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BRIEF COMMUNICATION

Cafeteria Behavior in the Rat After Hypothalamic Cholinergic and Adrenergic Stimulation[1]

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MONTGOMERY, R. B. AND S. ARMSTRONG. *Cafeteria behavior in the rat after hypothalamic cholinergic and adrenergic stimulation.* PHARMAC. BIOCHEM. BEHAV. 3(4) 709–711, 1975. – Norepinephrine, carbachol, or placebo was micro-injected into the perifornical region of the rat hypothalamus, via stereotaxically implanted cannulas. Ingestive behavior was observed in the hour after injection in a cafeteria situation in which water, milk, mash, powdered food, and lab chow were all freely available. After adrenergic stimulation, animals ingested significant amounts of mash only; after cholinergic stimulation, animals ingested significant amounts of water and milk, but water was significantly preferred to milk. These findings are seen as providing further support for the behavioral specificity of direct chemical stimulation of the brain, and as casting serious doubts on the interpretation of milk-ingestion as eating behavior.

Norepinephrine Carbachol Eating Drinking

OVER the past decade, evidence obtained from experiments involving direct chemical stimulation of the rat brain (CSB) has lead to the concept of a hypothalamic noradrenergic feeding system [2, 5, 7, 8, 16, 17]. However, the literature shows that different investigators have used a variety of laboratory foods on which to test the eating response induced by exogenous norepinephrine. For instance, Booth [2] in his investigation of hypothalamic loci for norepinephrine-elicited eating, in order to increase the strength of data on food intake and to diminish less specific oral effects, chose to use relatively unpalatable dry chow during drug administration tests, although at other times his rats had access to wet mash. Other authors for similar reasons have used a powdered diet.

In contrast, Miller *et al.* [16], when comparing intrahypothalamic adrenergic and cholinergic dose-level effects on consummatory behavior, used a Metrecal liquid diet in preference to dry food, in order to eliminate any effects indicative of gnawing rather than eating. Still other investigators have used Purina laboratory chow pellets [3, 10, 11, 12], Purina laboratory checkers [7,8] and milk [1, 13, 14]. These latter studies have led to a hypothalamic noradrenergic "satiety" theory [14].

The purpose of the present study was to investigate which food rats preferred to eat after the microinjection of norepinephrine into the lateral hypothalamus. In particular, the use of milk as a food and the interpretation of milk ingestion as eating were considered. Milk, at the concentrations used by previous authors [1,13], one part Borden's sweetened condensed milk, to two parts water, obviously consists mainly of water. Intrahypothalamically administered norepinephrine depresses deprivation-induced water drinking behavior [21]. Thus, in the present study, the effects of exogenous norepinephrine on consummatory behavior in a cafeteria situation were compared to those produced by carbachol, which has been shown to elicit water drinking.

METHOD

Animals

Eighteen male Wistar-derived rats weighing approximately 320 g served for the experiments described below. All rats were housed in individual cages in a room where temperature was controlled at 22°C. Up to the time of surgery, animals were fed laboratory chow and maintained on tap water ad lib.

Surgery

Rats were stereotaxically cannulated with bilateral im-

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plants [4] into the perifornical region, just posterior to the anterior hypothalamus, at stereotaxic co-ordinates A = 0.8 mm anterior to bregma, L = 1.9 mm lateral to the midline, and H = 8.5 mm below the dura [19].

Drugs

Stimulus solutions consisted of 325 $\times$ 10^{-4} M (10μg/μl) norepinephrine (l-arterenol bitartrate monohydrate, Sigma) and 18 $\times$ 10^{-4} M (0.3μg/μl) carbachol (carbamylcholine chloride, Merck) prepared in saline solution to be isotonic with the placebo, 0.154 M NaCl.

Procedure

Initial testing. Following implantation, rats were allowed one week to recover from surgery, with chow and tap water always available. Rats were then screened by functional tests for an adrenergically elicited increase in eating (a criterion increase of at least 0.8 g) and a cholinergically elicited increase in drinking (a criterion increase of at least 6.0 ml water). Of the 18 rats, 10 showed responses to both drugs in both cannulas.

These ten rats were maintained in a cafeteria situation, with an ad lib choice of water, milk (reconstituted, unsweetened, Nestle's powdered milk, at 160 g powder/liter of water), wet mash (800 g Mecon powdered rat diet/liter of water), dry mash (powdered diet only), and lab chow (Mecon rat and mouse cubes). Rats obtained milk or water by licking at holes drilled centrally in 200 ml bottles attached to the inside of the cage. Other substances were placed on the floor of the cage. All supplies were changed each morning and evening, and their positions around the cage varied randomly to control for any position effects.

Four weeks were allowed before testing, for rats to accustom themselves to the cafeteria situation, and to control for novelty effects.

Testing. On test days, rats were injected with 1 μl of one substance into each cannula, at about 11 a.m. Measurements of all 5 supplies were taken by weight prior to, and 1 hr after injection. Two days were allowed between test days, to control for cumulative effects of repeated adrenergic stimulation [5]. Treatment orders of placebo, norepinephrine, and carbachol were counterbalanced over rats. Treatments were then duplicated so that a rat's score for any one treatment condition was the mean of two such tests.

After the completion of this treatment, rats were maintained in the cafeteria situation, and one week later were again tested with placebo, carbachol or norepinephrine in counterbalanced order. However, this time, all substances except milk were removed from the home cage for the 1 hr drug-test period. Thus, the affects of cholinergic and noradrenergic stimulation on milk intake alone were ascertained. Eight rats survived this régime.

RESULTS

During the four weeks pre-experimental period, in the cafeteria situation, it was observed from daily food intake, that: (a) rats did not eat any powdered diet, and (b) as a whole did not eat cubes, although individual preferences for cubes were observed, possibly for gnawing rather than ingestion, and (c) consumed wet mash as their staple food substance; because the wet mash contained considerable amounts of water, (d) rats drank very little water from burettes, but (e) seemed to prefer to drink milk in preference to the water, for their fluid intake.

The intrahypothalamic injection of norepinephrine in the cafeteria situation, elicited significant eating of wet mash, but not of any other substance. Cholinergic stimulation elicited significant ingestion of both water and milk, but water was significantly preferred to milk; no other intake was significantly affected by cholinergic CSB.

The results are shown in Table 1. The data were subjected to a randomized blocks analysis of variance and Rodger's [20] planned contrasts applied, $F(14,126) = 14.8$, $\alpha = 0.01$).

When milk was presented alone, carbachol significantly enhanced milk intake by 4.5 g above placebo control intakes ($t = 13.02$, $df = 7$, $p<0.05$), whereas, following noradrenergic stimulation a mean increase of 0.3 g was found ($t = 0.38$, $df = 7$, $p>0.05$).

Histology

Rats were sacrificed by decapitation, the brains removed through the dorsal surface of the skull, and stored in a solution of 10% formal saline. The brains were then blocked in paraffin and sectioned frontally at 20 μ parallel to the cannula track and therefore in the plane of Pellegrino and Cushman's stereotaxic atlas [19]. Deparaffinized hypothalamic sections were stained in Luxol Fast Blue. Loci of

TABLE 1

MEAN INTAKES OF FIVE SUBSTANCES AFTER HYPOTHALAMIC CSB IN 10 SATIATED RATS

	Mean Intake (g)				
Injection	Water	Milk	Wet Mash	Dry Mash	Chow
Norepinephrine	1.27	1.20	6.54*	0.29	0.53
Carbachol	6.10*	2.49*	2.28	0.32	0.25
Placebo	1.01	0.99	2.69	0.46	0.18

*Significantly different from placebo, $p<0.01$

stimulation ranged from the following co-ordinates: anterior to posterior +1.0 to −0.9mm from bregma; lateral, 1.8 to 2.4mm from the midline; and horizontal −7.3 to −8.8mm from the top of the brain.

DISCUSSION

The findings in the cafeteria situation firstly provide further support for the behavioral specificity of the effects of chemical stimulation of the brain. After noradrenergic stimulation, the rats clearly preferred to eat wet mash, rather than ingest any of the other four substances available. Their preference for wet mash as food is in accord with their preference displayed during the four week pre-experimental period, and is typical of rats in our laboratory. This could suggest that the consummatory behavior elicited by noradrenergic stimulation of the brain had the properties of normal feeding. On the other hand, even though both the milk and wet mash contained considerable amounts of water, the rats preferred to drink water after cholinergic stimulation, although they also drank a significant amount of milk. This is in contrast to the pre-experimental period, when rats seemed to prefer milk to water.

The specificity effect is further demonstrated by the results obtained when only milk was available. Cholinergic CSB significantly increased milk-drinking over placebo levels, whereas adrenergic CSB had no significant effect. Investigators using other dipsogens [6,9] have proposed central regulatory extracellular and cellular thirst systems, a cholinergic system being implicated in the latter. Cholinergic CSB, as in this study, may have its primary effect on the latter system, which may preferentially involve water drinking.

Oatley [18] has contended that the role of each type of behavior should be sought (partly) in the life of the animal. Milk intake normally occurs only early in the life of most mammalian species, including the rat [22], and acts as a source of both water and nutrition.

Two main conclusions can be drawn from this study: First, it has been demonstrated that wet mash, the preferred food of our rats on ad lib conditions, is also the preferred choice under norepinephrine-induced eating. Thus, wet mash would seem to be the ideal choice of laboratory foods, for future experiments investigating norepinephrine-induced eating behavior. Second, milk, due to its dual nature as a source of both water and nutrition, is not a suitable test substance for gathering valid data on eating behavior.

It is therefore concluded that it is not justifiable to regard milk-licking as a definite eating response, and such studies do not cast light on the role of adrenergic systems in the control of eating behavior because of the possibility of the involvement of the cholinergic drinking system in the response observed as the dependent variable [1, 13, 14, 15].

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