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Original article:

Septal cholinergic neuromodulation of hoarding behaviour in male wistar rats

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ABSTRACT:

Background: Food hoarding, falling under the appetitive realm of ingestive behaviour, has been less researched. With food hoarding holding clinical implication in idiopathic human obesity, researching the root mechanisms underlying food hoarding is crucial. The present study aimed to investigate the role of acetylcholine as a septal neuromodulator in the hoarding behaviour of laboratory rats.

Material and Methods: Using stereotaxy in 20 male adult Wistar rats, acetylcholine was instilled in the septal region of the experimental group (n=10) and 0.9% normal saline was instilled in the septal region of the control group (n=10). The hoarding scores before and after instillation of the chemicals were compared.

Results: The results were that acetylcholine significantly increased ($p < 0.001$) the food hoarding scores (20.4 ± 7.1) as compared to the baseline hoarding scores (36.8 ± 6.49). The control group didn't show any significant change when the hoarding scores were compared before and after the injection of 0.9% normal saline.

Conclusion: This is indicative of intraseptal acetylcholine favourably impacting food hoarding behaviour.

Key words: septal, cholinergic, hoarding behavior, rats, neuromodulation.

Introduction:

Wallace Craig, the animal behaviorist, broadly divided behaviors into two sets of behavioural responses, namely appetitive and consummatory. The appetitive aspect deals with those behavioural responses which lead animals to come in contact with goal objects like food, water, mate, etc. The consummatory aspect consists of those behavioural responses involved in the concluding actions after the objects are obtained/cached, eg. eating/ drinking/ mating.[1] Food hoarding falls under the appetitive domain of ingestive behaviour. Food hoarding widely exists across animal taxa including humans. Humans do hoard food as seen from their actions of storing foodstuffs in refrigerators, freezers and storerooms. Hitherto food hoarding appears to be only an evolutionary selected trait.[2,3] Food hoarding in the laboratory was studied for the first time by Wolfe in the year 1939. He illustrated the quantifiable nature of this behaviour.[4] Being a common behaviour to many species of rodents, hoarding has been studied experimentally in hamsters, gerbils, mice, rats, etc. [5-9]

Most of the research on ingestive behaviour has stressed dominantly on the consummatory facet such as the amount of food eaten in response to treatment with various hormones, neuropeptides, and metabolic conditions. On the other hand, research dealing with the appetitive nature, such as the approach to food, the feeding upon unpalatable substances, or foraging and hoarding is still in its infancy.[10,11] With this background, presently, it is difficult to peculiarize the central/ peripheral factors that dictate the control of food hoarding.[2]

It has been previously noted that laboratory rats are not natural hoarders.[12,13] Yet, the food carrying by laboratory rats from the site of the located food to a secure place to eat, indeed claims some shared mechanisms with those mechanisms underlying natural food hoarding by species (generally animals enabled with cheek pouches that allow considerable quantities of food fetching towards home) where this behaviour is a noteworthy part of their ingestive behavioural pattern.[2]

Considering the clinical relevance of food hoarding in idiopathic human obesity, studying the basic mechanisms which underlie food hoarding (storing) in humans could help give deeper insights for pharmaceutical or behavioural interventions in both treating and preventing obesity.[2] Though extensive efforts have been put into translating hoarding behavior in animal models, yet a comprehensive model is unidentified.[14] Acetylcholine is one of the various neuromodulatory substances which reconfigure neuronal circuits underlying the mechanism of behaviour.[15,16] To the best of our knowledge, the neuromodulatory activity of acetylcholine influencing hoarding behaviour has not been broadly investigated in animal models. Hence, the present study was undertaken with an aim to explore the role of acetylcholine as a septal neuromodulator in the hoarding behaviour of laboratory rats.

Materials and methods:

Approval of the institutional animal ethics committee for the animal housing conditions and experimental procedures was obtained. 20 male adult Wistar rats weighing about 240 to 250 grams were included in the study. They were placed in separate home cages measuring 2 feet x 2 feet x 1 feet. Their food intake was recorded for three successive days. After noting down their basal food intake, the rats were subjected to a restricted food intake for a period of 8-10 days till their body weight decreased by 15-20 % of the basal readings. Only water was given for 24 hours. This was done to achieve a measurable hoarding score.[17]

After 10 days, their weights were recorded and the hoarding test was carried out. For the first few days, training was given to the animals with food pellets kept near the cage initially and later on gradually increasing the distance between the home cage and food pellets to 1.5 meters. Food pellets (weighing 5 grams each) were placed outside the home cage in an open maize setting, allowing the animals to have free access to it for 30 minutes. Only water was provided for 24 hours. This test was carried out in the morning between 9:00 a.m. to 9:30 a.m. Food intake was noted down as the number of pellets consumed. The hoarding score was calculated as the number of pellets hoarded inside the cage over a span of those 30 minutes of free access. This was continued for a few days till a stable hoarding score was achieved by the animals. After this, intraseptal implantation of cannula made up of 18 G needle about 1 cm in length was done in the animals with the help of steriotaxic techniques.

Cannulation procedure:

Cannulation procedure was carried out under phenobarbitone anaesthesia, using phenobarbitone in the dose of 35 mg/kg body weight for intraperitonial injection. When the animal was under anaesthesia, it was placed in position and properly assembled with steriotaxy. An incision was taken on the head in the midline so as to expose the bregma. The incision was extended to some extent anteriorly and posteriorly, and the bones were cleared to expose the bregma and the surrounding area with scalpel. Then with the help of steriotaxy, markings were made considering bregma as ‘O’ point. The septal site, that is 0.2 mm anterior and 0.5 mm lateral to the bregma was widened with a blunt instrument so that the cannula could pass easily through the bones. With the cannula held in position, it was inserted into the brain substance upto a depth of 5 mm. Then the cannula was fixed in position with the help of dental cement and stellet was inserted into the cannula so as to avoid blockage. This was followed by application of antibiotic (Neosporin) powder at the site of incision and the animal was kept in its home for 4-5 days. Only 5 gm food was given during these five days and water for 24 hours.

Then the animals were divided into two groups as:

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

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

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

Injection Procedure:

Acetylcholine was injected in the first group. Before injecting, stellet from the cannula was removed and the animal was held in position. The drug was taken into the "Hamilton's Micro Syringe" upto 2 µl mark. Then the tip of the syringe was inserted into the cannula and the drug was injected slowly over a period of 60 seconds. After injecting the drug, the syringe and the animal were held in position for another 60 seconds. In the control group (group no.2), 2µl of 0.90% saline was injected in the same way as above. Later, all the animals were replaced in their respective home cages. All were subjected to hoarding tests four days after the cannulating procedure and the readings were obtained for five days thereafter. The mean of these five readings was taken as the hoarding score postoperatively.

At the end of experiment, each of the septally cannulated rats was sacrificed using ether. When the animal was anaesthetized, 2µl of 1% ferric chloride was injected intraseptally. The thorax was opened. Tip of the needle attached to perfusion set of 0.9% normal saline bottle was introduced into the cavity of left ventricle and the drip was started. Another needle was put in the cavity of right ventricle to drain its content till the effluent fluid was clear. This was followed by 10% formal saline perfusion. After this, head of the animal was removed, skin was reflected and the skull was opened. The brain was taken out and kept out in 10% formal saline for hardening and later subjected to histological examination by Prussian blue reaction.

Histology:

1. Fixation:

This helps in hardening the organ so that the sectioning and staining is without distortion of tissue and is easier. The brain was cut into 10-mm blocks and processed further.

a) First the brain was washed under tap water for 1 hour.
b) Then kept in 50% alcohol for 2 hours.
c) Then kept in 70% alcohol for 6 hours.
d) Following this, the brain was kept in 90% alcohol overnight.
e) On day 2, this brain was kept in absolute alcohol I for 2 hours.
f) Then in absolute alcohol II for 2 hours.
g) Then in absolute alcohol III for 2 hours.
h) Then in Toluene I for 2 hours.
i) Then in Toluene II for overnight.
j) On day 3, it was kept in paraffin wax I for 2 hrs at 52°C to 56° C.
k) Next it was changed to paraffin II for 2 hours at 52°C to 56° C.
l) And then paraffin III for 2 hrs at 52°C to 56° C. Thus there was progressive replacement of water in the tissue by alcohol (i.e. dehydration).

2. Sectioning of paraffin blocks:

Using rotatory microtome, brain sections were made having thickness of 10 mm.

3. Mounting of Sections:

The sections were mounted on egg albumin coated with the help of a glass rod. The slides were dried overnight.

4. Staining:

First the sections were passed through xylene to remove paraffin. Three changes were given with xylene; each change was of 1 hour. Then the sections were washed with distilled water and treated with N/10 HCl followed again by washing with distilled water and treatment with eosin for 2 to 3 minutes. When the ferric chloride is injected into brain, ferric ions are deposited at the site of cannulation. This interacts with HCl to form ferric chloride, ferric ferro cyanide (prussian blue color) and potassium chloride. Thus the site of cannulation becomes visible and this confirms the site of the lesion histologically.

Statistical Analysis: Data was summarized in terms of mean and standard deviation and the effect of chemicals on hoarding behaviour was compared by applying paired 't' test. The effects were considered significant when p was less than 0.01.

Results:

The effect of septally administered acetylcholine on food hoarding behaviour in rats was studied in group no.1 (n=10, table.no.1). The results show that acetylcholine increases the food hoarding score as compared to hoarding score before injecting the acetylcholine. In the control

group (n=10, table.no.2), injection of 0.9% normal saline doesn't show any significant change in hoarding score as compared to the previous score obtained before the saline injection.

Table.no.1 : Effect of septal acetylcholine on hoarding behaviour in the rats (group I/ experimental).

Score before injection of acetylcholine (Mean±S.D.)	Score after injection of acetylcholine (Mean±S.D.)	Result
20.4 ± 7.1	36.8 ± 6.49	$p < 0.001$

Table.no.2 : Effect of septal normal saline (0.9%) on hoarding behaviour in the rats (group II/ control).

Score before injection of normal saline (Mean±S.D.)	Score after injection of normal saline (Mean±S.D.)	Result
23.88 ± 14.79	24.44 ± 15.48	$p > 0.05$

Discussion:

The present study was planned to examine whether intraseptal acetylcholine alters the food-hoarding behaviour. It was seen that acetylcholine increased the food hoarding score in the experimental group; whereas there was no significant difference in the hoarding scores before and after instillation of normal saline in the control group. This indicates that the change in hoarding score was not because of trauma during cannulation in the septal region, but because of the injected acetylcholine. There was no significant difference in the weight and the food intake between the two groups, hence implying that these two factors aren't accountable for the differences in the hoarding scores of the groups.

It has been found that the septum contains cholinergic, GABAergic and glutamatergic neurons projecting to the hippocampus via the fimbria/fornix.[18-20] In previous studies, discrete electrolytic lesions of ventral hippocampus have shown decrease in hoarding and the reverse was noted with dorsal hippocampal lesions. This proposes a distinctive function of the hippocampus which maybe working through the amygdala and/or hypothalamus both of which either individually or conjunctly govern the feeding and hoarding behaviour in an influential manner.[21] However, on chemical manipulation, both atropine (anticholinergic) and haloperidol (dopamine antagonist) upon instillment in the dorsal hippocampus depicted a reduction in hoarding while apomorphine (dopamine agonist) in the same area showed an increase in the hoarding score.[22] Determining the firing profile of anatomically and histochemically delineated septohippocampal cholinergic neurons is required which will help throw light on the action of acetylcholine in the hippocampus pertaining to behavioral mechanism.[23]

In addition, it has also been reported that ibotenic acid-induced lesions of the nucleus accumbens in rats resulted into increased abnormal hoarding behavior whereas their consummatory behavior was as usual; implying that the neural circuits regulating appetitive and consummatory behaviors act in distinct ways atleast to a certain extent.[24,25]

Hypothalamic neurons in the arcuate nucleus take information regarding the nutritional state of the organism from the periphery and signal it to various parts of the central nervous system.[26] Electrical stimulation of the lateral hypothalamus creates enormous hoarding venture in satiated rats, as occurs when food is deprived for a long time.[27] Proopiomelanocortin (POMC) neurons, a subset of arcuate nucleus neurons belong to the cholinergic phenotype.[26] Additionally, the nucleus accumbens and the amygdala receive POMC projections.[28]

It has also been reported that lesions of substantia nigra abates hoarding.[29]

Inspite of extensive studies on the neuromodulator acetylcholine, detailed profile of acetylcholine release in the hippocampus is still speculative. Bioengineering and genetic research methods together with transgenic animals can mediate the experiments working towards precisely defining the functions of the diffuse cholinergic pathways involved in hippocampal dependent behaviours.[16] The central convergence and integration of all these inputs geared towards modulating hoarding behaviour is unclear. However, all these findings hint that one of the possible mechanisms neuromodulating the hoarding behaviour may be cholinergic.

Conclusion:

Intraseptal acetylcholine augments food hoarding behaviour, suggesting that acetylcholine acts positively in the septal region to influence neural mechanisms controlling hoarding behaviour.

Recommendations:

There is a huge scope for research owing to the need to study the effect of instillation of various chemicals influencing hoarding in different areas of the brain to achieve a comprehensive evaluation of the mechanism of hoarding behaviour. Such approach will facilitate in providing targets in an effort to treat or prevent obesity.

Study limitations: -

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References:

1. Craig W. Appetites and aversions as constituents of instincts. Biol Bull. 1918;34:91–107.
2. Bartness TJ, Keen-Rhinehart E, Dailey MJ, Teubner BJ. Neural and hormonal control of food hoarding. Am J Physiol Regul Integr Comp Physiol. 2011;301(3):R641–55.
3. Rowland NE, Vaughan CH, Mathes CM, Mitra A. Feeding behavior, obesity, and neuroeconomics. Physiol Behav. 2008;93(1-2):97–109.
4. Wolfe JB. An exploratory study of food-storing in rats. J Comp Psychol. 1939;28:97–108.
5. Wong R, Jones CH. A comparative analysis of feeding and hoarding in hamsters and gerbils. Behav Processes. 1985;11(3):301-8.
6. Nyby J, Wallace P, Owen K, Thiessen DD. An influence of hormones on hoarding behavior in the Mongolian gerbil (Meriones unguiculatus). Horm Behav. 1973;4:283-8.
7. Beigneux F, Lassalle JM, Le Pape G. Hoarding behavior of AKR, C57BL/6 mice and their F1 in their home living space: an automatic recording technique. Physiol Behav. 1980;24(6):1191-3.
8. Fantino M, Cabanac M. Effect of a cold ambient temperature on the rat's food hoarding behavior. Physiol Behav. 1984;32(2):183-90.
9. Herberg LJ, Blundell JE. Non-interaction of ventromedial and lateral hypothalamic mechanisms in the regulation of feeding and hoarding behaviour in the rat. Q J Exp Psychol. 1970;22(2):133-41.
10. Buckley CA, Schneider JE. Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters.Am J Physiol Regul Integr Comp Physiol. 2003;285(5):R1021-9.
11. Keen-Rhinehart E, Dailey MJ, Bartness T. Physiological mechanisms for food-hoarding motivation in animals. Philos Trans R Soc Lond B Biol Sci. 2010;365(1542):961–75.
12. Whishaw IQ, Whishaw GE. Conspecific aggression influences food carrying: studies on a wild population of Rattus norvegicus. AggressBehav. 1996;22:47-66.
13. Takahashi LK, Lore RK. Foraging and food hoarding of wild Rattus norvegicus in an urban environment. Behav Neural Biol. 1980;29:527-31.
14. Nikray N, Karimi I, Siavashhaghighi Z, Becker LA, Mofatteh MM. An effort toward molecular biology of food deprivation induced food hoarding in gonadectomized NMRI mouse model: focus on neural oxidative status. BMC Neurosci. 2018;19(1).
15. Marder E. Neuromodulation of neuronal circuits: back to the future. Neuron. 2012;76:1-11.
16. Teles-Grilo Ruivo LM, Mellor JR. Cholinergic modulation of hippocampal network function. Front Synaptic Neurosci. 2013;5:2.
17. Gogate MG, Salgar DC, Brid SV, Wingkar KC. Dissociation of feeding and hoarding after bilateral destruction of lateral septal nuclei in rats. Indian J Physiol Pharmacol. 1989;33(1):59-62.
18. Freund TF, Antal M. GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature. 1988;336:170-3.
19. Müller C, Remy S. Septo–hippocampal interaction. Cell Tissue Res. 2018;373:565–75.
20. Naumann T, Linke R, Frotscher M. Fine structure of rat septo-hippocampal neurons. I. Identification of septo-hippocampal projection neurons by retrograde tracing combined with electron microscopic immunocytochemistry and intracellular staining. J Comp Neurol. 1992;325:207-18.
21. Borker AS, Mascarenhas JF. Variations in hoarding behavior following regional hippocampal lesions in rats. Indian J Exp Biol. 1990;28(1):91-2.
22. Borker AS, Mascarenhas JF. Role of acetylcholine and dopamine in dorsal hippocampus on hoarding behavior in rats. Indian J Physiol Pharmacol. 1991;35(1):71–73.
23. Simon AP, Poindessous-Jazat F, Dutar P, Epelbaum J, Bassant MH. Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. J Neurosci. 2006;26(35):9038-46.
24. Whishaw IQ, Kornelser RA. Two types of motivation revealed by ibotenic acid nucleus accumbens lesions: dissociation of food carrying and hoarding and the role of primary and incentive motivation. Behav Brain Res. 1993;55:285–95.
25. Berridge KC. Motivation concepts in behavioral neuroscience. Physiol Behav. 2004;81:179–209.
26. Meister B, Gomuc B, Suarez E, Ishii Y, Durr K, and Gillberg L. Hypothalamic proopiomelanocortin (POMC) neurons have a cholinergic phenotype. Eur J Neurosci. 2006;24:2731-40.
27. Herberg LJ, Blundell JE. Lateral hypothalamus: hoarding behavior elicited by electrical stimulation. Science. 1967;155(3760):349-50.
28. Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. Nutr Metab (Lond). 2007;4:18.

29. Borker AS, Mascarenhas JF, Gogate MG. Hoarding deficits in rats with lesions of ventral hippocampus and substantia nigra. Ind J of Expt Biol. 1988; 26:53-5.

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