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Effects of Intrastriatal Injections of Scopolamine on Appetitive Behavior

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NEILL, D. B. AND S. P. GROSSMAN. *Effects of intrastriatal injections of scopolamine on appetitive behavior.* PHARMAC. BIOCHEM. BEHAV. 1(3) 313–318, 1973.–Direct application of the anticholinergic drug scopolamine to the corpus striatum of food-deprived rats reliably inhibited the intake of solid food as well as food-rewarded lever pressing. Water intake and operant responding for water rewards in water-deprived rats were not affected, suggesting that the drug treatment did not interfere with swallowing or with the animals' ability to lever press. The intake of a liquid diet by food-deprived rats was also not affected by intrastriatal scopolamine, suggesting that the observed suppression of food intake and food-rewarded instrumental behavior may not be related to a loss of food motivation per se.

Food intake Water intake Corpus striatum Motivation Scopolamine

CONSIDERABLE experimental evidence suggests that the striatum may contribute importantly to the organization of ingestive behaviors. Morgane [14] reported more than a decade ago that lesions in the globus pallidus produce a syndrome of aphagia and adipsia which appears to be similar to that seen after damage to the lateral hypothalamus (LH). Krantz and Teitelbaum [see 24] have reported more recently that the effects of a unilateral lesion in the LH (a few days of aphagia and adipsia) sum with the equally temporary effects of a contralateral lesion in the globus pallidus to produce a persisting inhibition of both food and water intake. Striatal mechanisms have been implicated further by a recent report by Sorenson and Ellison [23] that total neodecortication did not result in persistent impairments in feeding behavior unless additional damage to the caudate-putamen complex was produced.

Morgane [14] suggested that the effects of lesions in the globus pallidus might be due to an interruption of the pallidofugal fiber system which connects the striatum with the brainstem. This interpretation has received support from the work of Gold [6] who reported that lesions in the brainstem tegmentum also produce aphagia and adipsia. Gold further found that the effects of a unilateral tegmental lesion (mild and temporary depression of food and water intake) sum with the equally transient effect of a contralateral LH lesion to produce long-term aphagia and adipsia.

Ungerstedt [25] has recently shown that electrolytic as well as chemical lesions in portions of the diencephalon which contain the nigrostriatal bundle (ascending dopaminergic projections from the substantia nigra to the striatum) also result in aphagia and adipsia. This suggests that a disruption of nigrofugal afferents to the striatum may be responsible for some or all of the effects of striatal lesions. This view receives support from a recent report by Oltmans and Harvey [19] that lesions in the LH which interrupt this fiber bundle produce transient hypophagia as well as apparently permanent and severe hypodipsia, the magnitude of the latter being correlated with the degree of dopamine depletion from the striatum.

We [9] have recently shown that microsurgical transections of the fibers which cross the lateral border of the hypothalamus result in long-term aphagia and adipsia even though there is little or no direct tissue damage in the hypothalamus or the striatum. This demonstrates unequivocally that the connections between the midbrain or diencephalon and the striatum are essential for the organization of ingestive behavior. Our results do not distinguish between the suggested role of the pallidofugal or nigrostriatal pathways since both are diffusely represented in the region of the transection.

The observations indicating that pathways which course through the hypothalamus on their way to or from the striatum may play an important role in the organization of feeding and drinking raise fundmental questions concerning the organization of the central pathways which mediate these behaviors. Largely on the basis of the observation that LH lesions result in long-term aphagia and adipsia, the

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hypothesis is widely accepted that the lateral hypothalamus contains a focal point of integration for feeding and drinking behavior. The demonstration of adipsia and aphagia following striatal or midbrain lesions or the interruption of fibers coursing between the striatum and diencephalon raises the possibility that LH lesions may produce aphagia and adipsia only because of incidental damage to fiber systems which course through it. Alternatively, it is possible that these fiber systems may synapse in the hypothalamus and that the striatum, LH, and midbrain may represent way-stations among a complex neural circuit which contribute distinct regulatory or organizational influences on feeding and drinking behavior.

We have recently investigated the role of the striatum in complex, aversively controlled behavior [16,18] and have incidentally observed changes in food intake following intrastriatal injections of compounds (such as atropine and scopolamine) which interfere with neural activity in cholinergic pathways. Many investigators [e.g., 19,25] have proposed that ingestive behavior may be influenced by dopaminergic components of the area, but this does not exclude the possibility that cholinergic mechanisms which are quite prevalent in this part of the brain [e.g., 13] may not also be involved. It is quite possible, for that matter, that an interference with the dopaminergic input to the striatum may modify the activity of cholinergic components of the region or vice versa [2]. The present experiments were therefore undertaken to systematically investigate the possible role of cholinoceptive components of the striatum in the organization of ingestive behaviors.

METHOD

Animals

Thirty-four adult male albino rats, obtained from Holtzman (Madison, Wisconsin) were used. All weighed 350–380 g at the time of surgery. The animals were individually housed in a continuously lighted colony.

Apparatus

The effects of intrastriatal injections of scopolamine on food and water intake were analyzed in free-access observation as well as operant conditioning situations. Eight 24 x 24 x 30 cm observation boxes, constructed of Plexiglas with fine stainless steel mesh floors, were used for the free-access tests. An inverted graduated cylinder, filled with tap water, was mounted on the outside of each box such that its spout protruded into the compartment. A second cylinder mounted in a similar fashion was filled with a liquid diet (Metrecal) on some tests. A known quantity of solid food (Teklad rat pellets) was placed on the floor of the boxes before each test.

Two standard operant conditioning boxes housed in sound-resistant enclosures (Lehigh-Valley, No. 1417) were used to examine drug effects on instrumental responding. Food (one 45 mg Noyes pellet) or water (0.1 cc) reinforcements were available on a variable-interval schedule of reinforcement which averaged one reward every 30 sec (VI-30). Lever presses and reinforcements were automatically recorded by counters and a cumulative recorder.

Procedure

Surgery. Double walled stainless steel cannulas (30 gauge inner, 23 gauge outer) were stereotaxically implanted bilaterally into either the dorsal or ventral head of the striatum of 34 rats (see [7] for details of this procedure) under Nembutal anesthesia. The tips of the dorsal implants were aimed at AP = 8.6; L = 2.7; H = 2.7, the tips of the ventral implants at AP = 8.6; L = 2.7; H = 1.0, using coordinates from the Pellegrino and Cushman [20] atlas of the rat brain.

Training and deprivation conditions. Beginning at least 1 wk after surgery, the animals were either starved to 85% of their free-feeding weight or placed on a 23 2/3 hr water deprivation schedule. They were then trained to (a) consume a sizeable portion of their daily food and water intake during a 30 min test in the observation boxes and (b) operate a lever to obtain food or water during a 30 min test in the operant conditioning apparatus. Only one such test (either in the observation cages or in the operant apparatus) was conducted each day. The food-deprived animals received sufficient additional food in the home cage approximately 1 hr after the daily test to maintain body weight at 85% of normal.

Drug tests. After the ingestive and operant behaviors had stabilized, drug and sham-injection control tests were begun. Before each daily test, the inner cannulas were removed, cleaned, and returned to the animal's head either empty (sham procedure) or after approximately 5–10 μg of crystalline scopolamine methyl nitrate (Sigma Chemical Co.) had been tamped into their tips. The doses were estimated by weighing similarly loaded inner cannulas on a microbalance before and after loading.

Four animals with ventral striatal implants were food-deprived and tested in the observation boxes with solid pellets. These animals were given 1.0 to 10.0 μg of crystalline *d*-amphetamine sulfate, dopamine hydrochloride, scopolamine methyl nitrate, haloperidol, and norepinephrine hydrochloride in order to provide some evidence concerning the specificity of the effects of the scopolamine treatments used in the principal experiments.

Evaluation of data. After all experiments were completed, the animals were sacrificed by a lethal dose of Nembutal and intracardially perfused with isotonic saline followed by 10% Formalin. After fixation, 50 micron frozen sections were cut through the area of the implant placements and stained with cresyl violet.

Analyses of variance of repeated measures [27] were used to obtain overall F ratios. The Newman-Keuls test [27] was used to assess the statistical significance of differences between individual groups.

RESULTS

Microscopic analysis of the histological material showed the location of the tips of the cannulas to be as depicted in Fig. 1. On the basis of these data and the results of previous experiments [16] which suggested functional differences between the dorsal and ventral striatum, 20 animals were classified as having ventral placements, 14 as having dorsal placements.

Food and water intake in food-deprived rats. The administration of scopolamine to the dorsal ($p<0.01$) or ventral ($p<0.01$) striatum of food deprived rats reliably reduced the intake of solid food in the free-access tests (see Fig. 2). No consistent differences between the effectiveness of the ventral and dorsal placements could be discerned. Three of 6 animals with dorsal cannula placements showed a dramatic concurrent increase in water intake. The water

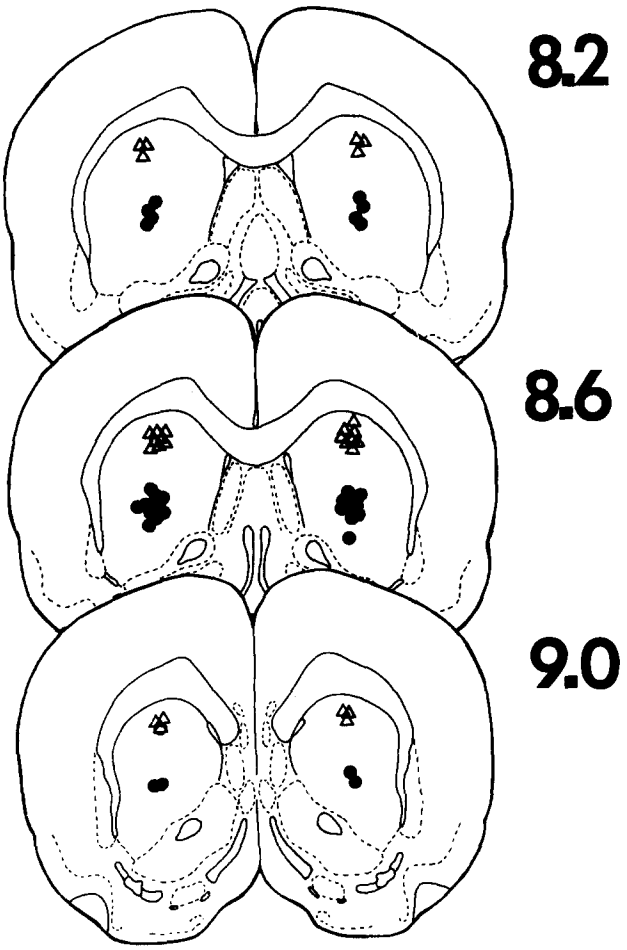

FIG. 1. Location of cannula tips in the dorsal or ventral striatum.

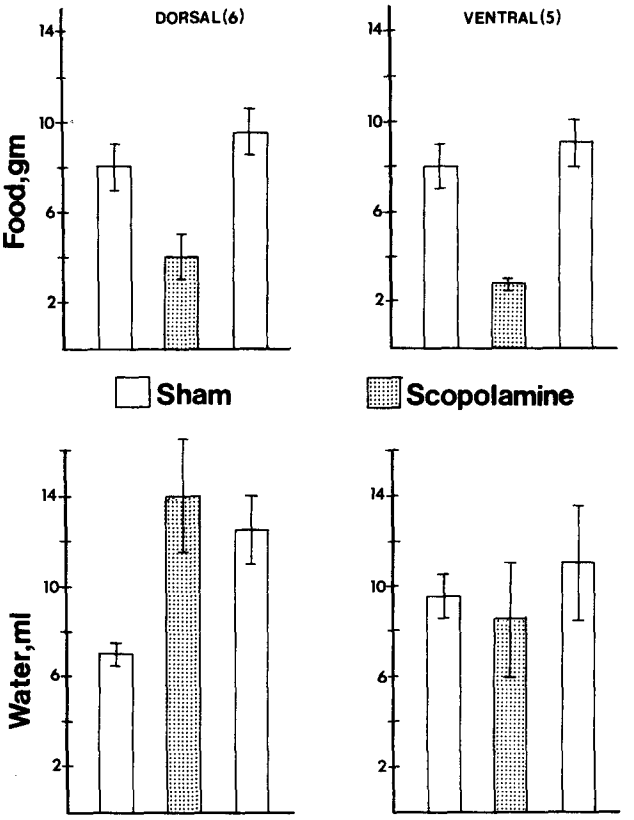

FIG. 2. Food and water intake of food-deprived rats in 30-min tests. Sham injections were performed on days immediately preceding and following the scopolamine treatments.

consumption of the remaining 3 animals was within normal limits. The water intake of the 3 animals which were hyperdipsic on the drug test remained elevated on the first control test and then dropped back to predrug level.

When the same animals were offered a liquid diet (Table 1), scopolamine had no reliable effect on either food or water intake ($F<1$ in both cases).

Operant responding for food rewards. The administration of scopolamine to the dorsal or ventral striatum markedly suppressed responding for food rewards presented on a VI-30 schedule of reinforcement ($p<0.01$; see Table 2). The drug effect was reliably larger ($p<0.05$) in animals with ventral cannula placements. An analysis of the response rate during the test period indicated that most of this difference between the dorsal and ventral placements was due to the fact that response suppression occurred more rapidly after administration of the drug to the ventral region.

Food and water intake in water-deprived rats. The application of scopolamine to the dorsal or ventral striatum had no reliable effect on water intake in water-deprived animals ($F<1$). The intake of dry food was significantly ($p<0.01$) inhibited (see Fig. 3).

Operant responding for water rewards. The administration of scopolamine to the dorsal or ventral striatum did not reliably affect lever pressing for water rewards ($F<1$) (see Table 2).

TABLE 1

MEAN INTAKE (ML WITH S. E.) OF A LIQUID DIET DURING 30 MIN TESTS AFTER INJECTION OF SCOPOLAMINE INTO THE STRIATUM

Placement	Control	Scopolamine	Control
Dorsal (n = 6)	28.8 ± 2.1	30.3 ± 3.2	26.7 ± 2.9
Ventral (n = 5)	27.8 ± 2.1	30.0 ± 4.5	28.8 ± 1.8

Effects of catecholaminergic drugs. As shown in Fig. 4, neither the catecholaminergic stimulants nor the adrenergic/dopaminergic blocking agent haloperidol reliably affected the intake of solid pellets. Scopolamine drastically ($p<0.01$) suppressed intake. Amphetamine and dopamine administrations resulted in hyperactivity, as previously noted [15].

DISCUSSION

The results of the present experiments indicate that components of the rat striatum may be specifically involved

TABLE 2

MEAN NUMBER OF RESPONSES (WITH S. E.) FOR FOOD OR WATER REINFORCEMENTS IN 30 MIN ON A VI-30 SEC SCHEDULE

Placement	Control	Scopolamine	Control
Food			
Dorsal (n = 5)	1147 ± 376	419 ± 108*	1050 ± 405
Ventral (n = 6)	1007 ± 182	194 ± 39*	992 ± 260
Water			
Dorsal (n = 5)	534 ± 83	543 ± 87	486 ± 123
Ventral (n = 5)	529 ± 65	548 ± 41	540 ± 29

*$p<0.01$

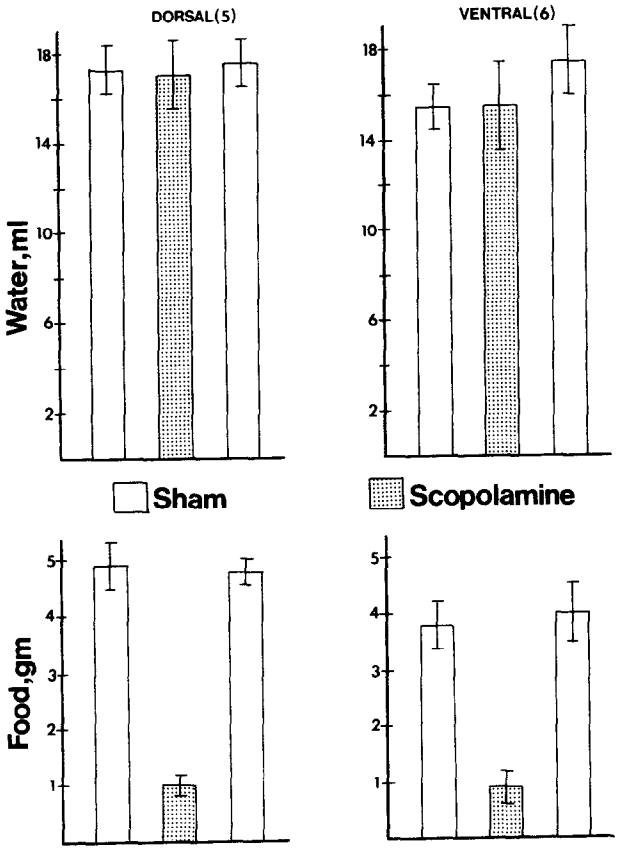

FIG. 3. Water and food intake of water-deprived rats in 30-min tests. Sham injections were performed on days immediately preceding and following the scopolamine treatments.

in the organization of feeding behavior. The nature of this relationship is not entirely clear at this time. Following intrastriatal injections of scopolamine, food deprived rats failed to eat dry food or work a lever for pellet rewards, but water deprived rats drank normal quantities of water and worked well for water rewards. This pattern of effects indicates that the scopolamine treated rats were capable of swallowing and lever pressing.

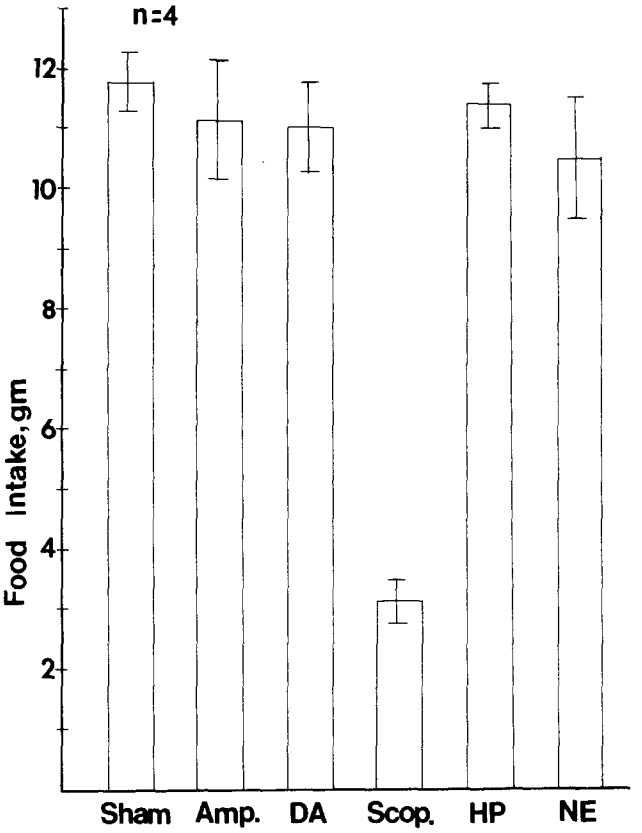

FIG. 4. Food intake of food-deprived rats in 30-min tests after intrastriatal administrations of amphetamine (Amp), dopamine (DA), scopolamine (Scop), haloperidol (HP), or norepinephrine (NE).

The scopolamine treated animals also consumed normal quantities of a liquid diet, suggesting that a loss of food motivation may not be responsible for the drug effect on feeding and on food-rewarded operant responding. It is, of course, not possible to discount the possibility that the scopolamine treated animals may have consumed normal quantities of the liquid diet purely on the basis of its palatability in the absence of normal hunger.

It appears more likely, however, that the drug interfered with motor functions (e.g., biting, chewing) which are essential to the ingestion of dry but not liquid foods. Such an interpretation is supported by incidental observations of the scopolamine treated animals' behavior. The animals picked up pellets of food and bit into them, but the biting and chewing appeared poorly coordinated and rarely resulted in the ingestion of food particles. Instead, the animals appeared to shred the pellets until small food particles littered the floor of the observation cages. The suggested interpretation of our results is supported by reports of chewing (but not consummatory response) in response to electrical stimulation of the striatum of rats [e.g., 12].

The possibility that motor functions specifically related to the ingestion of the dry food commonly used in rodent laboratories may be integrated by striatal mechanisms is of special significance in view of several recent reports which have implicated pallidofugal or nigrostriatal pathways in the

regulation of feeding behavior [6, 14, 25] and the growing concern [see 8] that an interruption of these pathways may contribute directly or indirectly to the aphagia seen after LH lesions. Rogers *et al.* [22] have shown that rats with lateral hypothalamic lesions not only fail to eat and drink voluntarily but also do not press a lever to obtain intragastric injections of a liquid diet. These observations are widely accepted as evidence that an impairment of motor functions specifically related to feeding may not be responsible for the effects of LH lesions on ingestive behavior. However, Levine *et al.* [12] have recently shown that lesions in the globus pallidus result not only in aphagia and adipsia but also in a marked impairment of brain stimulation rewarded lever pressing that appeared to be due to sensory-motor deficits. One of the major efferent connections of the globus pallidus, the pallidofugal fiber system, courses through the LH, and it has been suggested [14] that the effects of LH lesions may be related to an interruption of these connections. It is thus possible that the results reported by Rogers *et al.* may reflect a concurrent impairment of motor functions related to lever pressing and to biting, chewing, or swallowing.

Balagura *et al.* [1] have shown that the marked effects of LH lesions on posture and locomotion (rats with LH lesions appear to be in a state resembling catatonia) probably are not responsible for their effects on food and water intake, and Grossman and Grossman [9] have obtained similar effects in animals which sustained transections of the lateral connections of the diencephalon. These observations do not, however, demonstrate that motor functions specifically related to ingestive behavior may not have been lost or significantly impaired. It would appear essential that future investigations of the role of hypothalamic as well as striatal mechanisms in feeding and drinking pay more detailed attention to motor dysfunctions in order to demonstrate unequivocally the motivational influences commonly assumed to originate in the hypothalamus.

It may be useful, in this connection, to examine the possibility that the hypothalamus and striatum may interact to organize ingestive behaviors, and that this interaction may be approximated by models such as that proposed by Glickman and Schiff [5]. According to this model, motivated behaviors require an interaction of at least two neural systems – a motivational component that determines whether a stimulus is capable of eliciting a response and a motor component which organizes the required reaction to it.

The results of our limited control tests suggest that the components of the striatum which are responsible for the observed changes in feeding behavior may rely on cholinergic mechanisms either pre- or postsynaptically. This possibility is of special interest in view of recent suggestions that the connections of the striatum that mediate its effects on ingestive behavior may be dopaminergic. Aphagia and adipsia have been reported after injections of 6 hydroxydopamine (which selectively destroys aminergic nerve terminals) into the substantia nigra and along the course of the nigrostriatal fiber system [25]. Neill and Parker [17] have recently observed temporary aphagia following intrastriatal injections of this compound. However, we have consistently found that intrastriatal injections of dopamine or the aminergic blocking agent haloperidol do not interfere with feeding, and others [e.g., 4,26] have reported similar negative results. (It should be noted, however, that haloperidol does not go into solution as readily as other compounds, so that its failure to modify feeding behavior must be interpreted cautiously.) These observations suggest the possibility that the dopaminergic input to the striatum may modulate the activity of its cholinergic components, as Hornykiewicz [11] and others have suggested.

The observed dramatic effects of intrastriatal scopolamine on feeding behavior are surprising when viewed against the background of a large literature which consistently fails to report persisting changes in food intake after extensive damage to the striatum [e.g., 3, 10, 21, 28]. In previous investigations of the behavioral effect of striatal lesions, we have noted evidence of food-shredding but consistently found that animals with dmage to the ventral or dorsal striatum are capable of maintaining a normal body weight [16,18].

It is possible that scopolamine injections affect a larger proportion of the striatum than lesions or that the selective inhibition of cholinoceptive components of the region may produce an imbalance in some motor functions which is more disruptive than the concurrent elimination of dopaminergic and cholinergic mechanisms. It appears more likely, however, that rats are capable of learning to compensate for the impairments produced by striatal dysfunction if the problem persists after a lesion, but fail to do so when the impairment is temporary, as is the case in the present experiments.

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