemical detection was performed by a Coulochem 5100 A (ESA Inc.) with the guard cell set at +500 mV, the conditioning electrode at +100 mV, and the second electrode at −400 mV. The order of elution was DOPAC, DA, and HVA with retention times of 3.5, 5.2, and 10.5 min, respectively. Neurochemicals in each sample were measured by the ratio of unknown to standard peak heights, and the results expressed as pg/20 µl.

In Experiment 1, bilateral sulpiride injections in the pf-LH were tested for their effects on extracellular DA in the accumbens. Sixteen rats in four groups of four animals received one bilateral pf-LH microinjection of sulpiride (4, 8, or 16 µg/0.3 µl) or its vehicle (0.3 µl) while extracellular levels of DA and its metabolites DOPAC and HVA were measured in the NAc. The 16 µg dose was prepared by dissolving 27 mg of d-l-sulpiride (Sigma Chemical Co.) in 500 µl of vehicle containing 425 µl of Ringer and 75 µl of 1N acetic acid. The pH of the solvent was 2.0 but increased to 5.0 after the addition of sulpiride; therefore, for control injections, the vehicle was adjusted to pH 5.0 with NaOH. Lower concentrations of sulpiride were prepared by dilution.

In Experiment 2, sulpiride injections were made in the pf-LH on just one side, ipsilateral or contralateral to the NAc. Two groups of four rats received unilateral LH sulpiride (8 µg/0.3 µl), four rats on the right and four on the left, while microdialysis perfusates were assayed from the right NAc.

Experiment 3 tested the possibility that sulpiride could diffuse from the injection site in the LH into the ventricles or towards the VTA, where the drug might directly affect DA cell bodies through D₂ autoreceptor blockade that would increase activity of the meso-accumbens DA pathway. Five rats received bilateral microinjections of sulpiride (8 µg/0.3 µl) at a site 1.2 mm posterior and 0.8 mm medial to the LH coordinate used in the other experiments.

Self-Injection Procedure

In Experiment 4, the right pf-LH was used for self-administration of sulpiride. After recovery from surgery the animals were trained to work for food (45 mg Noyes pellets) in a test chamber (30 cm long × 10 cm wide) with one active and one blank lever installed at opposite ends and protruding

from the walls 3.5 cm above the floor. During the training phase food was used initially and then faded out as follows. In two overnight sessions food-deprived animals learned to press the active lever for food. The intended purpose of the food reward was simply to teach the animals how to press. During the first of several daytime sessions the deprived animals were connected by silica capillary tubing (47) via a swivel joint (46) to a remote 10-μl syringe. The active lever simultaneously triggered the food dispenser and a syringe pump delivering intrahypothalamic injections (21.5 ± 1.28 nl/2 s) of sulpiride (10 μg/μl). For the next two sessions animals were not deprived and received food concomitant with the injections, but only during the first 15 min. In sessions four and five the animals did not receive food at all, but a few priming injections were delivered by the experimenter to start intracranial self-administration.

The testing phase consisted of three sessions. The animals started pressing the lever spontaneously, and responses on the active lever were automatically recorded in 30-min periods and on the blank lever for the whole session. Intracranial self-administration was allowed during 2-h sessions on a continuous reinforcement schedule (i.e., fixed ratio, one response : one injection) with 3-day intervals between sessions.

Statistical Analysis

Because there was a large variation in DA baseline between animals, the data were normalized for statistical comparison by expressing the concentration in each sample as a percent of the mean of three samples (mean baseline). Within subjects analyses were performed to assess the effect of each treatment on the extracellular levels of DA, DOPAC, and HVA by comparing preinjection and postinjection samples using repeated measures ANOVA followed by means/regression coefficient comparisons. Linear regression analysis was carried out on data from the first experiment to assess the degree of correlation between the different doses of sulpiride and the maximal increases in the extracellular levels of DA, DOPAC, and HVA. Data obtained from the active and blank levers during the 2-h self-administration sessions were compared using one-way ANOVA.

Histology

After the experiments the rats were anesthetized with pentobarbital and the brains perfused with formalin. After at least 5 days of fixation, the brains were sliced and the tracks of the injector cannulas were localized.

RESULTS

Experiment 1: Bilateral pf-LH Sulpiride Releases DA in the NAc

Bilateral intrahypothalamic administration of sulpiride increased extracellular DA and its metabolites in the NAc at all the doses tested (Fig. 1). The maximal mean DA levels (second sample postinjection in top graph) for the 4.8 and 16 μg doses were respectively 179.7%, 268.5%, and 433.5%, $F(3, 5) = 18.2, p < 0.001, 12.5, p < 0.01,$ and $16.7, p < 0.001,$ respectively. Vehicle administration did not modify extracellular DA levels (96.4%). Linear regression analysis performed on the different doses and the corresponding DA levels of the second postinjection sample showed a positive significant correlation ($r = 0.74, F = 17.6, p < 0.001$), as depicted in Fig. 2.
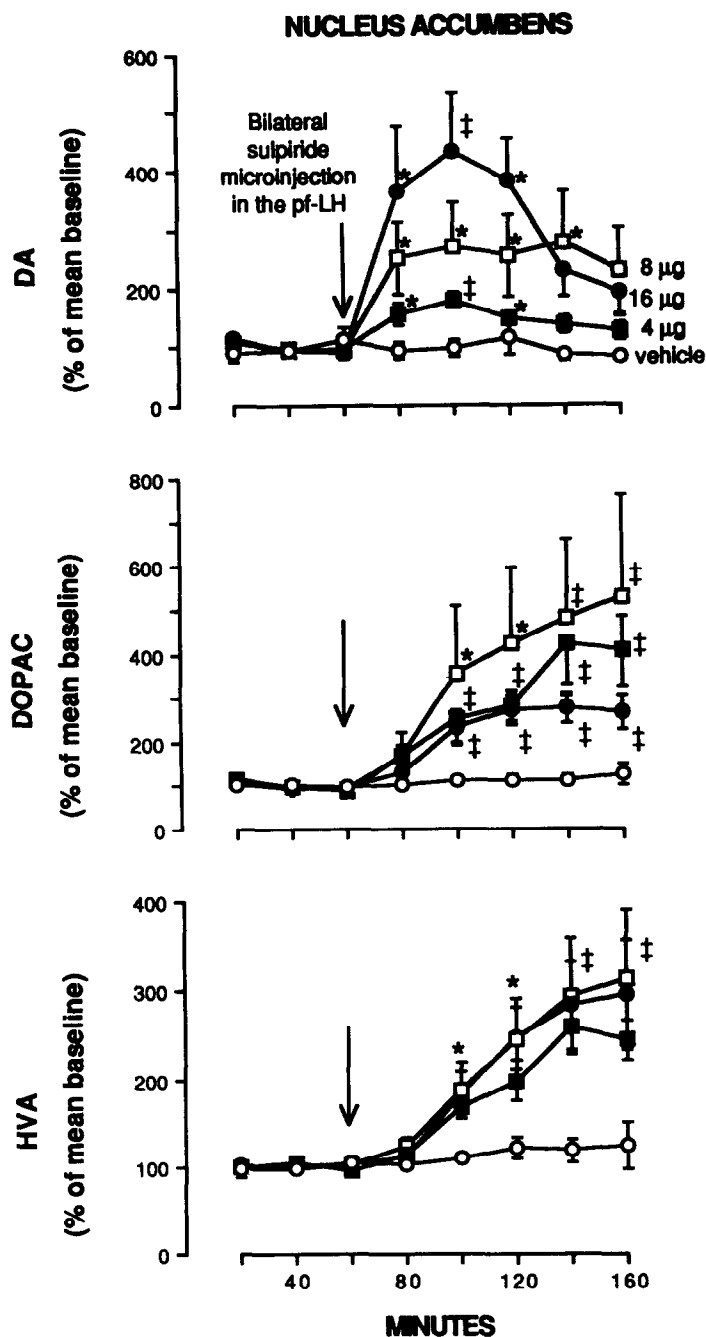
DOPAC (Fig. 1—middle graph) and HVA (Fig. 1—bottom

FIG. 1. Sulpiride injected bilaterally in the pf-LH increases extracellular DA in the NAc. Curves show extracellular levels of DA (top graph), DOPAC (middle graph), and HVA (bottom graph) in the right NAc of rats receiving bilateral pf-LH microinjections of sulpiride (filled squares, 4 μg/0.3 μl; open squares, 8 μg/0.3 μl; filled circles, 16 μg/0.3 μl; open circles, 0.3 μl of vehicle). Arrows mark the time of the injection. Symbols indicate statistically significant differences between a data point and its corresponding preinjection level (*$p < 0.01$; ‡$p < 0.001$). Data represent the mean ± SE for four different animals at each dose.

graph) increased steadily after sulpiride injections and reached their highest levels in the last sample taken in this experiment (fifth sample postinjection). DOPAC mean levels 100 min
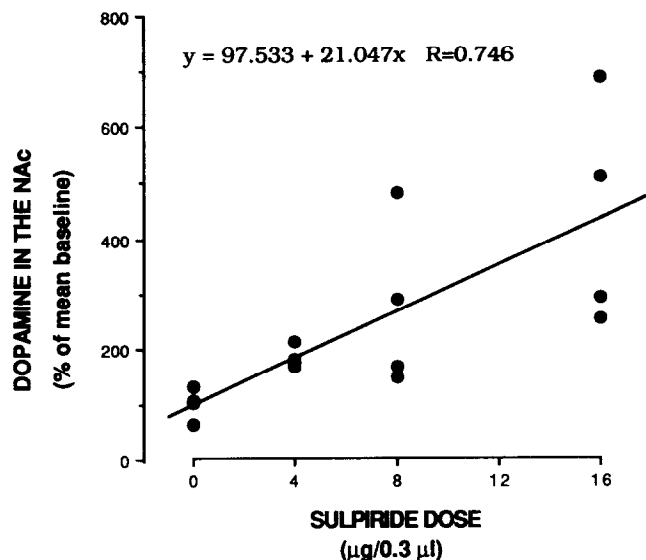
$$y = 97.533 + 21.047x \quad R=0.746$$

FIG. 2. Relationship between bilateral intrahypothalamic sulpiride dose and extracellular DA levels in the nucleus accumbens 40 min after the injections. Each point represents a different subject. Four animals were used for each dose.

after the three doses were 298%, 405.4%, and 266.7% ($p < 0.0001$ for each dose). After vehicle injection, DOPAC levels remained unchanged (107% in the last sample). Regression analysis showed no significant correlation between sulpiride doses and DOPAC levels of the last samples.

HVA mean levels in the last samples were 244.3%, 312.3%, and 296.1% ($p < 0.0001$ for each dose). The small increase in extracellular HVA level after the vehicle administration (128.5%) was nonsignificant, $F(3, 5) = 0.7$. The correlation between the different sulpiride doses and the final HVA levels was also nonsignificant.

Experiment 2: Either Ipsilateral or Contralateral pf-LH Sulpiride Increases DA in the NAc

Unilateral injection of 8 μg sulpiride in the right or left pf-LH increased extracellular DA and its metabolites in the right NAc (Fig. 3). DA concentration (top graph in Fig. 3) increased steadily after the ipsilateral injection of sulpiride and was still above the preinjection levels 80 min after the injection [214.7%; $F(3, 4) = 8.6, p < 0.05$]. Even contralateral sulpiride increased extracellular DA during the first three samples after the injection, with the peak effect during the first sample postinjection [173.2%; $F(3, 4) = 10.1, p < 0.01$].

DOPAC increased in the NAc and reached its maximal levels during the last sample recorded after the ipsilateral [179%; $F(3, 4) = 28.5, p < 0.001$] and contralateral [146.7%; $F(3, 4) = 11.7, p < 0.01$] sulpiride injection (Fig. 3—middle graph).

HVA levels also increased significantly (bottom graph in Fig. 3) with maximal levels reached during the last sample for the ipsilateral injection [192.1%; $F(3, 4) = 19.8, p < 0.001$] and the contralateral injection [185.5%; $F(3, 4) = 26.2; p < 0.001$].

Experiment 3: Sulpiride Effect Was Not Due to Diffusion to the VTA

As shown in Fig. 4, the bilateral administration of 8 μg of sulpiride in an area approximately equidistant between the LH and the VTA did not produce any modification in extracellular DA, DOPAC, or HVA in the NAc.
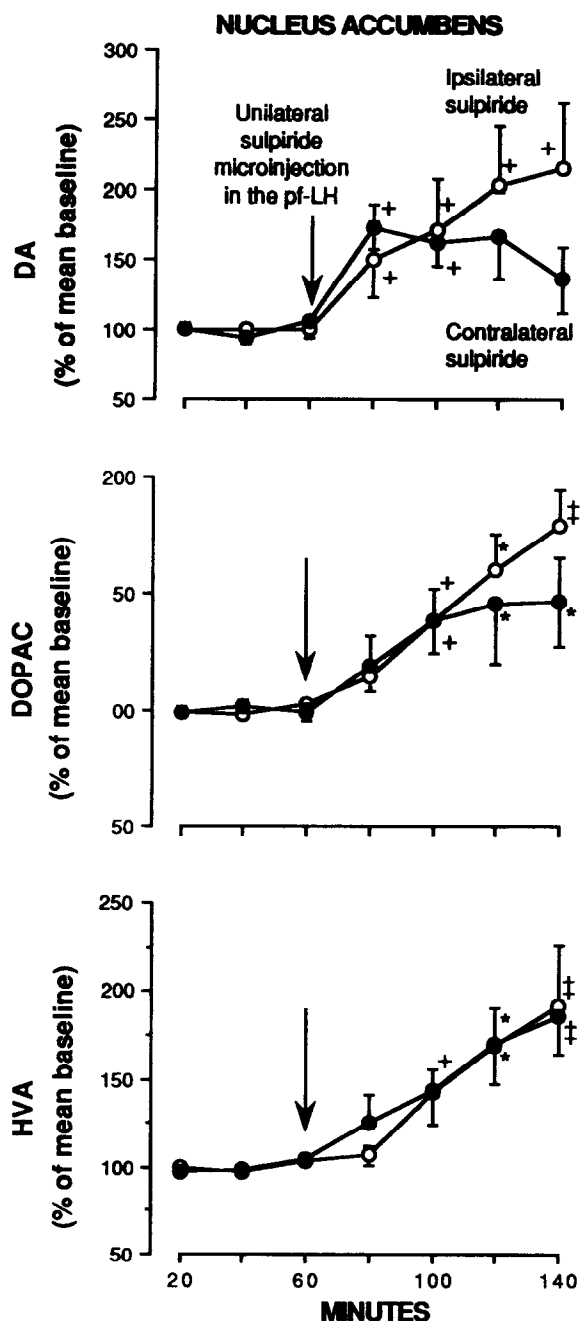

FIG. 3. Extracellular levels of DA (top graph), DOPAC (middle graph), and HVA (bottom graph) increase in the right NAc of rats receiving pf-LH microinjections of 8 μg/0.3 μl sulpiride (arrows) on the same side or opposite side of the brain. Symbols indicate significant differences between data point and its corresponding preinjection level ($+p < 0.05$; $*p < 0.01$; and $\ddagger p < 0.001$). Each point represents the mean $\pm$ SE of four animals.

NUCLEUS ACCUMBENS

preferred the lever that injected sulpiride to the blank lever that did not have any contingent value. The mean frequency of responses on the drug lever vs. the blank lever during the three 2-h sessions was 111 vs. 20 (each session individually as follows: 98 vs. 23, 108.4 vs. 29 and 127 vs. 34). The bottom graph in Fig. 5 shows that the frequency of lever pressing was homogeneously distributed in the 30-min periods of each session and each was statistically significant.

The histological analysis showed no substantial differences with our previous studies. The tips of the injector cannulas ended in the vicinity of the perifornical lateral hypothalamus as shown in those previous reports (38-40).

DISCUSSION

The present report shows an increase in extracellular DA, DOPAC, and HVA in the NAc as a consequence of sulpiride injections in the perifornical lateral hypothalamus, and not the posterior hypothalamus. These results give support to the idea that neurons in the LH modulate the activity of dopaminergic neurons linking the VTA to the NAc and strongly suggest that the mesolimbic DA system is involved in the expression of the behavioral repertoire induced by hypothalamic sulpiride. We do not know how or where the pf-LH neurons connect to the mesolimbic DA neurons, but those connections are in-
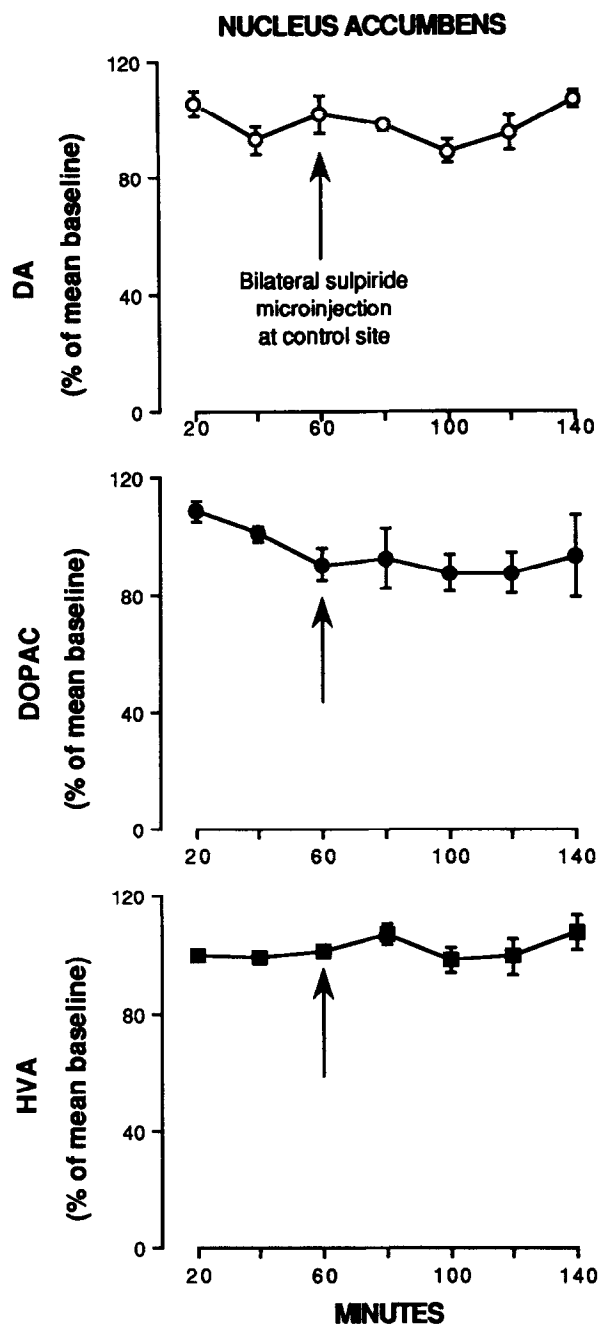

FIG. 4. Sulpiride injected at a control site closer to the VTA and third ventricle had no effect. Curves show extracellular levels of DA, DOPAC, and HVA in the right NAc of rats with bilateral microinjection of 8 μg/0.3 μl of sulpiride in an area 1.2 mm posterior and 0.8 mm medial to the coordinate used in the other experiments of this report (mean ± SE, n = 5).

Experiment 4: Rats Self-Inject Sulpiride in the pf-LH

The animals learned intrahypothalamic self-administration of sulpiride This behavior was maintained during several sessions though only data from the last three sessions without food or priming are reported and included in the graphs of Fig. 5. The top graph shows that the animals consistently
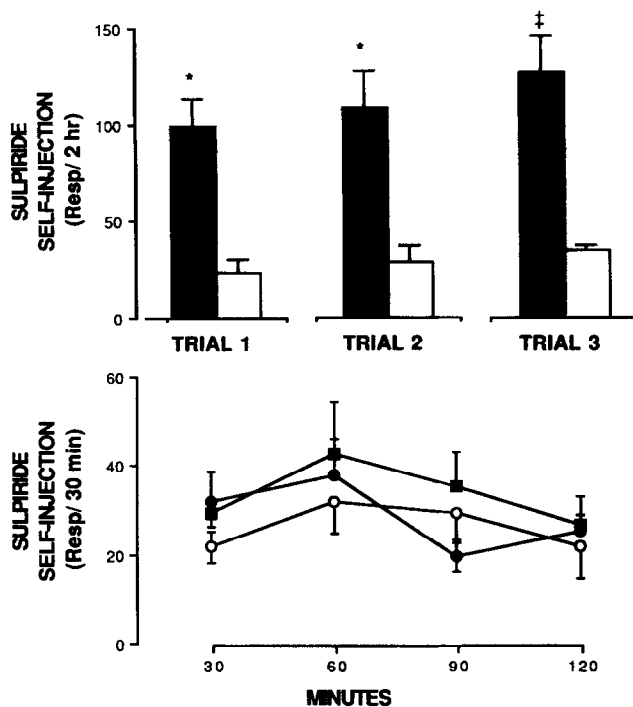
FIG. 5. Intrahypothalamic self-administration of sulpiride in five rats during sessions (trials) on 3 different days. Each press of the active lever delivered a 2 s injection of 21.5 ± 1.28 nl of 10 ng/nl sulpiride. Presses on the blank lever were recorded but they did not have any contingent value. Top graph: response frequency on the active lever (filled columns) and the blank lever (open columns) during each session. Symbols indicate significant differences between responses on the active and blank levers (*p < 0.01; and ‡p < 0.001). Bottom graph: response frequency on the active lever was consistent in all four 30 min periods during the first (open circles), second (filled circles), and third (filled squares) trials on three different days.

ferred from experiments showing that electrical stimulation of feeding areas in the LH modifies the activity of electrophysiologically characterized DA neurons in the VTA (36) and increases extracellular DA, DOPAC, and HVA in the NAc (11). Due to the nonspecific nature of electrical stimulation it may not discriminate well between local LH cell bodies and fibers of passage. Chemical stimulation of the kind produced by sulpiride, on the other hand, implies a specific action on local hypothalamic neurons. The D_2 family of receptors thought to be responsible for sulpiride's action could be on input terminals, local interneurons, or output cells in the lateral hypothalamus. Output neurons in the LH could reach DA terminals in the NAc or DA cell bodies in the VTA. Direct connections from the lateral hypothalamic cell bodies to the NAc were discovered using the retrograde transport of wheatgerm aglutinin (49) or Fluoro-gold (3), but there are also indirect connections, for example, via the hippocampus (27). Anatomical evidence also gives support to the idea that the increase in extracellular DA, DOPAC, and HVA in the NAc brought about by experimental manipulations of the lateral hypothalamus is produced by activation of a direct LH-VTA pathway that increases the activity of mesoaccumbens DA cell bodies. The anatomical evidence has been gathered from studies tracing axonal degeneration to the VTA after electrolytic lesions placed in the LH (18,62), anterograde transport of tritiated amino acids from the LH to the VTA (56), and retrograde transport of horseradish peroxidase from the VTA to the LH (48). Both ipsilateral and contralateral projections from the LH to the VTA have been described (48) and help to explain the findings of our second experiment showing increases of DA metabolism in the right accumbens after ipsilateral or contralateral injections of sulpiride in the LH. The contralateral effect of sulpiride may also be due to bilateral projections from the VTA to the NAc; just as bilateral nigral (7,51,52) or VTA (35) projections to the dorsal striatum have been well described. Indirect connections between the lateral hypothalamus and the VTA via the lateral habenula or the locus coeruleus have been proposed (11).

Lateral hypothalamic administration of sulpiride induces a complex repertoire of responses reminiscent of those seen with electrical stimulation of the same area. Behavioral effects are evident within a few seconds after the injection (42), which discounts the possibility of drug diffusion to neural elements relatively far from the injection site in the lateral hypothalamus. However, the sampling time (20 min) of our microdialysis experiments is so long that the drug could theoretically have diffused to areas relatively close to the LH to affect DA release in the NAc. One argument against this possibility is that sulpiride is a somewhat selective dopamine D_2 receptor blocker (22,25) and D_2 receptors are located close to the injection site in the pf-LH (50). However, the present experiments did not rule out the possibility that sulpiride could be blocking D_2 receptors in the dorsal hypothalamic zona incerta where D_2 receptors have been shown in relatively close proximity to the injection site (21). On the other hand, D_2 receptors in the VTA inhibit mesolimbic dopamine cells (24); so any blockade of those receptors by sulpiride reaching the VTA would explain DA release in the NAc. The result of Experiment 3 counters this possibility by showing that sulpiride injections between the pf-LH and VTA did not produce any detectable neurochemical modifications in the NAc. This makes it very unlikely that pf-LH sulpiride could have diffused to the VTA and directly activated DA neurons there. Diffusion 4 mm to the NAc is also unlikely, judging by previous experiments (44) in which bilateral injections of large doses of amphetamine (20 μg) in the pf-LH area did not increase locomotion as

would have been expected if amphetamine had reached DA terminals in the NAc. Diffusion via the ventricles is unlikely because the ineffective control site was closer to the third ventricle than the effective pf-LH site. Thus, our conclusion is that sulpiride acted within the pf-LH or the zona incerta to indirectly increase the activity of the meso-accumbens DA system, probably via an ascending connection to the NAc or a descending neural connection to the VTA.

Some decades ago it was postulated that approach behaviors and positive reinforcement are related and share a common neural mechanism embedded in the medial forebrain bundle (MFB), including the lateral hypothalamus (10). This idea was later elaborated in the context of addictive drugs and termed the psychomotor stimulant theory of addiction (60). Briefly stated, this theory postulates that drugs acting as positive reinforcers elicit forward locomotion and that both reinforcement and locomotion arise from activation of dopaminergic pathways traveling in the MFB. It is currently accepted that opiate reinforcement can occur either by activation of the mesolimbic DA system or by an independent action in the NAc or elsewhere (5,28), which suggests the existence of multiple substrates or a series of substrates for reinforcement in the brain. Given the evidence that hypothalamic sulpiride induces locomotion and activates the mesolimbic dopamine system, the psychomotor stimulant theory predicts that sulpiride can activate positive reinforcement mechanisms in the LH, and suggests that sulpiride be classified as a psychostimulant when it is locally applied to the pf-LH.

In previous articles (43,44) we reported that intracranial administration of sulpiride in the pf-LH was followed by intense locomotor and exploratory activity similar in magnitude to the locomotion triggered by a moderate dose of intraperitoneal amphetamine (44). That phenomenon was not the consequence of some nonspecific irritation because DA administered locally at the same site before sulpiride almost completely blocked the sulpiride effect (43). The microdialysis experiments in the present report show that mesoaccumbens DA neurons are activated by sulpiride injections in the same area where the drug induces locomotion. Experiment 4 confirms the prediction that the animals would self-administer sulpiride in the pf-LH, just as they self-administer amphetamine in the NAc (16).

The self-administration of sulpiride reported here does not include all the tests recommended to validate the specificity and rewarding value of intracranial self-administration (2,16, 61). In regard to the anatomical controls required (61), we have not yet mapped the self-injection effect; however, it can be noted that sulpiride triggered the reward process from an area that supports electrical self-stimulation and is widely recognized as an important component of a positive reinforcement system (12,13,19,34,38,39,57,59,60,63). It can also be predicted that sulpiride self-administration will prove anatomically specific because microinjections of the drug in an area about 2 mm away, and closer to the ventricles and VTA did not have any measurable effect on the meso-accumbens DA pathway (Experiment 3). The pharmacological specificity of sulpiride on hypothalamic DA receptors that trigger self-administration can be deduced from the facts that (a) sulpiride is known to be a relatively specific D_2-type antagonist (22,25), (b) D_2 receptors are present in the pf-LH (50), and (c) prior administration of DA suppresses sulpiride-induced locomotion (43). In terms of behavioral controls, lever pressing for sulpiride was maintained at a consistent rate throughout several sessions by animals that were nondeprived and no longer receiving food as a consequence of their responses and no longer primed to start the behavior. The homogeneous

distribution of responses during the four 30-min periods of each session argues against the possibility that the response rate on the active lever might be reflecting the extinction of responses associated with lever pressing for food. Once engaged in sulpiride self-administration the animals became progressively more hyperactive, and some responses were probably due to this hyperactivity. However, sulpiride injections had rewarding consequences because the animals consistently chose the lever associated with the infusion of the drug and displayed a relatively low frequency of responses on the blank lever.

This suggests that sulpiride applied locally caused a direct activation of some neurons originating in or near the pf-LH, the output of which activated DA neurons that originate in the VTA and that induce not only locomotion but also reinforcement. Because DA release in the NAc can occur during rewarding or aversive experiences, the fact that we demonstrated positive reinforcement suggests that the pf-LH sulpiride induced not only DA release, which may help reinforce any behavior, but also set up the conditions for reward as opposed to aversion. It is quite possible that the local hypothalamic neurons involved in sulpiride reinforcement belong to the set of neural elements activated during electrical self-stimulation of the pf-LH. It has been suggested that the cell bodies involved in the positive reinforcement of electrical self-stimulation of the MFB are mainly located in the region of the diagonal band and the lateral preoptic area (63). We cannot rule out sulpiride diffusion into those areas to produce its rewarding effects, but the existence of cell bodies subserving the descending reinforcement process linked to the electrical self-stimulation and located in the LH can be inferred from experiments showing that lesions of the anterior part of the MFB are less effective than posterior lesions to reduce reward related to LH self-stimulation (59).

The psychomotor and behavior reinforcement roles of DA in the NAc are widely accepted ideas, although it has only recently become recognized that NAc DA may be involved in both positive and negative reinforcement (55). It has long been known that pf-LH electrical stimulation produces both positive and negative reinforcement as reflected in self-stimulation and stimulation-escape. The relative proportion of each behavior depends in part on the animal's physiological or pharmacological condition (12). Somewhere, including the hypo-thalamus, there are systems that inhibit feeding and the reinforcement that is reflected in forward locomotion and self-stimulation. Hypothalamic DA receptors may be part of such systems.

Some dopamine receptors in the pf-LH clearly influence locomotion, appetitive behavior, and reward. At the pf-LH site where sulpiride induces locomotion, DA had the opposite effect, as it selectively blocked exploratory locomotion in food- and water-deprived rats but not locomotion in animals placed for the first time in a novel ambience (43). These data suggest that LH dopamine exerts an inhibitory modulation on locomotor mechanisms related in part to the search for positive reinforcer stimuli such as food or water. The present experiments also suggest that DA in the LH might also have an antireinforcement action, e.g., reward-reduction, or an aversive effect, or simply a lack of sensorimotor engagement. The anorectic action of exogenous DA applied locally in the pf-LH (32,33,41) might well have something to do with the fact that DA in the pf-LH apparently inhibits locomotion for food procurement and inhibits the reinforcement of ongoing behavior.

In summary, the present report shows that microinjections of sulpiride in the pf-LH increase extracellular DA, DOPAC, and HVA in the NAc in a dose-dependent manner, and that rats can learn to self-administer this dopamine receptor antagonist in the pf-LH. These results suggest the existence of neurons in the LH with influence over mesoaccumbens DA neurons. These LH neurons are characterized by their dopaminergic receptors, probably of the D_2 family. Apparently endogenous DA in the pf-LH normally inhibits these neurons thereby inhibiting some aspect of locomotion, eating, and drinking and inhibits the release of NAc dopamine. Disinhibition of these LH DA-sensitive cells seems to a) promote appetitive behaviors, b) promote locomotion, and c) do this in conjunction with accumbens release of DA. The conjunction of behavior plus accumbens DA may be sufficient to reinforce the behavior. Given that appetitive behaviors have been observed, it is presumably appetitive behaviors that would normally be reinforced.

REFERENCES

1. Björklund, A.; Lindvall, O.; Nobin, A. Evidence of an incertohy-pothalamic dopamine neurone system in the rat. Brain Res. 89: 29–42; 1975.
2. Bozarth, M. A. Intracranial self-administration procedures for the assessment of drug reinforcement. In: Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer Verlag; 1987:173–187.
3. Brog, J. S.; Salyapongse, A.; Deutch, A. Y.; Zahm, D. S. The pattern of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. J. Comp. Neurol. 338:255–278; 1993.
4. Chan-Palay, V.; Záborsky, L.; Köhler, C.; Goldstein, M.; Palay, S. L. Distribution of tyrosine-hydroxylase-immunoreactive neurons in the hypothalamus of rats. J. Comp. Neurol. 227:467–496; 1984.
5. Ettenberg, A.; Pettit, H. O.; Bloom, F. E.; Koob, G. F. Heroin and cocaine intravenous self-administration in rats: Mediation by separate neural systems. Psychopharmacology (Berlin) 78:204–209; 1982.
6. Fallon, J. H.; Moore, R. Y. Catecholaminergic innervation of the basal forebrain. IV: Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol. 182:545–580; 1978.
7. Fass, B.; Butcher, L. L. Evidence for a crossed nigrostriatal pathway in rats. Neurosci. Lett. 22:109–113; 1981.
8. Fibiger, H. C. Drugs and reinforcement mechanisms: A critical review of the catecholamine theory. Annu. Rev. Pharmacol. Toxicol. 18:37–56; 1978.
9. Fibiger, H. C.; Phillips, A. G. Dopamine and the neural mechanisms of reinforcement. In: Horn, A. S.; Westerink, B. H. C.; Korf, J., eds. The neurobiology of dopamine. New York: Academic Press; 1979:597–615.
10. Glickman, S. E.; Shiff, B. B. A biological theory of reinforcement. Psychol. Rev. 74:81–109; 1967.
11. Hernandez, L.; Hoebel, B. G. Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiol. Behav. 44:599–606; 1988.
12. Hoebel, B. G. Brain-stimulation reward and aversion in relation to behavior. In: Wauquier, A.; Rolls, E. T., eds. Brain-stimulation reward. Amsterdam: Elsevier; 1976:335–372.
13. Hoebel, B. G.; Rada, P.; Mark, G. P.; Hernandez, L. The power

of integrative peptides to reinforce behavior by releasing dopa-
mine. In: Strand, F. L.; Beckworth, B.; Chronwall, B., Sandman,
C. A., eds. Models of neuropeptide action. vol. 739. New York:
Annals of the New York Academy of Science; 1994:36–41.

14. Hoebel, B. G.; Hernandez, L.; Mark, G. P.; Schwartz, D. H.;
Pothos, E.; Steckel, J. M.; Stone, E. A. Brain microdialysis as a
molecular approach to obesity: Serotonin, dopamine, cyclic-
AMP. In: Bray, G. A.; Ricquier, D.; Spiegelman, B. M., eds.
Obesity: Towards a molecular approach. New York: Wiley-Liss;
1990:45–61.

15. Hoebel, B. G.; Leibowitz, S. F.; Hernandez, L. Neurochemistry
of anorexia and bulimia. In: Anderson, H.; Kennedey, S. H.,
eds. The biology of feast and famine: Relevance to eating disor-
ders. New York: Academic Press; 1992:21–45.

16. Hoebel, B. G.; Monaco, A. P.; Hernandez, L.; Aulisi, E. F.;
Stanley, B. G.; Lenard, L. Self-injection of amphetamine directly
into the brain. Psychopharmacology (Berlin) 81:158–163; 1983.

17. Hökfelt, T.; Mårtensson, R.; Björklund, A.; Kleinau, S.; Gold-
stein, M. Distributional maps of tyrosine-hydroxylase-immuno-
reactive neurons in the rat brain. In: Björklund, A.; Hökfelt,
T., eds. Handbook of chemical neuroanatomy. vol. 2: Classical
transmitters in the CNS, part I. New York: Elsevier Sci. Publ.;
1984:277–379.

18. Huang, Y. H.; Mogenson, G. J. Neural pathways mediating
drinking and feeding in rats. Exp. Neurol. 37:269–286; 1972.

19. Iversen, S. D.; Koob, G. F. Behavioral implications of dopamin-
ergic neurons in the mesolimbic system. Adv. Biochem. Psycho-
pharmacol. 16:209–214; 1977.

20. Jacobowitz, D. M.; Palkovits, M. Topographic atlas of catechol-
amine and acetylcholinesterase-containing neurons in the rat
brain. I. Forebrain (telencephalon, diencephalon). J. Comp. Neu-
rol. 157:13–28; 1974.

21. Janowsky, A.; Neve, K. E.; Kinzie, J. M.; Taylor, B.; De Paulis,
T.; Belknap, J. K. Extrastriatal dopamine D_2 receptors: Distribu-
tion, pharmacological characterization and region-specific regula-
tion by clozapine. J. Pharmacol. Exp. Ther. 261:1282–1290;
1992.

22. Jenner, P.; Elliot, P. N. C.; Clow, A.; Reavill, C.; Marsden,
C. D. A comparison of in vitro and in vivo dopamine receptor
antagonism produced by substituted benzamide drugs. J. Pharm.
Pharmacol. 30:46–48; 1978.

23. Jones, D. L.; Mogenson, G. J.; Wu, M. Injections of dopaminer-
gic, cholinergic, serotonergic and gabaergic drugs into the nucleus
accumbens: Effects on locomotor activity in the rat. Neurophar-
macology 20:29–37; 1981.

24. Kalivas, P. W. Neurotransmitter regulation of dopamine neurons
in the ventral tegmental area. Brain Res. Rev. 18:75–113; 1993.

25. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine.
Nature 277:92–96; 1979.

26. Kizer, J. S.; Palkovits, M.; Brownstein, M. J. The projection of
the A8, A9, and A10 dopaminergic cell bodies: Evidence for a
nigral-hypothalamic-median eminence dopaminergic pathway.
Brain Res. 108:363–370; 1976.

27. Köhler, C.; Haglund, L.; Swanson, L. W. A diffuse αMSH-
immunoreactive projection to the hippocampus and spinal cord
from individual neurons in the lateral hypothalamic area and
zona incerta. J. Comp. Neurol. 223:501–514; 1884.

28. Koob, G. F. Separate neurochemical substrates for cocaine and
heroin reinforcement. In: Commons, M. L.; Church, R. M.; Stel-
lar, J. R.; Wagner, A. R., eds. Biological determinants of rein-
forcement. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988:
139–156.

29. Koob, G. F.; Riley, S. J.; Smith, S. C.; Robbins, T. W. Effects
of 6-hydroxydopamine lesions of the nucleus accumbens septi and
olfactory tubercle on feeding, locomotor activity, and amphet-
amine anorexia in the rat. J. Comp. Physiol. Psychol. 92:917–
927; 1978.

30. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral
hypothalamus: Their role in the mediation of amphetamine an-
orexia. Brain Res. 98:529–545; 1975.

31. Leibowitz, S. F.; Brown, L. L. Histochemical and pharmacologi-
cal analysis of catecholaminergic projections to the perifornical

hypothalamus in relation to feeding inhibition. Brain Res. 201:
289–314; 1980.

32. Leibowitz, S. F.; Rossakis, C. Mapping study of brain dopamine-
and epinephrine-sensitive sites which cause feeding suppression in
the rat. Brain Res. 172:101–113; 1979.

33. Leibowitz, S. F.; Rossakis, C. Pharmacological characterization
of perifornical hypothalamic dopamine receptors mediating feed-
ing inhibition in the rat. Brain Res. 172:115–130; 1979.

34. Lestand, I.; Cardo, B.; Roy, M. T.; Velley, L. Electrical self-
stimulation deficits in the anterior and posterior parts of the me-
dial forebrain bundle after ibotenic acid lesion of the middle lat-
eral hypothalamus. Neuroscience 15:379–388; 1985.

35. Loughlin, S. E.; Fallon, J. H. Mesostriatal projections from ven-
tral tegmentum and dorsal raphe: Cells project ipsilaterally or
contralaterally but not bilaterally. Neurosci. Lett. 32:11–16; 1982.

36. Maeda, H.; Mogenson, G. J. A comparison of the effects of
electrical stimulation of the lateral hypothalamus on the activity
of neurons in ventral tegmental area and substantia nigra. Brain
Res. Bull. 7:283–291; 1981.

37. Mark, G. P.; Schwartz, D. H.; Hernandez, L.; West, H. L.;
Hoebel, B. G. Application of microdialysis to the study of moti-
vation and conditioning: Measurements of dopamine and seroto-
nin in freely behaving rats. In: Robinson, T. E.; Justice, J. B.,
Jr., eds. Microdialysis in the neurosciences. New York: Elsevier
Sci. Publ.; 1991:369–385.

38. Nassif, S.; Cardo, B.; Libersat, F.; Velley, L. Comparison of
deficits in electrical self-stimulation after ibotenic acid lesion of
the lateral hypothalamus and the medial prefrontal cortex. Brain
Res. 332:247–257; 1985.

39. Ono, T.; Nakamura, K.; Nishijo, H.; Fukuda, M. Hypothalamic
neuron involvement in integration of reward, aversion and cue
signals. J. Neurophysiol. 56:63–79; 1986.

40. Palkovits, M.; Brownstein, M.; Saavedra, J. M.; Axelrod, J.
Norepinephrine and dopamine content of hypothalamic nuclei of
the rat. Brain Res. 77:137–149; 1974.

41. Parada, M. A.; Hernandez, L.; Degoma, E. Serotonin may play
a role in the anorexia induced by amphetamine injections in the
lateral hypothalamus. Brain Res. 577:218–225; 1992.

42. Parada, M. A.; Hernandez, L.; Hoebel, B. G. Sulpiride injections
in the lateral hypothalamus induce feeding and drinking in rats.
Pharmacol. Biochem. Behav. 30:917–923; 1988.

43. Parada, M. A.; Hernandez, L.; Puig de Parada, M.; Paez, X.;
Hoebel, B. G. Dopamine in the lateral hypothalamus may be
involved in the inhibition of locomotion related to food and water
seeking. Brain Res. Bull. 25:961–968; 1990.

44. Parada, M. A.; Hernandez, L.; Santiago, C. An improved circu-
lar tilt-cage shows that intrahypothalamic injections of sulpiride
increase locomotion. Brain Res. Bull. 21:873–880; 1988.

45. Parada, M. A.; Hernandez, L.; Schwartz, D.; Hoebel, B. G.
Hypothalamic infusion of amphetamine increases serotonin, do-
pamine and norepinephrine. Physiol. Behav. 44:607–610; 1988.

46. Parada, M. A.; Puig de Parada, M.; Hoebel, B. G. A new triple
channel swivel for fluid delivery in the range of intracranial (10
nl) and intravenous (100 μl) self-administration volumes and also
suitable for microdialysis. J. Neurosci. Methods 54:1–8; 1994.

47. Parada, M. A.; Puig de Parada, M.; Hoebel, B. G. A remote
insertion technique for intracerebral microinjections in freely
moving animals. J. Neurosci. Methods 50:237–241; 1993.

48. Phillipson, O. T. Afferent projections to A10 dopaminergic neu-
rons in the rat as shown by the retrograde transport of horserad-
ish peroxidase. Neurosci. Lett. 9:353–359; 1978.

49. Phillipson, O. T.; Griffiths, A. C. The topographic order of in-
puts to nucleus accumbens in the rat. Neuroscience 16:275–296;
1985.

50. Pizzi, M.; Coen, E.; Memo, M.; Missale, C.; Carruba, M. O.;
Spano, P. F. Evidence for the presence of D_2 but not D_1 dopamine
receptors in rat hypothalamic perifornical area. Neurosci. Lett.
67:159–162; 1986.

51. Pritzel, M.; Huston, J. P. Neural and behavioral plasticity:
Crossed nigrothalamic projections following unilateral substania
nigra lesions. Behav. Brain Res. 3:393–399; 1981.

52. Pritzel, M.; Sarter, M.; Morgan, S.; Huston, J. P. Interhemi-

spheric nigrostriatal projections in the rat: Bifurcating nigral projections and loci of crossing in the diencephalon. Brain Res. Bull. 10:385–390; 1983.

53. Pulvirenti, L.; Swerdlow, N. R.; Hubner, C. B.; Koob, G. F. The role of limbic-accumbens-pallidal circuitry in the activating and reinforcing properties of psychostimulant drugs. In: Wilner, P.; Scheel-Krüger, J., eds. The mesolimbic dopamine system: From motivation to action. Chichester: John Wiley & Sons; 1991:131–140.

54. Radhakishum, F. S.; van Ree, J. M.; Westerink, B. H. C. Scheduled eating increases dopamine release in the nucleus accumbens of food-deprived rat as measured with on-line brain dialysis. Neurosci. Lett. 85:351–356; 1988.

55. Salamone, J. D. Behavioral pharmacology of the dopamine system: A new synthesis. In: Wilner, P.; Scheel-Krüger, J., eds. The mesolimbic dopamine system: From motivation to action. Chichester: John Wiley & Sons; 1991:599–613.

56. Saper, C. B.; Swanson, L. W.; Cowan, W. M. An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. J. Comp. Neurol. 183:689–706; 1979.

57. Schwartzbaum, J. S. Electrophysiology of taste, feeding and reward in lateral hypothalamus of rabbit. Physiol. Behav. 44:507–526; 1988.

58. Staton, D. M.; Solomon, P. R. Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differen-tially affect stereotypy and locomotion in the rat. Physiol. Psychol. 12:159–162; 1984.

59. Stellar, J. R.; Neeley, S. P. Reward summation function measurements of lateral hypothalamic stimulation reward: Effects of anterior and posterior medial forebrain bundle lesions. In: Hoebel, B. G.; Novin, D., eds. The neural basis of feeding and reward. Brunswick, ME: Haer Institute; 1982:431–444.

60. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.

61. Wise, R. A.; Hoffman, D. C. Localization of drug reward mechanisms by intracranial injections. Synapse 10:247–263; 1992.

62. Wolf, G.; Sutin, J. Fiber degeneration after lateral hypothalamic lesions in the rat. J. Comp. Neurol. 127:137–156; 1966.

63. Yeomans, J. S. Principles of brain stimulation. New York: Oxford University Press; 1990.

64. Yoshida, M.; Yokoo, H.; Mizoguchi, K.; Kawahara, H.; Tsuda, A.; Nishikawa, T.; Tanaka, M. Eating and drinking cause increased dopamine release in the nucleus accumbens and ventral tegmental area in the rat: Measurement by in vivo microdialysis. Neurosci. Lett. 139:73–76; 1992.

65. Young, A. M. J.; Joseph, M. H.; Gray, J. A. Increased dopamine release in vivo in nucleus accumbens and caudate nucleus of the rat during drinking: A microdialysis study. Neuroscience 48:871–876; 1992.