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Immobilization of Rats Modifies the Response of Striatal Neurons to Dexamphetamine

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WARENYCIA, M. W. AND G. M. McKENZIE. *Immobilization of rats modifies the response of striatal neurons to dexamphetamine*. PHARMACOL BIOCHEM BEHAV **21**(1) 53–59, 1984.—The effect of dexamphetamine (DEX) on striatal multi-unit activity was examined in freely moving rats and again 24 or 48 hr later during immobilization. Animals which in the freely moving state responded with striatal activation following DEX, 1 mg/kg IP, did not respond to this dose of DEX after immobilization. Similarly with DEX 2.5 mg/kg IP, the incidence of excitatory responses seen in freely moving animals decreased to 18% after immobilization, and the incidence of inhibition and biphasic responses increased from 0% in freely moving animals to 52% in immobilized preparations. The results suggest that the response of striatal neurons to DEX is dependent upon the behavioural state of the animal. Furthermore, these findings indicate that the central actions of DEX are more complex than previously believed, and raises the speculation that the excitatory effects of DEX on striatal neurons may be mediated through excitatory striatal afferents.

Dexamphetamine Multi-unit activity Freely moving Immobilized Excitatory afferents Striatum

SOME controversy has arisen as to whether dexamphetamine (DEX) results in activation or inhibition of neurons in the striatum. By studying single unit discharges in immobilized, artificially respired rats it has been concluded that the predominant response of striatal neurons to DEX, 0.5–5.0 mg/kg IP, consists of inhibition [1, 8, 15, 16, 17]. In contrast to these findings are the results of others which demonstrate with both multi-unit and single-unit recording that in freely moving animals DEX, 1–10 mg/kg IP, produces excitation of striatal neurons [10, 13, 21].

The reason for these apparently divergent results is not easily reconcilable. Studies can be cited to support any one of several speculative hypotheses; however, the first approach must be a comparison of DEX treatments in the same animal, during the freely-moving state and again during immobilization, using the same recording procedures. We report here that whereas DEX consistently produces neuronal activation in the striatum of freely moving rats, during immobilization these animals no longer respond predictably with striatal activation.

METHOD

Long-Evans male rats, weighing 250–350 g were used throughout these experiments. Bipolar recording electrodes were implanted unilaterally or bilaterally in the striata of a total of 49 rats (58 electrode placements) during halothane anesthesia as described previously [10]. Co-ordinates for electrode placement were taken from the atlas of Pellegrino and Cushman [14] and ranged as follows: 2.2–3.0 mm anterior to bregma, 2.5–3.0 mm from the midline and 4.0–5.5 mm ventral from the surface of the dura. Following recovery, animals were individually housed, kept on a 12-hr light-dark cycle and given unrestricted access to food and water. Animals were allowed 5–7 days to recover prior to experimentation and all experiments were carried out at approximately the same time each day.

Recording of Striatal Neuronal Activity

Neuronal firing in the striatum was recorded as described by Hansen and McKenzie [10]. For discrimination, windows were set well above the background noise level; signal-to-noise ratios were 2.5:1 or greater. Spontaneous multi-unit activity was constantly monitored and cumulative 4-min counts printed on an Ortec printer. Baseline activity, recorded for 30–40 min prior to drug injection, was used to determine the mean pre-injection firing rate for each animal and was defined as 100%. All data points were then expressed as percent of control firing rate.

Experimental Protocol

Immobilization procedure. Animals were anesthetized with halothane and a tracheal cannula inserted. This surgical procedure required 10–15 min for completion. Each animal was paralyzed with 0.5 ml of a 100 mg/ml solution of succinylcholine (in 0.9% saline) injected subcutaneously and halothane anesthesia discontinued. Respiration was maintained by a rodent respirator and analgesia maintained by the gas mixture of 70%N_2O/30% O_2. Body temperature was monitored with a YSI telethermometer and maintained at 37.5±0.5°C with a circulating hot water heating pad (Hamil-

TABLE 1

PAIRED COMPARISONS OF SPONTANEOUS STRIATAL MULTI-UNIT ACTIVITY UNDER DIFFERENT EXPERIMENTAL TREATMENTS

Treatments Being Compared	Mean spikes/ 4-min interval†	N	S.E.D.*	Significance‡
1 Freely moving vs. Freely moving in 70% N_2O/30% O_2	6941 ± 965 6787 ± 951	7(6)	184	N.S.
2 Freely moving in 70% N_2O/30% O_2 vs. Immobilized and respired with 70% N_2O/30% O_2	9829 ± 1795 9070 ± 1576	8(8)	243	N.S.
3 Freely moving vs. Immobilized and respired with 70% N_2O/30% O_2	6205 ± 696 6966 ± 1021	8(8)	400	N.S.

*Standard error of the difference.

†Neuronal activity is expressed as the mean (±SEM) number of spikes obtained over 8 successive 4-min intervals just prior to drug treatments.

N=number of recordings used for comparison; number of animals is indicated in parentheses.

‡Statistical difference was not observed between any of the conditions ($p<0.05$), calculated according to the 2-tailed Student's t-test for paired observations.

ton Industries, Cinn., OH). Lidocaine hydrochloride ointment was applied to wound surfaces and warm physiological saline was periodically applied to the eyes to prevent corneal discomfort. Halothane-induced depression of neuronal activity reversed within 20–30 min after discontinuation of anesthesia, at which time recording of baseline striatal activity commenced.

Effects of Nitrous Oxide and Immobilization on Spontaneous Striatal Neuronal Activity

Three sets of conditions were compared:

Freely moving vs. freely moving in the presence of 70% N_2O/30% O_2. Animals (N=6) were allowed to freely move in the test cage while baseline neuronal activity was recorded for 30–40 min. The test cage was then enclosed and a mixture of 70% N_2O/30% O_2 was introduced into the cage through a side port at a flow rate of 1500 ml/min. It was calculated that 8 min was sufficient to fill the test enclosure with the gas mixture. Baseline striatal activity was then recorded in the presence of 70% N_2O/30% O_2 for 30–40 min.

Freely moving in the presence of 70% N_2O/30% O_2 vs. immobilized. Baseline neuronal activity in the presence of 70% N_2O/30% O_2 was recorded for 30–40 min. Animals (N=8) were then immobilized as described in the Method section and baseline striatal neuronal activity recorded for an additional 30–40 min.

Freely moving vs. immobilized. Baseline neuronal activity was recorded in freely moving animals (N=8) for 30–40 min, followed by immobilization and recording of baseline neuronal activity for another 30–40 min.

Experiments with DEX

After appropriate baseline intervals were established, freely moving animals were given DEX, 1.0 mg/kg IP (N=5) or 2.5 mg/kg IP (N=11), and subsequent neuronal responses recorded for 2–4 hr. In follow-up immobilization experiments 48 hr later, animals were allowed to freely move for 30–40 min while baseline neuronal activity was again recorded. Animals were then immobilized and after an appropriate baseline interval had been re-established, DEX 1.0 or 2.5 mg/kg IP was given.

In another group of freely moving animals (N=15) baseline neuronal activity was recorded for 30–40 min before and after the introduction of 70% N_2O/30% O_2 to the test chamber. DEX, 1.0 mg/kg IP, was then given and subsequent neuronal activity recorded for 2 hr in the presence of 70% N_2O/30% O_2. Follow-up experiments were carried out 48 hr later at which time animals were immobilized and given DEX, 1.0 mg/kg IP. One group of animals (N=5) was retested 24 hr later as freely moving in the presence of 70% N_2O/30% O_2.

In the case of immobilization experiments with drug-naive animals (N=13), animals were allowed to freely move for 30–40 min while baseline neuronal activity was recorded, and then immobilized. After an appropriate baseline interval had been re-established, DEX, 2.5 mg/kg IP was given. An identical protocol was followed for animals (N=5) receiving saline instead of DEX. Saline controls were not carried out on freely moving animals as previous work [10] had shown that saline injection was without effect.

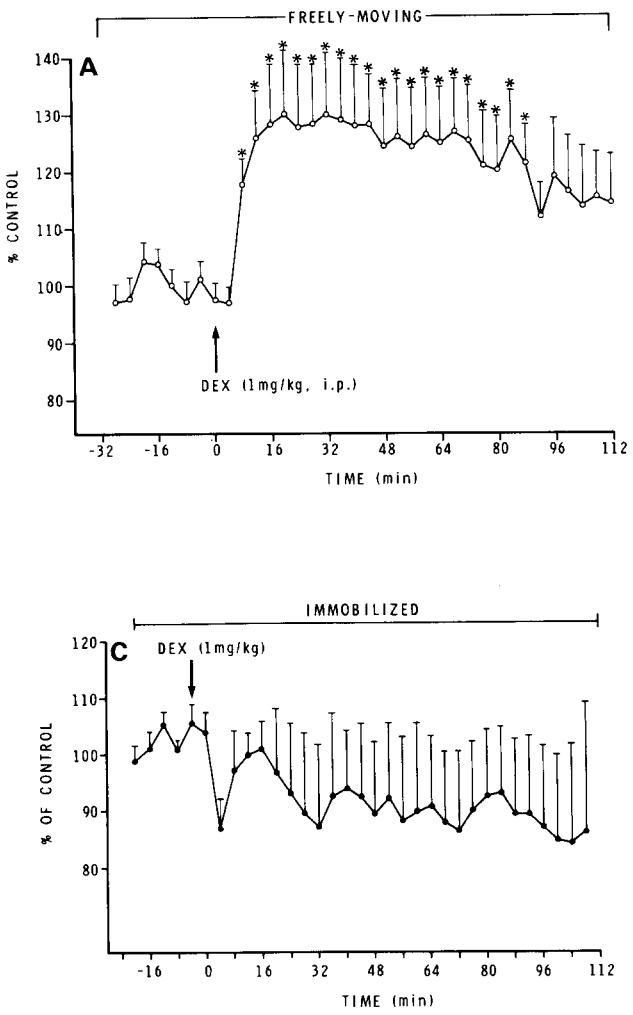
Histology

Upon completion of experimentation each animal received a lesioning current through the striatal electrodes. Animals were then administered a lethal dose of pentobarbital and perfused with saline followed by 10% formalin.

Electrode tip placements were verified by examining cryostat-obtained sections with a projector microscope (Reichert Instruments, Austria).

Statistical Analysis

Spontaneous striatal neuronal activity data were analyzed using Student's t-test for paired observations. Drug effects were analyzed using a randomized analysis of variance and treatment means were compared using the Student-Neuman-Keuls Multiple Range test. P values of less than 0.05 were considered significant. Differences in responses to 2.5 mg/kg DEX between drug-naive animals and those re-



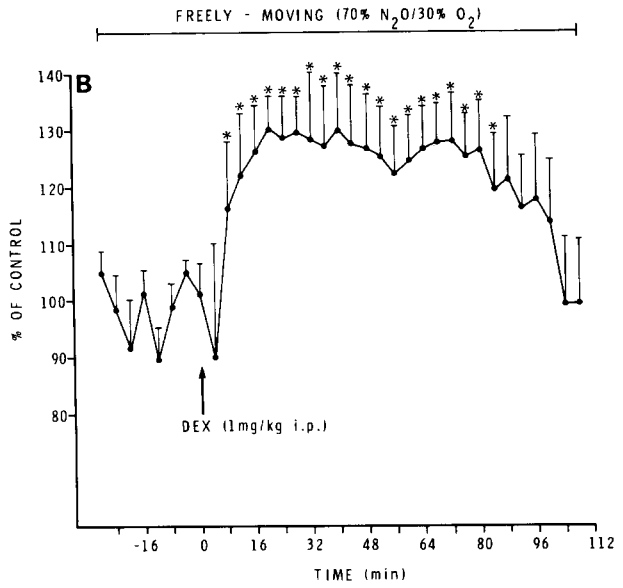


FIG. 1. Changes in the multi-unit activity of rat striata following DEX, 1 mg/kg IP. A. Freely moving animals (N=5). Control intervals preceding drug were pooled (N=10) from experiments where either 1.0 or 2.5 mg/kg doses of DEX were administered. All intervals between and including the asterisks were significantly different from controls ($p<0.05$). B. Freely-moving animals (N=5) in the test chamber containing 70% N_2O/30% O_2. As in above, control data pooled (N=10). All intervals between and including the asterisks were significantly different ($p<0.05$). C. Immobilized animals (N=5), all of which during freely-moving conditions responded with striatal activation following DEX, 1 mg/kg IP with a peak increase of 135±8.6% control (mean±SEM). Firing rates following DEX were not statistically different from control ($p<0.05$). In all instances, firing rates following DEX were compared to the pre-drug control period immediately preceding drug injection and statistically evaluated using analysis of variance and the Student-Neuman-Keuls Multiple Range Test.

ceiving a second dose of DEX 48 hr later were compared using a 4×2 contingency table and the Chi-square test for independence.

RESULTS

Spontaneous Striatal Multi-Unit Activity in Freely Moving vs. Immobilized Animals

Spontaneous multi-unit activity did not change significantly following introduction of 70% N_2O/30% O_2 in freely moving animals, nor did it change significantly as a result of immobilization (Table 1).

Effects of DEX on Striatal Multi-Unit Activity in Freely Moving vs. Immobilized Animals

The response of striatal neurons in freely-moving animals (N=5) to DEX, 1.0 mg/kg IP, is shown in Fig. 1A. In these animals, DEX increased neuronal firing to 130.2±10.7% (mean±SEM). The introduction of 70% N_2O/30% O_2 to the test chamber caused an apparent increase in the variability of the spontaneous activity of striatal neurons, which was not statistically significant (Fig. 1B). However, the response to DEX was essentially identical to that observed in animals without N_2O/O_2 treatment. Peak increase in neuronal activity was 129.8±10.9%, occurring 24 min after DEX and returning to baseline levels by 88 min. Immobilization of the same animals in Fig. 1A, 24 hr after the initial test with DEX, resulted in total abolition of the response to DEX (Fig. 1C).

Administration of a higher dose of DEX, 2.5 mg/kg IP, to freely moving animals (total of 18 electrode placements) resulted in the predictable increase in striatal multi-unit activity. The results of five animals are shown in Fig. 2A. Peak effect occurred between 40 and 50 min post drug, and reached 171.8±19.7% control discharge rate (mean±SEM). By 230 min, discharge rates no longer differed from control levels. Upon immobilization, animals which had previously demonstrated excitation 48 hr earlier responded with a multiplicity of neuronal responses consisting of either excitation (N=4), no change .(N=5), inhibition (N=3) or biphasic (N=6) (Table 2). Examples of these responses, in the same animal, both freely-moving and immobilized, are shown in Fig. 3. When the results of the no-change responses (N=5) were compared to saline treatment (N=5), the two groups were not statistically different (Fig. 2B). However, in the latter group of animals, ketamine, 50 mg/kg IP, produced striatal activation, having peak increases ranging from 180–240 percent of control and durations of 100–140 min.

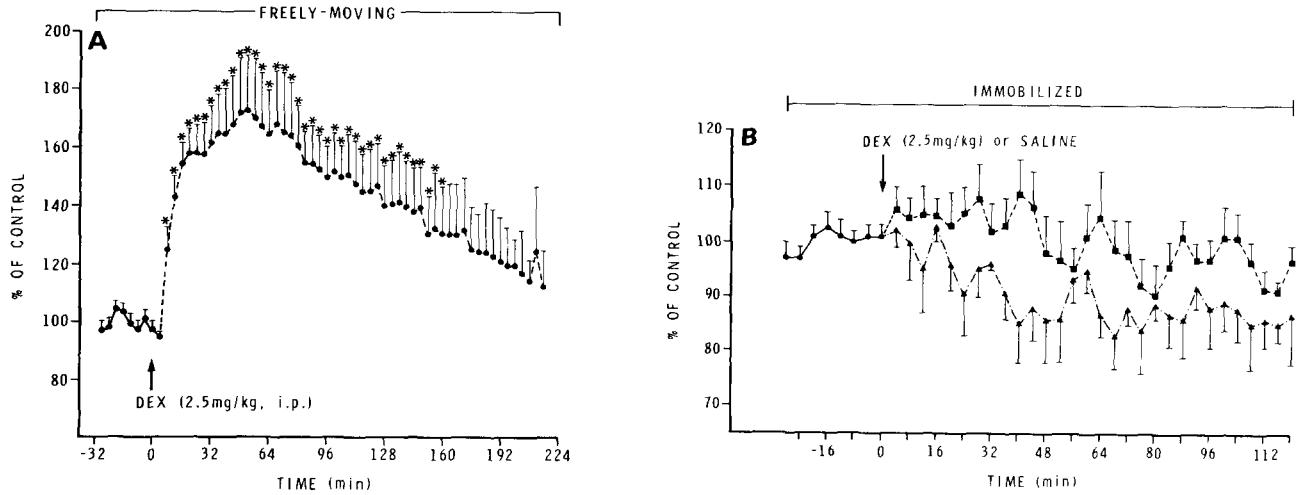


FIG. 2. Changes in the response of striatal neurons to DEX, 2.5 mg/kg IP, following immobilization. A. Freely-moving animals (N=5). *Statistically different from pooled control values (N=10), using analysis of variance and Student-Neuman-Keuls Multiple Range Test ($p<0.05$). B. Immobilized animals receiving DEX (▲, N=5) or saline (■, N=5). Firing rates following either DEX or saline were not different from pre-injection controls (pooled, N=12), nor from each other using analysis of variance and the Student-Neuman-Keuls Multiple Range Test. Note—difference in ordinate and abscissa scale between A and B.

TABLE 2

SUMMARY OF STRIATAL RESPONSES TO DEX IN IMMOBILIZED ANIMALS

	Excitation*	Inhibition†	No Change‡	Biphasic§	Total Recording Sites
Drug-Naive (N=13)**	3	7	6	4	20
Drug-Experienced¶ (N=11)**	4	3	5	6	18

*Sustained increase of 120% of control.
†Sustained decrease of 80% of control.
‡Remained between 80% and 120% of control.
§Combinations of the above responses.
¶Animals first tested with DEX (2.5 mg/kg IP) in the freely moving state, immobilized 48 hr later, and tested a second time with the same dose of DEX.
**Chi-square analysis showed no significant differences between the two groups ($p<0.05$) of animals.
N=number of animals.

Lack of Effect of Previous DEX Treatment on Striatal Multi-Unit Response to Subsequent Administration of DEX

Freely moving animals were given DEX, 1.0 mg/kg, IP followed by a 24-hr interval at which time the same dose was readministered. No significant change in striatal neuronal responsiveness to DEX could be demonstrated with this drug schedule (Table 3). Similarly, using a dose of DEX, 2.5 mg/kg IP, and a dosing interval of 48 hr, no significant change in the DEX response could be detected with the second administration (Table 3).

Electrode Location

All electrodes were situated in the anterior striatum, with 12 sites in the dorsomedial quadrant, 15 in the dorsolateral quadrant, 12 in the ventromedial quadrant and 19 sites in the ventrolateral quadrant.

DISCUSSION

The spontaneous activity of striatal neurons in freely moving rats did not change significantly as a result of subsequent immobilization. This is somewhat unexpected for two reasons. First, immobilization necessarily eliminates behaviour and therefore sensory feedback arising from behaviour, thus arguing in favour of a reduction in striatal activity during immobilization. Secondly, a comparison of results from single-unit studies in several laboratories indicates that compared to striatal neurons in freely moving preparations [21,23] striatal neurons in immobilized animals have

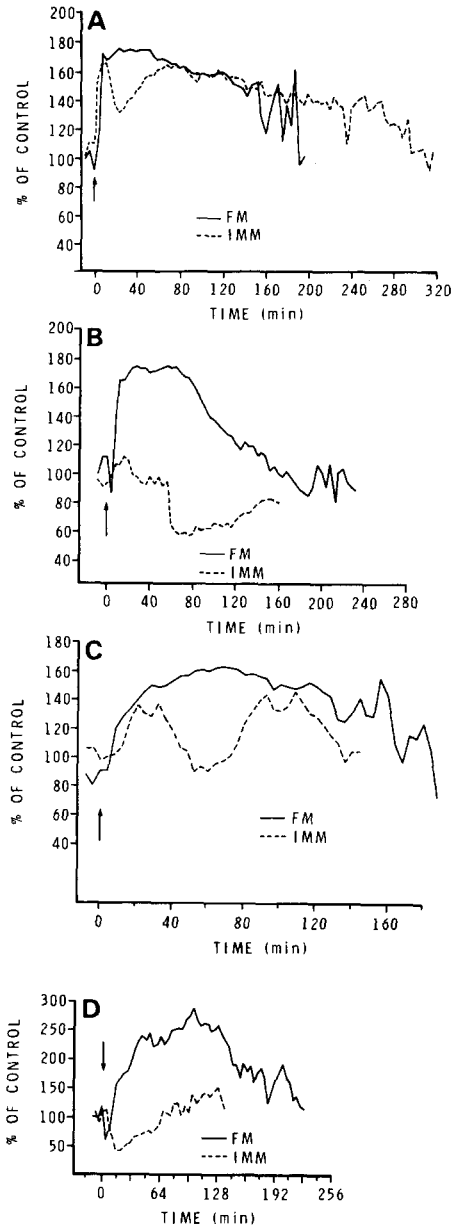


FIG. 3. Representative responses of striatal neurons to DEX, 2.5 mg/kg IP, in freely moving (FM) animals, and again in the same animal immobilized (IMM) 48 hr later. Note that DEX produced striatal activation in all animals, which upon immobilization responded to DEX with either excitation (A), inhibition (B), or biphasic responses (C) and (D). Note—ordinate and abscissa scales vary from A to D.

lower discharge rates [1,18]. On the other hand, our immobilization procedure differs from that used by others, by the inclusion of analgesic concentrations of nitrous oxide; and the presence of nitrous oxide may have altered the response of striatal neurons to immobilization. Indeed, it has been demonstrated that in addition to its analgesic action [4], nitrous oxide reduces biological responses to immobilization stress [5, 6, 7]. Consistent with this idea are the findings in this study which show that nitrous oxide administered to freely moving animals, a relatively non-stressful paradigm, had no significant effect on either spontaneous neuronal activity or behavioural activity. Ideally, a comparison should be made between immobilized animals with and without nitrous oxide. Unfortunately, immobilization without analgesia is no longer an acceptable procedure in many laboratories.

Freely moving animals which had responded with neuronal excitation following DEX, 1.0 mg/kg IP, did not respond to DEX in the follow-up immobilization experiments. Similarly, no consistent response to DEX was seen in immobilized animals receiving the higher dose of DEX, i.e., 2.5 mg/kg IP. Although DEX-induced inhibition was observed in 10 out of 38 electrode placements in immobilized animals, the predominance of other response patterns is in contrast to the consistent inhibition observed by others [1, 8, 15]. It has been reported that higher doses of DEX, 5.0 and 7.5 mg/kg, produce more consistent striatal activation in the immobilized rat [18], whereas freely moving animals demonstrate striatal activation throughout the dose range of 0.5–10 mg/kg [10]. Thus, it appears that, in the freely moving state, behaviour produced by low doses of DEX can modify the striatal response to DEX.

It may be argued that the absence of consistent inhibitory responses in immobilized animals in this study was due to the previous DEX treatment. However, parallel studies on other animals demonstrated that the excitatory response to subsequent DEX treatment was unchanged whether 24 hr or 48 hr separated treatments or whether 1 mg/kg or 2.5 mg/kg of DEX was used. It is also most unlikely that the presence of nitrous oxide *per se* was responsible for the difference between freely moving and immobilized animals in their response to DEX, since nitrous oxide did not alter either the magnitude or time course of either the behaviour or striatal activity following DEX in freely moving animals. In this regard, other investigators have shown that nitrous oxide does not significantly alter either cerebral metabolism [6] or neuronal excitability [22]. Finally, an overall non-specific decrease in neuronal responsiveness following immobilization was not the cause of the failure to respond to the low dose of DEX as ketamine was still able to consistently elicit marked increases in striatal firing rates in immobilized animals.

The present study shows that the excitatory response of striatal neurons to low doses of DEX is, 82% of the time, abolished by immobilization. Work by other investigators, on other neuronal systems, suggests that this is not uncommon. For example, it has been demonstrated that iontophoretic application of somatostatin in urethrane-anesthetized rats decreases neuronal excitability of cortical neurons [19], whereas, in freely moving animals, somatostatin produces marked excitation of neurons in the somatosensory cortex [11]. Similarly, other authors [2] have recently questioned the accepted view that 5-hydroxytryptamine is an inhibitory striatal neurotransmitter. As regards dopamine, the traditional view of an inhibitory role in the striatum, based on data derived from immobilized and/or anesthetized preparations, has been challenged on many occasions by numerous authors ([9,25] for review). Indeed, the excitatory response observed following DEX is consistent with an excitatory role for dopamine in the striatum.

The relative absence of inhibitory responses following DEX in freely moving animals may be a consequence of sensory feedback generated by DEX-induced behaviour. Although the neural substrate subserving feedback to the

TABLE 3

COMPARISON OF SUCCESSIVE DEX TREATMENTS IN THE SAME ANIMAL

Paradigm	Peak Response (% of control)* 1st trial	2nd trial	N	S.E.D.†
DEX, 1.0 mg/kg IP, 24 hr apart; freely moving under 70% N_2O/ 30% O_2 in each trial.	135.2 ± 5.3‡	139 ± 4.4‡	5(6)	2.7
DEX, 2.5 mg/kg IP 48 hr apart; animals freely moving in 1st trial and immobilized in 2nd trial	158.5 ± 6.7‡	160.3 ± 12.9‡	4(4)	8.8

*Peak response is defined as the maximal increase in striatal multi-unit activity following DEX. N refers to number of animals; number of electrode placements is given in parentheses.

†Standard error of the difference.

‡Peak responses were not statistically different as evaluated by the 2-tailed Student's t-test for paired observations ($p<0.05$).

striatum has not been defined, it is known that there are excitatory striatal afferents, originating in cortical and thalamic areas [3], which probably utilize glutamate and acetylcholine, respectively, as neurotransmitters [12,20]. Activity in these or other excitatory afferents may override local striatal inhibition produced by inhibitory striatal afferents. Consistent with this view is the recent report that freely moving rats, having ablations of cortical areas known to project to the striatum, respond to DEX with predominantly striatal inhibition rather than excitation [24].

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