Original Article

Effect of bilateral lesions of nucleus accumbens on consummatory behaviour in Wistar rats

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Abstract

Background: Nucleus accumbens (NAcc) is known to be involved in reward and addictive functions. In the present study we tested parameters of ingestive activity and alcohol intake following lesions of nucleus accumbens in order to establish the role of this nucleus unequivocally.

Materials and methods: Male Wistar albino rats were divided into groups, and were subjected to Sham lesion and bilateral lesion of nucleus accumbens. The consumption of water, ethanol and food were recorded daily and their body weight was noted weekly, for one week before lesion and 3 weeks following lesion. In another set, two bottle free choice test was carried out between 10% alcohol and potable water, rest of the procedures were maintained same as for the other set.

Results: The lesioned rats showed increase in intake of alcohol (p < 0.01). There was no significant change in food intake or body weight. In two bottle free choice test, the rats consumed increased volume of fluid. There was highly increased water intake (p < 0.001). There was no significant change in their alcohol intake. The increase in water intake was significantly more than the increase seen in the alcohol intake in the group provided only alcohol.

Conclusions: Bilateral lesions in nucleus accumbens did not change the alcohol intake in two bottle choice test. But they showed increased water intake in two bottle choice suggesting increased thirst. The alcohol intake increases only when there is no water provided, suggesting the pivotal role of this centre in ingestive behaviour.

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1. Introduction

Addiction\(^1\) is a well-known social problem affecting large section of population worldwide. In USA as much as 9.2% of people aged above 12 years have either had or have one or other incidence of substance abuse.\(^2,3\) Nucleus accumbens (NAcc) situated deep in grey matter in the forebrain, is believed to have effects on the consumption of water and other ingestive activities.\(^4\) This nucleus is also involved in the mesolimbic reward circuit.\(^5\)–\(^7\) Accumbens also had been shown to have role in alcohol consumption. Bilateral stimulation of NAcc led to reduced alcohol intake in alcohol preferring rats.\(^8\) Both stimulation of core or shell part of NAcc was effective in reducing the intake of alcohol in the rats.\(^9\) Previously reported dopamine antagonist effects on the reinforcing effect of ethanol in rats\(^10\)–\(^12\) and by ethanol conditioned appetitive responses.\(^13\)–\(^15\) Intra-AcbSh dopamine antagonist was reported to reduce expression of Conditioned Place Preference (CPP) induced by an intra-cerebroventricular ethanol injection in rats.\(^16\) This is contradicted by other reports.\(^17\)

Addiction to other agents such as cocaine, were also affected by the NAcc. It was shown that the stimulation of NAcc attenuated the cocaine seeking behaviour.\(^18\) The available literature on the role of nucleus accumbens indicated a profound influence on addictive behaviour and reward.\(^19\) There appears to be separate circuits involved in the food reward and the addiction to drugs in the nucleus accumbens.\(^20,21\) The role of nucleus accumbens on control of ingestive behaviour is far from clear. Therefore, in the present study we attempted to elucidate the effect of large bilateral lesions of NAcc on parameters of feeding behaviour and voluntary alcohol consumption in rats.

2. Materials and methods

Wistar albino strain male rats (n = 28) were selected for this experiment (body weight 230 ± 30 g at the time of selection). They were housed in separate plastic cages in a temperature controlled laboratory, with normal day–night cycle. Food (rat feed pellets) and potable tap water were made available ad lib. Ethyl alcohol was provided to drink ad lib. as per the requirement to respective groups. The experiments were conducted in separate groups of animals. The animals were divided into 4 groups. Group 1 with 14 animals were again subdivided into Group 1a (n = 6) Sham lesioned control group and Group 1b (n = 8) was lesioned group. Similarly Group 2 was also subdivided into sham lesioned control group (Group 2a, n = 6) and lesioned group (Group 2b, n = 8). Two animals from each group were left out from the statistical analysis of data because in Group 1b death occurred after surgery, and in Group 2b, one animal died and another did not receive proper bilateral lesion which was detected by histological examination.

3. Experimental procedure

The rats were maintained for one week before the lesion, providing them with known quantity of food and fluids. Their water & food consumption were measured every day and noted. Measurements of intake of alcohol and food were done at 10.00 AM every day. Since rodents are known to be more active during night time, the measurements were taken in the morning. The alcohol bottle and food pellets were topped up after measurements. Body weight was noted at the end of the week. The rats were subjected to surgery under Ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) anaesthesia. The electrolytic lesion of NAcc was done by passing current of 2 mA for 20 s, bilaterally with Grass (USA) lesion maker, by inserting a stainless steel electrode insulated except the at the tip, using rat stereotaxic co-ordinates.\(^22\) The DC current was passed only in the ‘lesioned’ group, while the Sham lesioned group underwent all surgical procedures and no current was passed. The rats were given an injection of Penicillin and maintained for three weeks, meticulously measuring the parameters – measurements were done every day and body weight measured at the end of every week, leaving 2 days of recuperation period after the surgery.

At the end of each experiment, the animals were sacrificed by overdose of ether and transfused with formal saline and the brains were dissected out and preserved in formalin. Subsequently they were processed by dehydration and paraffin embedded brain was cut into sections of 5 microns. Histological examination was done by staining the sections with H&E to confirm the site of lesion. Only those animals receiving reasonably symmetrical bilateral lesion was accepted for statistical evaluation.

3.1. Experiment 1: alcohol consumption following lesion of NAcc

The rats were provided with 10% alcohol to drink, along with food. The pre lesion data collection was done for 7 days before

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gr Ia Sham lesion (n = 6)</th>
<th>Gr Ib lesion (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prelesion</td>
<td>Post lesion</td>
</tr>
<tr>
<td></td>
<td>7D</td>
<td>14D</td>
</tr>
<tr>
<td>Food</td>
<td>12.75 ± 0.30</td>
<td>12.25 ± 0.79</td>
</tr>
<tr>
<td>Alcohol</td>
<td>17.33 ± 0.95</td>
<td>18.33 ± 0.55</td>
</tr>
<tr>
<td>Body weight</td>
<td>252.66 ± 3.90</td>
<td>259.83 ± 4.67</td>
</tr>
</tbody>
</table>

Prelesion vs post lesion **p < 0.01.
the lesion. The post lesion data collection was carried out for 3 weeks after the recuperation period of two days following surgery.

### 3.2. Experiment II: water and alcohol consumption in two bottle free choice situation

Sham lesioned control rats and lesioned rats were tested for intake of 10% ethanol and water in two bottle free choice situation. Ethanol consumption, water consumption was measured and tabulated. Their food intake and body weight too were noted. All the measurements and surgical procedures were similar to that explained above. The results were analysed by Mann Whitney U test and Wilcoxon signed rank sum test, and \( p < 0.05 \) was accepted as significant variation. Ethical clearance was obtained from the institutional ethical committee for animal experiments and all the procedures were done by maintaining highest ethical standards for laboratory animals.

### 4. Results

The data were analyzed by applying Non parametric Mann Whitney ‘U’ test. Bilateral lesions of NAcc showed significant increase in alcohol intake in the post-operative period of week 1, week 2 and week 3 when compared to pre-operative period \( (p < 0.01) \). The consumption of alcohol in lesioned animals was significantly more when compared to sham lesioned control groups. There was no significant increase in food intake and body weight during post lesion period of three weeks when compared to the pre lesion period. There was a marginal decrease in body weight, which was not statistically significant (Table 1).

The results of this group showed that there was increased water intake following the lesion of NAcc \( (p < 0.01) \). But the intake of alcohol did not show any statistically significant difference. The total fluid intake increased. Food intake and body weight did not show any significant difference when compared to their prelesion levels.

### 5. Discussion

Reward and punishment were known to be two most important factors concerned with the process of cognition. Reward could be the basis of addiction.1 Frontal cortex and prefrontal areas were implicated in the decision making process.23,24 The overlap of decision making and associative learning caused addiction.25,26 Nucleus accumbens has been recognized as an important location for the circuitry controlling the addictive behaviour. It also is believed to have excitatory inputs from Amygdala facilitating reward seeking behaviour.20,27 In the present study we found that the intake of 10% alcohol increased in the lesioned rats (Table 1). But when the rats were tested with 2 bottle free choice with alcohol and water, then the rats showed increased preference towards water (Table 2), showed a highly significant increase in water consumption. A role for NAcc has been suggested in the alcohol induced behaviour.28 But the lesion of NAcc did not show a specific preference to alcohol. Even though there was increase in the intake of ethanol in the lesioned rats, when ethanol alone was provided to drink, the increase was not as great as the increase seen in intake of water in a two bottle choice test. Therefore such an increase was probably due to increase in the desire to drink more fluid, which is a thirst response. Earlier documented reports also suggested that NAcc neuronal populations will be modulated by the inputs from other structures such as Ventral tegmental area (VTA).22,31 Therefore it can be concluded that the lesion effect of NAcc could be predominantly be effective on the quantity of fluid intake rather than alcohol intake per se. Role of other neuronal circuitry which could be involved in the concerned circuitry of addiction must be investigated to reveal the interrelationships among the centres.

### Conflicts of interest

All authors have none to declare.

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