ORIGINAL ARTICLE

Noradrenergic Neuromodulation of Septal Circuitry Mediating Hoarding Behaviour in Male Wistar Rats

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Abstract:

Background: Ingestive appetitive behaviour has been less delved into in research. Considering the connection of food hoarding with energy balance, unperplexing its exact neurophysiological mechanism will help in combating obesity. With evidence of noradrenaline as a neuromodulator in the septum, it may act on the septal circuitry to influence hoarding behaviour. *Aim and Objectives:* To evaluate the role of noradrenaline as a septal neuromodulator in the hoarding behaviour of laboratory rats. *Material and Methods:* Adult male Wistar rats (n=20) were employed in this study. Using stereotaxic techniques, noradrenaline was injected in the septum of the experimental group (n=10), and 0.9% normal saline was injected in the septum of the control group (n=10). Hoarding scores before and after injecting the chemicals were recorded. *Results:* It was found that noradrenaline significantly decreased ($p < 0.01$) the food hoarding scores (7.75 ± 3.95) as compared to baseline hoarding scores (24.12 ± 10.69). The control group did not show any significant change when the hoarding scores were compared before and after the injection of 0.9% normal saline. *Conclusion:* Intraseptal noradrenaline modulates septal network activity with inhibitory effect on hoarding behaviour.

Keywords: Noradrenaline, Appetitive Ingestive Behaviour, Obesity, Septal Region

Introduction:

Animals may show morphological, physiological or behavioural adaptation in order to evolve by natural selection. The adaptive approaches used may be migrating, gaining weight, hibernating, storing more fat and hoarding behaviour [1-2]. The food-hoarding paradigm is a fascinating model to deal myriad of interdisciplinary questions; attempting an integration of ecology, psychology, physiology and neurobiology within the purview of evolution. Breakthrough research in neurobiology has well-equipped us to dive into the mechanisms regulating such behaviours [3]. Over many years, hoarding has been studied experimentally, mostly in chipmunks, gerbils, hamsters, rats, mice, etc [4-8].

Food hoarding comes under the dominion of ingestive appetitive behaviour [9]. Though Craig, an animal behaviourist, constructed the labels “appetitive” and “consummatory” in 1917; Sherrington, in 1906, had first clearly articulated the behavioural differentiation in the appetitive/ consummatory nomenclature [10]. Appetitive behaviours depict the more variable, searching period of a behavioural sequence, whereas consummatory behaviours represent the stereotypic activities resulting in the termination of a behavioural sequence [11]. Experimentally, depiction by decerebrate rats of intact neuro-endocrine regulation of intraoral food intake but with an inability to express any appetitive behaviours [12], illustrates that the appetitive and consummatory phases of ingestive behaviour operate separately. These two phases are induced by different environmental settings through different neuroendocrine factors playing on separate neural substrates [9-10]. However, the appetitive aspect, though conferring huge scope for exploration, has not managed to grab much attention in ingestive behaviour research [13].

Obesity rates are shooting up worldwide. Probing into the neuroendocrine regulation of fundamental appetitive ingestive behaviours like food hoarding will help shed light on the modus operandi used by animals to maintain energy balance in their native milieus [9, 14]. The direction of 'energy flux' is thought to be a unifying element in the control of food hoarding [13]. Research in this direction might also give rise to evincible hypotheses in order to combat the obesity epidemic.

Noradrenergic afferents from different stem structures (alongwith afferents of other biochemical nature like serotoninergic and cholinergic fibers) arrive at the septum along the medial anterior fascicle of the spinal cord, thus rendering a neuroanatomical substrate for a septal noradrenergic pathway [15-16]. Though evidence hints that the central action of noradrenaline is inhibitory [17-18], it has also been put forth that the central nervous system comprises of alpha as well as beta receptors causing excitatory and inhibitory effects, respectively [19-20]. The apparent inconsistencies in between the different studies need to be resolved [20]. Although studies have evinced morphological and physiological basis for the action of noradrenaline as a modulator in the septum, its actual mechanism of action still needs to be enravelled [15, 21]. In view of this background, this study was carried out in laboratory rats hypothesizing that the septal circuitry governs food hoarding through the noradrenergic system.

Material and Methods:

Twenty male adult Wistar rats weighing about 240 to 250 g were involved in the experiment. Institutional Ethics Committee approval for the animal housing conditions and experimental procedures was obtained. They were lodged in separate home cages which measured 2 feet × 2 feet × 1 feet. The food intake of all the rats was recorded for three successive days. Once the basal food intake was recorded, the rats were then put on a restricted food intake for about 8-10 days in order to reduce their body weight by 15-20% of the basal readings. Water was given for 24 hours. This was executed to achieve an optimal hoarding score [22]. After a span of 10 days, their weights were rerecorded and the hoarding test was implemented. The animals were trained with the feed placed close to the cage initially, and later increasing the distance between the home and the feed gradually to one and a half meters. Food pellets of 5 g each were kept outside the home cage within an open maize situation. The animals were let free access to food for half an hour, and water ad libitum. This test was conducted in the morning hours between 9:00 a.m. to 9:30 a.m. The number of pellets eaten was considered as their food intake. The hoarding score was taken as the number of food pellets stored inside the cage within the half an hour duration of free access. This was repeated for initial few days until a stable hoarding score was accomplished by the animals. This was followed by implementation of steriotaxy in the animals for intraseptal implantation of cannula made up of 18 G needle and a length of 1 cm.

Cannulation procedure was done under phenobarbitone anaesthesia, with phenobarbitone used in the dose of 35 mg/kg body weight for intraperitonial injection. With the animal anaesthetized, it was properly assembled in position with steriotaxy. A midline incision was taken on the head in order to expose the bregma. Extending the incision anteriorly and posteriorly, the bones were cleared away to uncover the bregma along with the surrounding area with the help of a scalpel. Then employing steriotaxy and taking bregma as 'O' point, markings were made. The septal site, which is 0.2 mm anterior and 0.5 mm lateral in relation to the bregma, was widened using a blunt instrument in order to allow a cannula pass through the bones with ease. Then holding the cannula in position, it was inserted into the brain upto a depth of 5 mm. Fixing the cannula in position using dental cement, a stellet was inserted into the cannula for avoiding any block. Antibiotic (Neosporin) powder was then applied at the incision site and the animal was replaced in its home cage for 5 days. In these five days, only 5 g food was supplied with water *ad libitum.*

The animals were then grouped into two as follows:

Group I: Experimental group for intraseptal injection of noradrenaline (n=10)

Group II: Control group for intraseptal injection of 0.9% normal saline (n=10)

Noradrenaline was given as a dose of 2 µg of the drug dissolved in 2 µl of 0.9% normal saline. (1 mg of the drug dissolved in 1 ml of 0.9% normal saline gives 1µg of drug in 1 µL of 0.9% saline).

The stellet from the cannula was taken out and the animal was held in position. The drug noradrenaline was taken into the "Hamilton's Micro Syringe" upto 2 µl mark, and injected in the first group. Then inserting the syringe tip into the cannula, the drug was injected slowly over a span of one minute. After this, the syringe and the animal were held in position for another one minute. In the control group, 2 µl of 0.90% saline was injected in the same way as for noradrenaline. The animals were again lodged in their respective homes. All the rats were subjected to hoarding tests four days after the cannulating procedure and the readings were noted down for five days thereafter. The mean of these five readings was considered as the hoarding score postoperatively.

In the end, each of these rats was sacrificed with ether. With the animal anaesthetized, 2µl of 1% ferric chloride was injected intraseptally. Opening the thorax, the tip of the needle attached to perfusion set of 0.9% normal saline bottle was put into the cavity of left ventricle and the drip was set in. Another needle was inserted into the cavity of right ventricle to drain its content until clear effluent fluid was seen. After this, 10% normal saline perfusion was done. Then the animal's head was removed and the skin was reflected. Opening the skull, the brain was removed out and kept in 10% normal saline for hardening. Later, it underwent histological examination by Prussian blue reaction and the localisation of the tip of cannula was confirmed.

After summarising the data in terms of mean and standard deviation, the effect on hoarding behaviour before and after injection of noradrenaline and normal saline was compared by applying paired 't' test, considering the effects significant when p was less than 0.01.

Table 1: Effect of Septal Noradrenaline on Hoarding Behaviour in the Rats (Group I/ Experimental)

Score before injection of noradrenaline (Mean ± SD)	Score after injection of noradrenaline (Mean ± S.D)	*P*
24.12 ± 10.69	7.75 ± 3.95	< 0.01*

Table 2: Effect of Septal Normal Saline (0.9%) on Hoarding Behaviour in the Rats (Group II/ Control)

Score before injection of normal saline (Mean ± SD)	Score after injection of normal saline (Mean ± SD)	*P*
25.7 ± 14.59	23.1 ± 13.05	> 0.05[NS]

*NS- Not significant, *- significant at p<0.01*

Results:

The effect of septally administered noradrenaline on food hoarding behaviour in rats was studied in group no.1 (n=10, Table 1). The results showed that noradrenaline decreases the food hoarding score as compared to hoarding score before injecting noradrenaline. In the control group (n=10, Table 2), injection of 0.9% normal saline doesn't show any significant change in hoarding score as compared to previous score obtained before the saline injection.

Discussion:

This study was planned to investigate whether intraseptal noradrenaline alters the food-hoarding behaviour. Our study results showed that the hoarding score after intraseptal injection of noradrenaline significantly decreased (p <0.01) as compared to the baseline scores (before injecting noradrenaline); but there was no significant change in the hoarding scores before and after injection of normal saline. This reflects that the alteration in the hoarding score was not due to trauma induced while cannulating the septal region, but because of the injected noradrenaline. The weight and the food intake showed no significant difference in between the two groups, hence inferring that these two factors are unaccountable for the difference in the hoarding scores seen in between the groups.

A noradrenaline-embodied fibre system emanates in the mammalian brainstem, and culminates in all the areas of the forebrain [23-24]. The report by Arnold *et al*. heralded interest in the catecholamine system. The findings of this study revealed that both Reserpine, a catecholamine depleter, and 6-hydroxydopamine (6 OHDA), an agent selectively damaging catecholaminergic fibers, highly facilitated amygdala kindling. Besides, catecholaminergic circuitry seemed to act in antagonism to cholinergic circuitry [25].

In the study model used by Zhadina *et al*. to investigate the action of noradrenaline on the septal neurons using in vitro incubated slices of the guinea pig's brain, all extrastructural influences were eliminated. Here, it was found that in response to noradrenaline, septal neurons with non-regulatory activity became highly reactive with predominant activation effects; whereas those cells with regulatory activity showed comparatively less reactivity but with modulation of the endogenous pacemaker mechanism [15].

The feeding-hoarding interlinkage gets disrupted by bilateral lesions of lateral septal nuclei, as learnt from the finding that the rats could maintain their food intake and body weight but not food hoarding as previous. The lateral septal nuclei may possibly govern hoarding behaviour through their links with hypothalamic nuclei [26]. A few from the lot of topographically arranged septal inputs to mammillary bodies, have been proposed to synapse with the hypothalamic neurons while travelling through medial forebrain bundle [26-27]. Also, noradrenaline terminals have been reported to be in direct contact with septohippocampal neurons [28].

Noradrenaline has excitatory actions on septohippocampal GABAergic neurons which by virtue of local connections and hippocampal projections would impact both septal and hippocampal functioning [21]. The norepinephrine-mediated inhibition, accompanied with GABA-induced inhibitory effects at the soma and in the dendrites, has been shown to bring about discrete modulation of the working of hippocampal pyramidal cells. The noradrenergic inhibition results in decreased spontaneous activity, decreased outcome of slowly increasing excitation, while letting only brief excitations to pass substantially unchanged [20]. In vivo, iontophoretically applied norepinephrine was found to cause inhibition of hippocampal pyramidal firing [29-30]. Also, it has been put forward that the primary impact of noradrenaline may not be excitation or inhibition by itself, but preferably to alter the processing of synaptic input by the hippocampus. Like in the cerebellum, where noradrenaline has exhibited to cause an increase in both inhibitory and excitatory synaptic inputs while decreasing Purkinje cell spontaneous discharge; it may play its role as a 'neuromodulator' in the rat hippocampus as well. This neuromodulatory mechanism maybe by way of membrane hyperpolarization and decreasing membrane conductance [31-32].

In the central nervous system, noradrenaline may predominantly act to create a bias altering post synaptic response to conventional transmitter systems, which themselves are likely to be more directly involved with elaborate information transfer [32].

Overall, this can explain our finding of decreased food hoarding score after intraseptal injection of noradrenaline. Though there is supportive evidence indicative of the inhibitory role of the noradrenergic system acting on the septal circuitry via the hippocampal-septum-hypothalamic complex in the regulation of food hoarding, the central amalgamation of various inputs modulating hoarding behaviour needs to be worked out further to give a clearer picture.

Conclusion:

Intraseptal noradrenaline decreases food hoarding, indicating that noradrenaline has an inhibitory influence on the septal circuitry mediating hoarding behaviour.

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