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# Effects of Intrahypothalamic Administration of Norepinephrine on the Feeding Response of the Rat under Conditions of Light and Darkness[1]

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ARMSTRONG, S. AND G. SINGER. *Effects of intrahypothalamic administration of norepinephrine on the feeding response of the rat under conditions of light and darkness.* PHARMAC. BIOCHEM. BEHAV. 2(6) 811–815, 1974. – Intrahypothalamic injections of norepinephrine were given to rats in high and low states of hunger, under conditions of light and darkness. A significant interaction between state of hunger, condition of photoperiod and drug administration was found. Norepinephrine significantly facilitated feeding in the dark; this effect was more marked in the low hunger state than in the high hunger state. Conversely, norepinephrine significantly depressed feeding in the light, the depression being most marked in the high hunger state. Thus, condition of light is an important determinant of the effects or norepinephrine on feeding. Norepinephrine depressed food-associated drinking under all conditions.

Norepinephrine Hunger Photoperiod Eating Drinking

INTRACRANIAL injections into the lateral hypothalamus and associated limbic system structures of the rat have indicated that a brain noradrenergic system is involved in feeding behavior. Intrahypothalamic injection of norepinephrine facilitates feeding in food satiated rats [14, 16, 25] and food deprived rats [14]. In contradiction to these findings, norepinephrine has also been demonstrated to depress feeding in food satiated rats [21,22] and in food deprived rats [5, 8, 17]. From these experiments a noradrenergic feeding theory [14, 16, 25] and an opposing noradrenergic satiety theory [21,22] have been proposed. Condition of hunger therefore seems to be an important variable influencing the effect of exogenous norepinephrine on eating behavior.

More recently, a second major variable, that of photoperiod, has been reported to be involved in these studies [23]. Norepinephrine injected at the start, in the middle, and towards the end of the dark portion of a 12 hour light/dark cycle depressed feeding. Norepinephrine injected at similar times in the light portion facilitated feeding.

All evidence supporting the noradrenergic feeding theory has been obtained from experiments carried out in the light (or else the authors fail to mention the lighting conditions), while experiments supporting the satiety theory were carried out in the dark [21,22]. It is possible that the noradrenergic feeding and satiety theories are based on findings resulting from differential drug action under two photoperiodic conditions [23].

There is, however, a confounding of two sets of variables in the circadian study [23]; one being photoperiod, and the other being levels of hunger. In terms of general activity and eating responses, rats are predominantly nocturnal animals, and under laboratory conditions they eat 60% to 70% of their total 24 hour food requirement in the dark period of the cycle [2, 3, 20, 27]. Thus using meal size, duration of meal intakes, and length of intermeal interval as indices, rats raised in laboratories may be said to be in a high state of hunger during the dark and in a low state of hunger during light. This is of importance for the interpretation of the effects of intrahypothalamic norepinephrine on the circadian feeding response because of the different actions of intrahypothalamically injected norepinephrine which were discussed earlier.

The present study was an attempt to manipulate the photoperiod and hunger variables, so that both high and low hunger states were present under both light and dark

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conditions. A third variable involved in the controversy of the noradrenergic theories relates to the palatability of different food substances offered to rats after noradrenergic stimulation. Examination of this variable has been reported elsewhere [26] and in this experiment the palatability factor was controlled by using dry mash only.

## METHOD

### *Animals*

Experimental animals consisted of thirty-four naive, male Wistar-derived rats, 90–120 days old and weighing approximately 300 g at the time of surgery. From weaning up to the time of surgery rats had been housed in groups of 6, fed ad lib Mecon rat cubes, and supplied with tap water. All rats had previously been maintained on a 14 hour dark, 10 hour light cycle. Seventeen rats were used in the dark part of the experiment and a further 17 rats in the light. After surgery, rats were housed individually in wire mesh cages measuring 20 × 23 × 40 cm. The temperature of the room was thermostatically controlled at 72 ± 2° F and a 12 hour light/dark cycle was maintained.

### *Surgery*

Rats were starved for 24 hours prior to surgery and were anaesthetized by a chloral hydrate/nembutal intraperitoneal injection. During surgery, two stainless steel cannulae [7] were bilaterally implanted with the aid of a stereotaxic instrument, at the level of the lateral hypothalamus. The coordinates for the intended loci for the cannula tips were A +0.8 mm, H −8.5 mm, and L ±1.9 mm, relative to bregma [27]. This is an area where electrical stimulation elicits feeding and drinking [24] and where noradrenergic stimulation has elicited eating and cholinergic stimulation has elicited drinking [25]. Both alpha- and beta-adrenergic receptors are present [12, 17, 29] and noradrenergic depression of feeding has been demonstrated in this area [21, 22, 23]. The perifornical area thus seemed ideal for a preliminary reexamination of the noradrenergic feeding and satiety theories. Rats were given one week to recovery from surgery.

### *Procedure*

*Deprivation cycle.* Previous unpublished work from this laboratory has shown that rats subjected to a 20 hour food deprivation cycle, water ad lib, i.e. 4 hr feeding per day, eat 60% to 80% of their daily food requirements within the first 3 hr of the 4 hr feeding period. Thus by the end of the first 3 hr of feeding rats can be said to be approaching a state of low hunger analogous to that found towards the beginning of the light period of previous reports [23] i.e. have consumed 60–80% of their daily food intake.

Conversely, after 20 hr food deprivation, at the beginning of the 4 hr feeding period, rats may be said to be in a state of high hunger, analogous to that found at the beginning of the dark cycle. In the present experiment rats were maintained on a 20 hr food deprivation schedule and were operationally defined as being in a state of hunger in the first hour of the 4 hr feeding period, and as being in a state of low hunger in the last hour of the 4 hr feeding period. Intrahypothalamic injections were given at these times.

*Light cycle.* The first group of 17 rats was maintained on a reverse light cycle (lights on at 9:30 p.m. and off at 9:30 a.m.). While the second group of 17 rats was kept under the opposite conditions to those of the first group (lights on from 9:30 a.m. to 9:30 p.m.). Water was supplied ad lib via 100 ml E-MIL Goldline burrettes graduated to 0.2 ml, and attached to the back of the rats' cages. Mecon laboratory chow was presented for 4 hr per day from 1:30 p.m. to 5:30 p.m., i.e. for the first group in the middle of the dark period, and for the second group in the middle of the light period. Three weeks were allowed for rats to become accustomed to the 20 hr food deprivation and the light/dark cycle.

*Injection procedure.* Each rat was given approximately 5 min handling per day, for the 3 days prior to the first injection. Rats received 4 intrahypothalamic injections over 4 test days, each test day being separated by 2 nontest days to avoid cumulative drug effects [10]. The injection technique has been described previously [1] and the injection sequence for both groups of rats was randomized over the 4 injection days. The injections consisted of each rat receiving 1 $\mu$l into each hemisphere of one of the following treatments: norepinephrine 32.5 mM (10 $\mu$g/$\mu$l) (L-arterenol bitartrate monohydrate, Sigma, made isotonic to cerebrospinal fluid by addition of NaCl to 0.154M) or placebo (0.154M NaCl). Injections were given at two times in the feeding cycle, either at the start of the feeding period, before food presentation; or at the end of the third hour of the feeding period. At the beginning of the testing period, rats received preweighed food, which was reweighed every hour, all spillage being collected and included in the weighings. Water readings were taken at the beginning of the testing period, and then hourly. Three days after the last drug testing session 24 hr water intakes for 3 consecutive days were recorded at the following times: 9:30 a.m., 1:30 p.m., 5:30 p.m. and 9:30 p.m. For the dark-fed rats, all testing was carried out under two red lights, one of which was placed near the weighing balance and injection apparatus; the other was placed behind the rats' cages in order to record burette water readings.

### *Histology*

Upon completion of experimentation each group of rats was sacrificed by decapitation, the brain removed through the dorsal surface of the skull, and stored in a solution of 10% formal-saline. The brains were blocked in paraffin and sectioned frontally at 20 $\mu$, parallel to the cannula tracts, in the plane of the stereotaxic atlas [27]. Deparaffinized hypothalamic sections were stained in Luxol Fast Blue; locus of stimulation was determined by placing slides in a photographic projector and adjusting the image to that of the stereotaxic atlas.

## RESULTS

### *Food Intakes*

The data shown in Table 1 were subjected to an analysis of variance for a split-plot design [9], a three-factor experiment with repeated measures on two factors (hunger and drug administration).

Both photoperiod and hunger produced significant differences in food intake, $F(1,32) = 12.4$, $p<0.05$ and $F(1,32) = 168.4$, $p<0.05$, respectively. The drug administration effect was not significant, $F(1,32) = 2.4$, $p>0.05$, but there was a significant interaction between photoperiod and

TABLE 1

SUMMARY TABLE OF MEANS AND VARIANCES (IN PARENTHESIS) FOR FOOD INTAKES (g) AFTER L-NOREPINEPHRINE OR PLACEBO ADMINISTRATION

| | High Hunger | | Low Hunger | |
|---|---|---|---|---|
| | Placebo | Norepinephrine | Placebo | Norepinephrine |
| Light Group | 8.86 | 6.64 | 3.96 | 3.73 |
| (N = 17) | (2.17) | (3.23) | (1.63) | (2.41) |
| Dark Group | 10.28 | 11.11 | 4.97 | 6.40 |
| (N = 17) | (2.57) | (2.94) | (2.29) | (2.54) |

TABLE 2

SUMMARY TABLE OF MEANS AND VARIANCES (IN PARENTHESIS) FOR FOOD ASSOCIATED DRINKING (ml) AFTER L-NOREPINEPHRINE OR PLACEBO ADMINISTRATION

| | High Hunger | | Low Hunger | |
|---|---|---|---|---|
| | Placebo | Norepinephrine | Placebo | Norepinephrine |
| Light Group | 2.37 | 1.58 | 3.07 | 0.83 |
| (N = 17) | (2.75) | (2.10) | (1.56) | (1.28) |
| Dark Group | 3.04 | 0.97 | 3.88 | 1.17 |
| (N = 17) | (2.29) | (1.38) | (1.54) | (0.93) |

drug administration, $F(1,32) = 13.55$, $p<0.05$. Norepinephrine depressed feeding in the light and facilitated feeding in the dark. The other two second order interactions were not significant: (photoperiod and levels of hunger, $F(1,32) = 1.31$, $p>0.05$ and drug administration and levels of hunger $F(1,32) = 1.44$, $p>0.05$). The third order interaction between the three treatment variables was significant, $F(1,32) = 9.44$, $p<0.05$.

*Food-associated Drinking*

The data were analyzed in the same way as for food intake [9]. There was a significant difference between placebo and norepinephrine on water intake, $F(1,32) = 71.94$, $p<0.05$. All other differences and interaction effects were nonsignificant ($p>0.05$). Rats drank a mean of 3.09 ml under all placebo conditions and 1.14 ml under norepinephrine conditions. Thus, norepinephrine always depressed water intake irrespective of photoperiod or level of hunger conditions as can be seen in Table 2.

*24 Hour Water Intakes*

Two important points may be noted on the pattern of 24 hr water intakes. First, both the dark-fed and the light-fed groups showed essentially the same pattern of 24 hr drinking. Dark-fed rats drank 69% of their total 24 hr water requirements at feeding time; light-fed rats drank 66%. In the 4 hr preprandial period, neither group drank much water (3.5%). However, during the 12 hr dark period, the light-fed group drank 13.5% of its total 24 hr water needs as compared to the 3.1% drunk by the dark-fed group in the 12 hr light. The circadian drinking rhythm may still have been operating to a certain extent for animals fed in the light, in spite of the 20 hr food deprivation schedule.

Second, the dark-fed group drank more than the light-fed group (mean 24 hr intakes 26.6 ml and 19.9 ml, respectively). This difference is mainly accounted for in the 4 hr feeding period and the 4 hr postprandial period.

*Anatomical Localization*

Histological verification of cannula placements was carried out on all animals. The range of coordinates for loci of cannula tips are as follows [27]: anterior posterior +1.0 to −0.8; lateral +1.4 to +3.0; and horizontal −7.0 to −9.4. The histological data show that within the anterior posterior plane there was a differentiation of the sites for facilitation and depression of feeding. The best placements for facilitation of feeding in the dark were mainly concentrated posterior to bregma, extending from +0.4 to −0.8. Depression of feeding by animals fed in the light were mainly concentrated anterior to bregma, extending from bregma itself to +1.0. The extent of cannula damage is shown in Fig. 1.

## DISCUSSION

The significant difference between food intake of light-

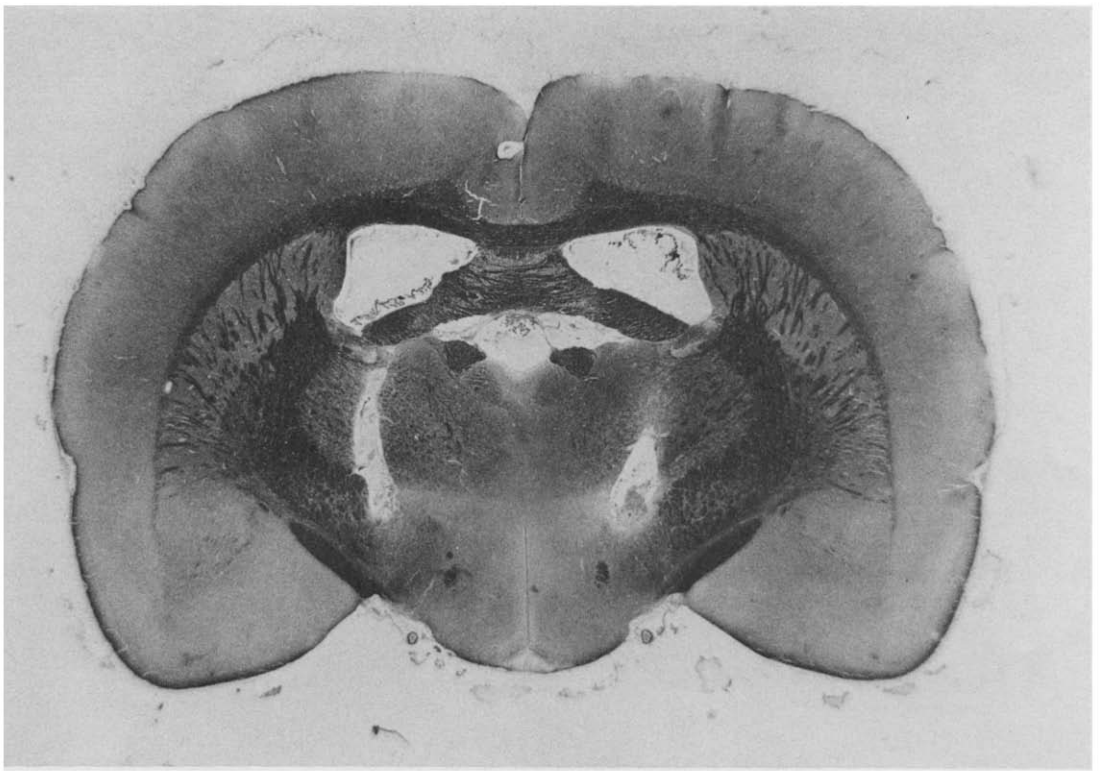

FIG. 1. Typical cannula track.

fed and dark-fed groups, as well as the significant difference between states of high and low hunger were to be expected. Rats ate 80% (dark) and 78% (light) of their total 24 hr food intake within the first 3 hr of the 4 hr feeding period. Rats ate more under dark conditions (mean = 16.4 g) than light conditions (mean = 11.6 g). Further, the nonsignificant interaction between the hunger and photoperiod variables showed that the differences between high and low states of hunger were not attributable to the light/dark conditions, and vice-versa.

While the placebo/drug effect was nonsignificant there was a significant photoperiod and drug administration interaction. In comparison to placebo, norepinephrine depressed feeding in the light and potentiated feeding in the dark; thus, the overall drug/placebo means showed no significant differences, as can be seen in Table 1. The difference between high and low hunger states was large in comparison to the placebo/drug difference.

It is possible to extrapolate certain trends as to the direction of drug induced change from the mean values and from number of rats exhibiting the change. As far as can be ascertained, the facilitation of eating by intrahypothalamic norepinephrine administered in the dark to hungry animals has not been reported before. Twelve out of 17 animals showed this increase under high hunger conditions and the same number under low hunger. While comparison of means shows that this facilitation was not as striking under high hunger as low hunger, the main point is that norepinephrine was clearly having the opposite effect under hunger in the dark as compared to light.

Norepinephrine depressed feeding in the light; 14 out of 17 animals showed this depression in a state of high hunger, while 8 out of 17 showed this in a state of low hunger. Thus, the depression of feeding in the light is mainly attributable to the condition of high hunger, which supports previous findings [6]. On the basis of previous work one would have expected an increase in eating behavior under low hunger in the light [4, 13, 25, 30], but this was not found in the present study. It is possible to question the operational definition of low hunger in this experiment in comparison to the ad lib feeding conditions used by other investigators [4]. However, any criticism of the operational definition must explain why rats under low hunger conditions in the dark increased their food intake under norepinephrine administration.

It may be that any testing carried out in the light classifying rats into eaters and noneaters involves experimenter bias, for the present results indicate that testing in the dark under low hunger elicits a greater occurrence of increased food consumption than in the light.

The results of the present study lend no support to the norepinephrine satiety hypothesis [23] but there are several methodological procedures employed which differ radically from those employed in the present study and it is possible that these differences may explain the conflicting findings. These include difference in dose of norepinephrine administered, form of norepinephrine (liquid versus crystalline), presentation of milk as food and procedure of milk presentation, which may involve a novelty effect.

The significant third order interaction which was found is difficult to interpretet beyond the prediction that a rat's eating response under placebo or drug varies with its level

of hunger, which in turn varies with the environmental condition of light and darkness.

Norepinephrine reduced water intake in all cases, when compared to saline injections. This confirms and extends previous findings [15,30]. There were no significant interactions found between photoperiod or hunger variables on water intake under drug administrative conditions. While the experiment was not specifically designed to answer questions on water intake per se, (the animals being under ad lib water conditions), because water and food consumption are known to be linked [11], one might have expected some reciprocity of drug action on water intake with the differences in food intake found under photoperiod, and hunger variables. This was not the case.

As might be expected, there was no significant effect on food-associated drinking between the two levels of hunger for on the 20 hr food deprivation schedule, water ad lib, water intake is fairly evenly distributed over the 4 hr feeding period.

The fact that norepinephrine always reduced water intake is again pertinent to the use of milk, with its liquid properties, as a food [23], and the previous finding that norepinephrine reduced milk intake in 4 out of 6 tests conducted at different times over the circadian feeding rhythm. The present results lend no support for previous reports that stimulation of a noradrenergic system increases [18,19] water intake, but support other contradictory findings [29].

In this study, it would seem that the condition of photoperiod per se is the dominant variable influencing the drug effect for the one dose of drug used. The effect on the eating response of intrahypothalamically administered norepinephrine is mainly dependent on the condition of light or darkness, whereas the effect of norepinephrine on food-associated drinking appears to be independent or photoperiod.

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