

# AMYGDALAR INJECTIONS OF VASOPRESSIN AND ITS ANTAGONIST DO NOT DISRUPT THE CIRCARDIAN RHYTHM OF FOOD AND WATER INTAKE IN THE RAT

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**Summary :** There is an indication that areas of the brain other than the suprachiasmatic nuclei (SCN), the known neural circadian pacemaker, are involved in the control of circadian rhythms. The present study investigated the role of amygdala in the circadian rhythms of food and water intake. Vasopressin and its antagonist  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  were injected into the amygdala bilaterally through chronically implanted stainless steel cannulae. The results of the study have shown that neither vasopressin nor its antagonist  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  alters the circadian rhythm of food and water intake thereby showing that vasopressinergic neurons/projections to amygdala are not involved in the control of circadian rhythms of food and water intake and amygdala is not likely to be an additional oscillator.

**Key words :** amygdala  
rhythm

vasopressin  
food intake

circadian  
water intake

## INTRODUCTION

The existence of some of entrained and free-running rhythms after the complete destruction of the suprachiasmatic nuclei (SCN) (4,20) suggests that areas other than SCN, a known neural pacemaker (10,21) may be responsible for the regulation of circadian rhythms. Moore-Ede *et al.* (11) suggested that the mammalian circadian system is made up of two pacemaker "X" and "Y" and one of them the "Y" pacemaker is located in the SCN and the other "X" pacemaker outside SCN. The anatomical location of the "X" pacemaker is not known with certainty at present.

Retinal projections which are a pre-requisite for the entrainment of circadian rhythms by way of providing photic information have been found not only in the SCN but also in other areas outside SCN (14, 18). The ventromedial hypothalamus (VMH) was suggested



as a circadian pacemaker on the basis that VMH lesions could eliminate (6,9) the entraining effects of a restricted feeding schedule. However, Inouye (7) could not identify a self sustained oscillator in VMH. It was also shown by Rietveld *et al.* (17) that bilateral lesions of VMH do not eliminate the circadian feeding rhythms.

This study was undertaken to investigate the role of amygdala in the regulation of the circadian rhythm of food and water intake. The selection of amygdala was primarily because of its known role in the regulation of food and water intake. The effect of lesions of amygdala on food and water intake varies with species and in rats aphagia and adipsia (22) to hyperphagia (3) have been reported. However, there is no report regarding the role of amygdala in the control of the circadian rhythm of food and water intake inspite of the above mentioned role of amygdala in the ingestive behaviour. Another equally important reason for selecting amygdala is the presence of Vasopressinergic neurons in the amygdala (2) and to and fro projections from SCN to amygdala (12,13).

#### MATERIAL AND METHODS

Male rats of the Wistar strain within a weight range of 200-250 g were used in this study. The animal room had a constant temperature of  $23 \pm 1^\circ\text{C}$ . Each animal was kept in a separate cage and was maintained under a Light Dark (LD) cycle of 12:12. All animals had a habituation period of 7 days at the end of which the experimental procedure started. Animals were provided with food in the form of pellets and tap water *ad libitum*.

For injection purposes stainless steel cannulae were implanted into the bilateral amygdala (Portions of the medial amygdaloid nuclei) using the following co-ordinates. AP 1,8 mm from bregma, L 3.5 mm and V 8.3 mm from dura (8). The animals which underwent the surgical operation were allowed to recover for at least 5 days after returning them to their cages.

Measured quantity of food and water were presented to the animals during the period of observation. The food and water were given at the beginning of each light and dark phase and the food and water consumption was calculated by weighing the remainder at the end of each phase.

The animals were divided into three groups for injection purposes. One group of animals ( $n=6$ ) had injections of vasopressin ( $30 \text{ ng}/\mu\text{l}$ , Sigma Chemical Company, U.S.A.) while the other group ( $n=6$ ) had injection of vasopressin antagonist  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ . The third group ( $n=5$ ) had injection of 0.9% saline and served as controls. The quantity injected in each case was  $0.5 \mu\text{l}$  on either side (total  $1 \mu\text{l}$ ). For injection, an injector cannula of 27 gauge connected to a  $2 \mu\text{l}$  syringe by way of a polyethylene tube of about 50 cm in



length was used. This was introduced into the guide cannula at the time of injection. Details of the procedure is given elsewhere (16). Any morphological and/or chemical changes that may have occurred at the site of injection due to repeated injection is avoided by each animal having only one injection (19).

Histological verification of the site of injection was carried out using ferric chloride injection (an equivalent amount,  $1 \mu l$ ) followed by intracardiac perfusion first with saline and subsequently with potassium ferrocyanide in formal saline. The sections were cut and identified. (Fig. 1). Histological examination showed that injections were made into the portions of the medial amygdaloid nuclei.

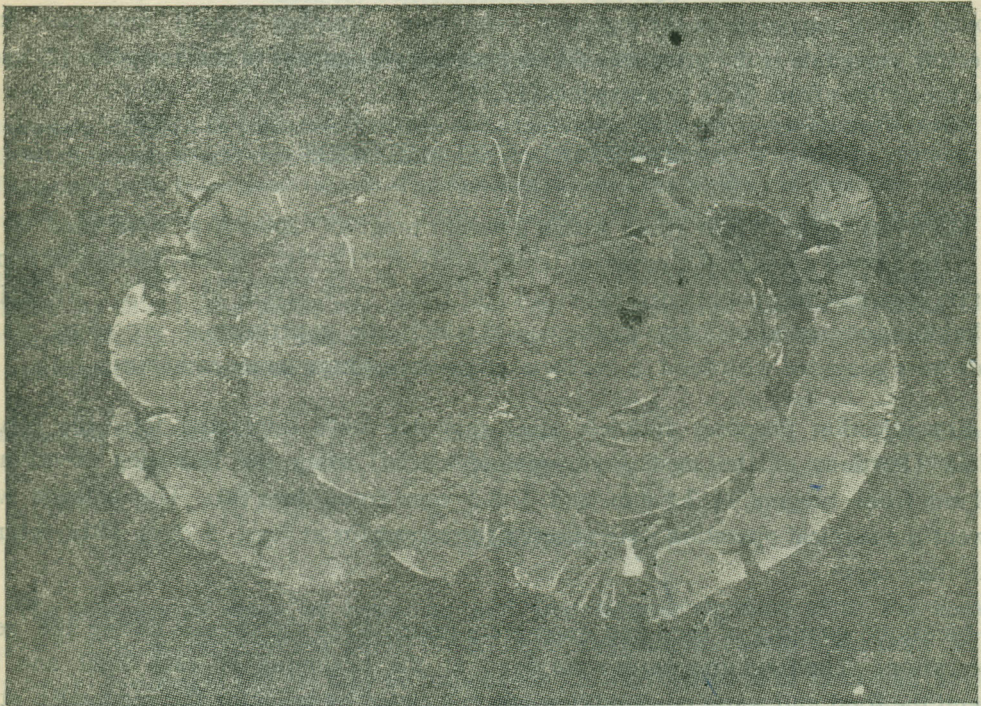


Fig. 1 : Coronal section of the rat brain. Arrows indicate the bilateral areas of the amygdala where injection was made.

## RESULTS

The results of the present study which was aimed to investigate the role of amygdala in the control of the circadian rhythm of food and water intake showed that injection of AVP or its antagonist  $d(CH_2)_5Tyr(Me)AVP$  is without any effect on food and water intake. The



TABLE I : Effect of bilateral injection of AVP, AVP antagonist-d(CH<sub>2</sub>)<sub>5</sub> Tyr(Me)AVP and saline injection on the circadian rhythm of food 9 in *gms* and water (in *ml*) intake.

Substance injected	Phase of injection	Food intake		Before injection water intake		Food intake		After injection Water intake	
		L	D	L	D	L	D	L	D
AVP (30 ng $\mu$ l)	L	3.8 $\pm$ 0.3	9.1 $\pm$ 0.2	12 $\pm$ 0.5	32 $\pm$ 0.1	3.6 $\pm$ 0.1	9.2 $\pm$ 0.1	12.2 $\pm$ 0.6	31.7 $\pm$ 1.4
AVP antagonist (2 $\mu$ g/ $\mu$ l)	L	4.4 $\pm$ 0.2	9.8 $\pm$ 0.2	12.8 $\pm$ 0.8	28.8 $\pm$ 1.4	4.0 $\pm$ 0.1	9.5 $\pm$ 0.1	11.3 $\pm$ 0.4	31.1 $\pm$ 1.0
Saline	L	3.2 $\pm$ 0.1	9.6 $\pm$ 0.1	12.0 $\pm$ 0.4	33.4 $\pm$ 0.9	3.4 $\pm$ 0.1	9.5 $\pm$ 0.1	12.5 $\pm$ 0.1	34.8 $\pm$ 0.5

Values are mean  $\pm$  SEM. L—Light, D—Dark



pre injection food intake ratio of  $3.8 \pm 0.2$  g ( $29.1\% \pm 1.5$ ) in the light and  $9.1 \pm 0.2$  g ( $70.9\% \pm 1.5$ ) in the dark phase did not alter significantly after either AVP or its antagonist injection. The circadian pattern of food intake also did not show any delayed disruption. The water intake also followed the same line as that of food intake and maintained its circadian pattern after the injection of AVP and its antagonist. The pre, post injection values of food and water intake expressed in g and ml respectively is given in Table I. Saline injection into the same area of the amygdala also did not produce any disruption. The total daily food intake in each case remained same. It was same with the water intake as well.

## DISCUSSION

The efferent vasopressinergic projections of SCN with amygdala (5,15) and the suggestion that vasopressin may act as a neurotransmitter in SCN and/or in its efferent projections (23) indicated the possibility that injections of AVP and its antagonist into the amygdala may produce alterations in the circadian rhythm if amygdala has any role. In a recent report from this laboratory (16) it was shown that the  $V_1$  receptor antagonist of AVP  $d(CH_2)_5Tyr(Me)AVP$  could disrupt the circadian rhythm of food intake on suprachiasmatic injection, while vasopressin itself failed to produce any modification of the circadian rhythm of food intake on injection. Differential effect of severing of efferent connections of SCN and different efferent connections controlling different circadian rhythms have been reported (1,15). However, the results of the present study has ruled out any role of amygdala by way of its vasopressinergic mechanism in the control of the circadian rhythm of food and water intake. It is also worth noting that studies by Inouye (7) also failed to locate a self sustained oscillator in VMH in spite of the report that VMH lesions could abolish the entraining effects of a restricted feeding schedule. Thus the present study appears to be an instance in which a brain region other than SCN, but having connections with SCN that too by way of vasopressinergic projections showing no definite role in the regulation of circadian rhythm-circadian rhythm of food and water intake.

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