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Selective Involvement of Dopamine in the Nucleus Accumbens in the Feeding Response Elicited by Muscimol Injection in the Nucleus Raphe Dorsalis of Sated Rats

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BENDOTTI, C., C. BERETTERA, R. INVERNIZZI AND R. SAMANIN. *Selective involvement of dopamine in the nucleus accumbens in the feeding response elicited by muscimol injection in the nucleus raphe dorsalis of sated rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1189–1193, 1986.—Muscimol injection (100 ng) in the nucleus raphe dorsalis (NRD) caused intense eating in non-food-deprived rats. At a dose (10 μg) blocking dopamine mediated responses (examined by increased locomotion or stereotypy caused by systemically injected d-amphetamine), fluphenazine injected in the n. accumbens, but not in the striatum, significantly reduced the eating response elicited by muscimol in the NRD while food intake of deprived rats was not significantly modified by fluphenazine injected in either area. Fluphenazine (20 μg) in the striatum reduced eating in both conditions, but the animals showed marked sedation which obviously interfered with the feeding response. Dopamine release and synthesis, measured respectively by 3-methoxytyramine and accumulation of dihydroxyphenylalanine after aromatic amino acid decarboxylase inhibition, were significantly reduced in the n. accumbens, but not in the striatum, of muscimol treated animals. The metabolism of serotonin was reduced in both areas of muscimol treated rats. It is suggested that changes in dopamine receptor sensitivity, together with changes in serotonin function, might be involved in the feeding response caused by muscimol injection in the NRD.

Feeding behaviour Muscimol Nucleus raphe dorsalis Dopamine Nucleus accumbens Caudate putamen

INJECTION of muscimol, a GABA receptor agonist, in the nucleus raphe dorsalis (NRD) of sated rats induce consistent eating associated with motor activation which was counteracted by the GABA antagonist bicuculline or picrotoxin given systemically or locally [18]. Since a significant reduction of serotonin metabolism was found in the hypothalamus and striatum after muscimol injection in the NRD, it was suggested that the increased food intake could be due to the inhibitory action of muscimol on serotonin neurons in this area. Recent data, however, showed that the lesion of serotonin neurons in the NRD by the serotonin neurotoxin 5,7-dihydroxytryptamine failed to modify the hyperphagic response to muscimol [6].

Unlike food intake by deprived rats, the hyperphagia induced by muscimol was inhibited by systemically injected penfluridol, a dopamine receptor blocker, suggesting that dopamine contributes to this behaviour [6]. There is evidence that nigrostriatal and mesolimbic dopamine systems are involved in the feeding responses elicited respectively by tail pinch [2] and by electrical stimulation of the lateral hypothalamus [16]. Moreover, some authors have noted stimulation of eating in sated rats after an injection of amphetamine into the striatum [22]. In the light of these data, it was interesting to investigate the involvement of mesolimbic and nigrostriatal dopamine systems in the hyperphagia induced by muscimol injected into the NRD. The present study examined whether fluphenazine, a dopamine receptor antagonist injected into the caudate putamen (CP) or nucleus accumbens (NA) at doses reported to block DA-mediated responses, modified the eating induced by muscimol injection in the NRD. The synthesis and metabolism of dopamine in these two brain areas were also determined after the muscimol injection in the NRD. The effect of fluphenazine on muscimol induced hyperphagia was compared with that on starvation-induced eating.

METHOD

Male CD-COBS rats (Charles River, Italy) weighing 230–280 g at the time of surgery were used. They were housed 4 per group at a constant room temperature (21 ± 1°C) and relative humidity (60%) with food and water provided ad lib. A standard formula diet (Altromin MT pellets for rats, Rieper, Italy) were used for all experiments.

Implantation of Cannulae

Under ethyl-ether anesthesia rats were stereotaxically implanted with chronic bilateral guide cannulae constructed from 23 gauge stainless-steel tubing in Plexiglas holders, placed with their tip 2 mm above the target area. Stainless-steel stylets, 30 gauge, as long as the guide, kept the guides patent until the animals were given intracerebral injections 7–10 days later.

The rats were accustomed to handling before testing and on the day of the test the stylets were withdrawn and replaced by bilateral injection units (30 gauge stainless-steel tubing) terminating 2 mm below the tip of the guides. The following stereotaxic coordinates from König and Klippel [15] rat brain atlas were used: A 9410, L ±1.0, V −1 for nucleus accumbens (NA) and A 8620, L ±2.2, V +0.6 for caudate putamen (CP). Each cannulated rat was tested once only.

Muscimol Injections in the NRD

Seven or ten days after brain cannula implantation, rats were anesthetized with ethyl-ether and positioned on the stereotaxic instrument for muscimol injection into NRD. The following stereotaxic coordinates were used: A=0.35, L=0, V=−0.6 [15]. The effect of muscimol on food intake was much less evident when it was injected in various sites of periaqueductal grey about 1 mm from the NRD. Lidocaine was applied on the suture to prevent local pain that might influence the rat's behaviour. 100 ng muscimol (Biosearch, San Raphael, CA) dissolved in distilled water was injected through an Agla syringe in a volume of 0.5 μl in 1 minute. Control animals received an equal volume of the vehicle.

Food Intake in Muscimol-Injected or Food Deprived Rats

Twenty minutes after muscimol injections the animals were put singly in cages with a grid and blotting paper on the floor on which a weighed amount of food was freely available. The food consumed in 30 minutes and 1 hr was measured to the nearest 0.1 g.

For experiments with food-deprived animals, the subjects were trained for 15 days to eat their food during 4 out of 24 hr (from 11.00 a.m. to 3.00 p.m.). On days 6–7 of this training they were stereotaxically implanted with bilateral chronic cannulae according to the methods described above.

On the day of testing, day 15, the rats were put singly in cages with a grid and blotting paper on the floor on which a weighed amount of food was freely available; intake during the first 30 min and 1 hr after was measured to the nearest 0.1 g. In both experiments the amount of food eaten was corrected for spillage.

Motor Hyperactivity and Stereotyped Behaviour Induced by d-Amphetamine

Two groups of rats implanted with bilateral cannulae in the NA or CP were used to verify the effectiveness of 10 μg of fluphenazine in reducing dopaminergic function in these brain areas. Motor hyperactivity and stereotypy induced by intraperitoneal injection of d-amphetamine were taken as models of dopamine hyperfunction respectively in NA and CP.

Locomotor activity was evaluated in an open field by measuring the total number of squares crossed in 5 min, 30 min after intraperitoneal injection of 1.5 mg/kg of d-amphetamine sulphate. Fluphenazine 10 μg or vehicle were bilaterally injected in NA just before amphetamine. Stereotyped behaviour was evaluated using the scoring system of Costall *et al.* [8] at different times after intraperitoneal injection of 10 mg/kg of d-amphetamine sulphate. Fluphenazine 10 μg or vehicle were injected bilaterally in CP just before amphetamine. Only data regarding the peak effect (60 min after drug injection) are reported.

Biochemistry

For biochemical experiments naive male CD-COBS rats (Charles River, Italy) were injected with muscimol in NRD under light ether anesthesia as described above. The animals were caged singly for measurement of food intake during the last fifteen minutes of the experiment. Forty-five minutes after muscimol injection the rats were killed by microwave irradiation focused on the head (1.3 KW at 2.45 GHz for 4.25 sec) using a commercial microwave unit adapted by Medical Engineering Consultants (Lexington, MA). The rat brains were removed and dissected into striatum and NA according to Glowinski and Iversen [13]. Homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were measured by high performance liquid chromatography (HPLC) with electrochemical detection according to Achilli *et al.* [1]. The HPLC method of Ponzio *et al.* [17] was used for 3-methoxytyramine (3MT) determination. The NA of 3 rats were pooled to measure 3MT.

In another experiment the animals injected with muscimol in NRD were treated intraperitoneally with 100 mg/kg of the decarboxylase inhibitor m-hydroxybenzylhydrazine (NSD 1015) 30 minutes before decapitation. The brains were rapidly removed and striatum and NA were dissected for biochemical assay. l-Dihydroxyphenylalanine (l-DOPA) accumulation was measured by the HPLC method of Benfenati *et al.* [5].

Drugs

Fluphenazine HCl was selected on the basis of its marked water solubility and good selectivity as dopamine receptor blocker [12]. Doses were calculated as salts. All solutions were delivered bilaterally through a 10 μl Hamilton microsyringe coupled to injection units in a volume of 2 μl each side in 2 min just before access to food. Control animals received an equal volume of the vehicle.

Histological Examinations

After completion of the experiments, animals were killed by decapitation, brains were immediately frozen in minced dry ice and 40 μm sections cut in a cryostat. In these conditions the trace of the needle and of cannulae was clearly seen and served to locate the site of injection. In preliminary experiments the injection coordinates were estimated by injection of dye solution (Gentian violet 0.015%) into the target areas. The area of diffusion of 2 μl dye injected into the n. accumbens or caudate putamen was about 2 mm around the tip of the cannula with a slight spread of dye dorsally through the area surrounding the injection unit. The spread of 0.5 μl dye injected in the dorsal raphe nucleus was less than 1 mm surrounding the tip of the needle.

The injection sites were located within the following range of coordinates: A:9820 to 9410; L: 1.4 to 0.8 and H: −0.6 to −1.2 for NA; A: 8380 to 7470; L: 2.6 to 1.8 and H: 1.0 to 0 for caudate putamen and A: 350 to P 100; L: 0; H:

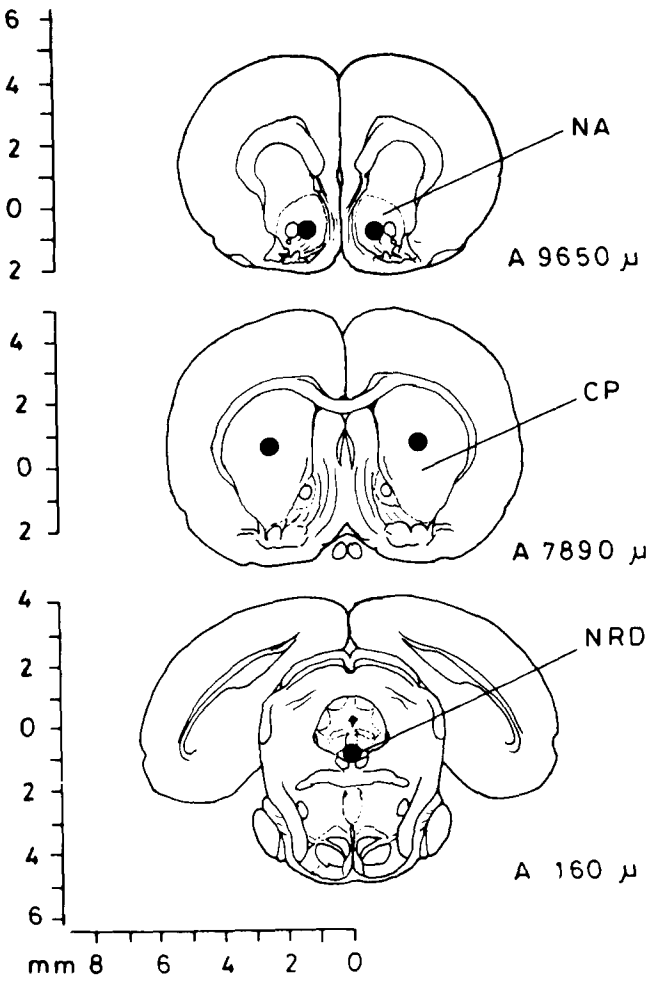

FIG. 1. Schematic diagram of representative frontal sections of the rat brain from the stereotaxic atlas of König and Klippel [15] showing the placement of injections sites (●) (see the Method section for the range of coordinates used to select correctly injected animals). NA=Nucleus accumbens; CP=Caudate putamen; NRD=Nucleus raphe dorsalis.

−0.6 to −1.0 for NRD. Data from animals in which the tip of cannula was not placed within this range were not included in the results.

Statistics

Food intake data were analyzed by Dunnett's two-tailed test for multiple comparison. Biochemical data were analyzed by Student's two-tailed *t*-test.

RESULTS

As previously reported [6,18], muscimol injected in the NRD caused intense eating associated with strong locomotion, rearing, stereotyped sniffing and occasional gnawing and licking the floor of the cage. None of these behavioural signs was observed in the rats injected with vehicle in the NRD, except for rare bouts of eating (mean food intake of all controls was 0.5±0.2 g). The sites of injection in NA, CP and NRD are represented schematically in Fig. 1.

TABLE 1

EFFECT OF FLUPHENAZINE, INJECTED BILATERALLY IN THE NUCLEUS ACCUMBENS OR CAUDATE PUTAMEN, ON EATING INDUCED BY STARVATION OR MUSCIMOL INJECTION IN THE NUCLEUS RAPHE DORSALIS

Treatment	Dose (μg/2 μl)	Eating (g/30 min) induced by Muscimol	Starvation
Nucleus accumbens			
Vehicle	—	6.4 ± 0.9	6.8 ± 0.4
Fluphenazine	5	7.7 ± 1.0	—
Fluphenazine	10	2.5 ± 0.9*	6.8 ± 1.3
Caudate putamen			
Vehicle	—	5.6 ± 0.6	6.1 ± 0.7
Fluphenazine	10	4.6 ± 1.0	4.9 ± 0.3
Fluphenazine	20	0.3 ± 0.0*	3.6 ± 0.3*

Values are mean ± S.E. of 4 or 5 rats for each group.
*$p<0.01$ vs. vehicle (Dunnett's test).

As shown in Table 1, bilateral injections of 10 μg of fluphenazine into the NA produced a 60% decrease of d-amphetamine induced hypermotility (vehicle=101±16 counts/5 min; fluphenazine 41±14 counts/5 min, $p<0.05$ Student's *t*-test) and significantly reduced the feeding responses elicited by muscimol injection in NRD. No effect was observed on food intake of deprived rats.

Bilateral injections of 10 μg of fluphenazine into the striatum completely blocked the stereotyped behaviour-induced by 10 mg/kg d-amphetamine (vehicle 3.7±0.3; fluphenazine 0.7±0.4, scoring system used by Costall *et al.* [8], $p<0.01$ Smirnov's test). The same dose failed to modify the eating induced by muscimol injection in NRD or by starvation. A significant reduction of eating in both conditions was observed with 20 μg of fluphenazine into the striatum; this dose, however, had marked sedative effects which obviously interfered with feeding behaviour. Table 1 gives the mean food intake measured during the first 30 min after fluphenazine injections, but results were similar 1 hr after fluphenazine (data not shown). No attempt was made to quantitate the behavioural activation caused by muscimol, but during the feeding test there were no obvious modifications by fluphenazine injections in NA or striatum.

The effects of muscimol injection in the NRD on dopamine and serotonin metabolites are shown in Table 2. Muscimol raised DOPAC in striatum and NA, the effect in the latter being more marked and significant. HVA levels in the striatum and NA were not modified by muscimol injections. Muscimol lowered 3MT by 40% in the NA but had no effect on this metabolite in the striatum. A decrease in 5HIAA was observed in the striatum and NA of rats injected with muscimol in the NRD. As shown in Table 3, l-DOPA accumulation was significantly decreased in the NA of rats injected with muscimol in the NRD but no effects were seen in the striatum.

DISCUSSION

At doses blocking DA-mediated responses, fluphenazine in the NA, but not in the striatum, significantly reduced the eating response elicited by muscimol injection in the NRD suggesting that dopamine in NA is selectively involved in the

TABLE 2

LEVELS OF DOPAMINE METABOLITES (HVA, DOPAC, 3MT) AND 5HIAA IN THE NUCLEUS ACCUMBENS AND STRIATUM AFTER MUSCIMOL INJECTION IN THE NUCLEUS RAPHE DORSALIS OF RATS

	Metabolites (ng/g tissue)							
	N. accumbens				Striatum			
Treatment	HVA	DOPAC	3MT	5HIAA	HVA	DOPAC	3MT	5HIAA
Vehicle	766 ± 30	1184 ± 30	5.5 ± 0.6	939 ± 44	602 ± 29	651 ± 23	12.5 ± 1	603 ± 31
Muscimol (100 ng/0.5 μl)	693 ± 42	1501 ± 54†	3.3 ± 0.3†	651 ± 29†	637 ± 26	759 ± 38*	12.5 ± 1	464 ± 16†

Each value is the mean ± S.E. of 5 rats.
Rats were killed 45 min after muscimol injection.
*$p<0.05$, †$p<0.01$ vs. vehicle. Student's t-test.

mechanism by which muscimol stimulates feeding in sated rats. A role of mesolimbic DA in eating behaviour was recently suggested by Mogenson and Wu [16] who found the feeding response caused by electrical stimulation of the lateral hypothalamus was reduced by injecting spiroperidol in the NA.

Involvement of the nigrostriatal DA systems has been suggested for the overeating caused by tail pinch [2]. Moreover, amphetamine injection in the striatum was reported to stimulate eating in free feeding rats [22], although the long latency to this effect suggests that drug diffusion to the NA could have occurred in this study. It seems therefore that DA in striatum and NA may be involved in overeating by rats, depending on the experimental conditions.

The fact that fluphenazine injection in NA failed to modify the food intake of deprived rats suggests that DA of this area is not involved in the eating induced by nutritional deficit but plays a role in particular forms of eating associated with behavioural activation, as was observed for muscimol-induced eating. That behavioural arousal induced by a moderate increase of dopamine function in NA may elicit feeding in sated rats is also suggested by the evidence that muscimol injected in the caudal ventral tegmental area, site of origin of DA neurons innervating the NA, produced dopamine-mediated hyperlocomotor activity associated with an increase of food intake in satiated rats [3].

The specificity of NA involvement in the present study was confirmed by the fact that DA release and synthesis, measured respectively by 3MT levels and DOPA accumulation, were changed in the NA but not in the striatum of muscimol injected animals. However, surprisingly a reduction in DA release and synthesis was observed. A similar biochemical effect on DA release and synthesis has been found after dopamine receptor stimulation by dopaminergic agonist injection as a result of a feed-back control on presynaptic dopaminergic neurons [7,10]. Since in this study the DA mesolimbic function appears to be increased in muscimol treated rats, the effect on DA release and synthesis may be secondary to an increase in post-synaptic receptor sensitivity. It should be underlined however that there was also an increase in DOPAC, not easily reconcilable with an increased activity of postsynaptic DA receptors. Thus other mechanisms are probably involved in the biochemical, and perhaps behavioural, changes caused by muscimol injection in the NRD.

In view of the decreased metabolism of serotonin seen in the striatum and NA of muscimol treated rats, changes in 5HT function should receive particular attention. 5HT injections in the NA inhibited the hyperactivity caused by locally applied DA [9] and increased DA metabolism in this area [19], whereas stereotyped movements induced by high doses of apomorphine, an effect of direct activation of postsynaptic DA receptors in the striatum [11] were little or not affected [4,20] by agents which enhance 5HT transmission.

These and other data suggested that 5HT acts as a blocker of DA activity on postsynaptic neural elements in the NA but not in the striatum [21]. A similar suggestion for 5HT in the NA was made by Jones *et al.* [14]. Moreover, injections of 8.8 μg 5HT in the NA blocked eating caused by muscimol in the NRD (unpublished results). Although the exact relation between 5HT and DA mechanisms in the NA is not known, it is likely that changes in 5HT function partly contributed to changes in behaviour and DA dynamics found in animals injected with muscimol in the NRD.

In conclusion, muscimol injection in the NRD caused eating associated with behavioural activation which seem to involve specific changes of DA mechanisms in the NA. Although further studies are needed to clarify the exact mechanisms, it is suggested that changes in DA receptor sensitivity, together with changes in 5HT function, are involved.

TABLE 3

l-DOPA ACCUMULATION INDUCED BY DECARBOXYLASE INHIBITION AFTER MUSCIMOL INJECTION IN THE NUCLEUS RAPHE DORSALIS IN THE RAT

	l-DOPA (ng/g tissue)	
Treatment	N. accumbens	Striatum
Vehicle	1204 ± 151	953 ± 86
Muscimol (100 ng/0.5 μl)	727 ± 117*	758 ± 110

Values are mean ± S.E. of 5 rats.
Rats were killed 45 min after muscimol injection.
*$p<0.05$ Student's t-test.

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REFERENCES

1. Achilli, G., C. Perego, F. Ponzio and S. Algeri. Simultaneous determination of DA, NA, 5HT and their metabolites in several rat brain areas by HPLC with electrochemical detection. *Res Commun Chem Pathol Pharmacol* **40:** 67–72, 1983.
2. Antelman, S. M., H. Szechtman, P. Chin and A. E. Fisher. Tail pinch-induced eating, gnawing and licking behavior in rats: Dependence on the nigrostriatal dopamine system. *Brain Res* **99:** 319–337, 1975.
3. Arnt, J. and J. Scheel-Kruger. GABA in the ventral tegmental area: Differential regional effects on locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. *Life Sci* **25:** 1351–1360, 1979.
4. Bendotti, C., F. Borsini, M. G. Zanini, R. Samanin and S. Garattini. Effect of fenfluramine and norfenfluramine stereoisomers on stimulant effects of d-amphetamine and apomorphine in the rat. *Pharmacol Res Commun* **12:** 567–574, 1980.
5. Benfenati, F., P. Ferretti, C. Ferretti, F. Ponzio and S. Algeri. Determination of L-dopa in brain tissue using high-performance liquid chromatography with electrochemical detection to study the activity of central dopaminergic neurons. *IRCS Med Sci* **10:** 425–426, 1982.
6. Borsini, F., C. Bendotti, B. Przewłocka and R. Samanin. Monoamine involvement in the overeating caused by muscimol injection in the rat nucleus raphe dorsalis and the effects of d-fenfluramine and d-amphetamine. *Eur J Pharmacol* **94:** 109–115, 1983.
7. Carlsson, A. Receptor-mediated control of dopamine metabolism. In: *Pre- and Postsynaptic Receptors*, edited by E. Usdin and W. E. Bunney, Jr. New York: Marcel Dekker, 1975, pp. 49–65.
8. Costall, B., R. J. Naylor and J. E. Olley. Stereotypic and anticataleptic activities of amphetamine after intracerebral injections. *Eur J Pharmacol* **18:** 83–94, 1972.
9. Costall, B., R. J. Naylor, C. D. Marsden and C. J. Pycock. Serotoninergic modulation of the dopamine response from the nucleus accumbens. *J Pharm Pharmacol* **28:** 523–526, 1976.
10. Di Giulio, A. M., A. Groppetti, F. Cattabeni, C. L. Galli, A. Maggi, S. Algeri and F. Ponzio. Significance of dopamine metabolites in the evaluation of drugs acting on dopaminergic neurons. *Eur J Pharmacol* **52:** 201–207, 1978.
11. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
12. Fielding, S. and H. Lai. Behavioral actions of neuroleptics. In: *Handbook of Psychopharmacology, Vol 10, Neuroleptics and Schizophrenia*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1978, pp. 91–128.
13. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. *J Neurochem* **13:** 655–669, 1966.
14. Jones, D. L., G. J. Mogenson and M. Wu. Injections of dopaminergic, cholinergic, serotoninergic and GABAergic drugs into the nucleus accumbens: Effects on locomotor activity in the rat. *Neuropharmacology* **20:** 29–37, 1981.
15. König, J. F. R. and R. A. Klippel. *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: Williams & Wilkins, 1963.
16. Mogenson, G. J. and M. Wu. Neuropharmacological and electrophysiological evidence implicating the mesolimbic dopamine system in feeding responses elicited by electrical stimulation of the medial forebrain bundle. *Brain Res* **253:** 243–251, 1982.
17. Ponzio, F., G. Achilli and S. Algeri. A rapid and simple method for the determination of picogram levels of 3-methoxytyramine in brain tissue using liquid chromatography with electrochemical detection. *J Neurochem* **36:** 1361–1367, 1981.
18. Przewlocka, B., L. Stala and J. Scheel-Kruger. Evidence that GABA in the nucleus dorsalis raphe' induces stimulation of locomotor activity and eating behavior. *Life Sci* **25:** 937–946, 1979.
19. Pycock, C. J., R. W. Horton and C. J. Carter. Interactions of 5-hydroxytryptamine and gamma-aminobutyric acid with dopamine. In: *Advances in Biochemical Psychopharmacology, Vol 19*, edited by P. J. Roberts, G. N. Woodruff and L. L. Iversen. New York: Raven Press, 1978, pp. 323–341.
20. Rotrosen, J., B. M. Angrist, M. B. Wallach and S. Gershon. Absence of serotonergic influence on apomorphine-induced stereotypy. *Eur J Pharmacol* **20:** 133–135, 1972.
21. Spampinato, U., R. Invernizzi and R. Samanin. Evidence of serotonin involvement in the effect of morphine on dopamine metabolism in the rat nucleus accumbens but not in the striatum. *Pharmacol Res Commun* **16:** 519–523, 1984.
22. Winn, P., S. F. Williams and L. J. Herberg. Feeding stimulated by very lose doses of d-amphetamine administered systemically or by microinjection into the striatum. *Psychopharmacology (Berlin)* **78:** 336–341, 1982.