Evaluation of Weight, Caloric Intake, and Behavior of Rats After Bilateral Ablation of the Nucleus Accumbens Shell

ABSTRACT

Introduction: Deep brain stimulation and ablation of certain regions of the brain are being widely used in research aiming to link some region of the cortex with certain psychiatric disorders. The nucleus accumbens, for example, belonging to the basal ganglia, responsible for modulating the reward system and limbic-motor integration, according to studies, is related to the pathophysiology of alterations in neurotransmitters and neuronal connections in anorexia nervosa. Research in animals and humans reinforces this theory. Objective: To evaluate whether even in healthy rats, it is possible to observe changes in eating patterns and behavior that would justify such relationship. Materials and methods: A total of 24 female Wistar rats were randomly divided into experimental group (n=20) and control group (n=4). The rats in the experimental group underwent surgery with bilateral ablation of the nucleus accumbens shell using a stereotactic-guided radiofrequency needle. After surgery, the rats had their weights and caloric intake measured daily. Their behavior was empirically observed and then compared with that exhibited by the control group. Results: Regarding behavioral changes, it was observed an exaggerated increase in grooming, sniffing, searching/exploration, and avoidance/escape. Aggressiveness was present in only one animal of the experimental group. Regarding weight gain and caloric intake, a statistically significant relationship was found between them in relation to the time after the surgical intervention, when comparing the experimental and control groups. Discussion/Conclusion: In this study we investigated whether even in healthy rats, performing an inhibition – ablation of the Nucleus Accumbens – would cause an increase in caloric intake and consequently weight of the rats, and whether the procedure would cause changes in their behavior. From the results obtained we can infer that such assumption is true. Moreover, other research, both in humans and animals, support the data and the connection of the Nucleus Accumbens given by the reward system, to psychiatric disorders, in this case, anorexia nervosa. More investigations are necessary to further elucidate such interactions and consequences.

Keywords: Anorexia nervosa; Brain atlas; Brain lesions; Psychosurgery; Stereotactic surgery
Ablation or deep brain stimulation (DBS) are highly investigated techniques for their therapeutic use in psychiatric disorders, possibly being a new practice in refractory cases.\(^1\)

Nucleus Accumbens (NAcc), similarly, is one of the most highly researched brain structures, theorizing and having tested its functions in psychiatric disorders, mainly Anorexia Nervosa (AN), in animal models.\(^2,3\) A significantly advanced knowledge, allowed open trials in humans.\(^4,5\) NAcc, responsible for the reward system,\(^6\) is part of the basal ganglia\(^7,8\) and acts as an interface of the limbic and the motor system.\(^9,10\) It is divided into two regions, due to histochemical and neuronal pathway differences. It receives glutamatergic afferences from prefrontal cortex, amygdala, thalamus, hippocampus, and dopaminergic afferences from ventral tegmental area and substantia nigra; and, GABAergic afferences differs as the sub-region of origin, the core projects to extrapyramidal system, and the shell to limbic structures, mainly lateral hypothalamus.\(^11–16\)

Regarding AN, studies have shown that there is increased neuronal activation in the NAcc region with an imbalance in the secretion of neurotransmitters, especially serotonin and dopamine.\(^17–20\) Therefore, the use of DBS or ablation aims to inhibit that disproportionate activity, and its experimentation in humans and animals has brought promising results, with both the patient and animal subject gaining weight.\(^2–5\)

Based on those assumptions, we performed a bilateral ablation of the NAcc shell guided by stereotactic surgery, a very accurate technique,\(^21\) with a radiofrequency needle in healthy rats. The NAcc shell was chosen as the surgical target for its neuronal pathways and its use as a surgical target in animal models.

The aim of the study is to evaluate whether even in healthy rats, an inhibition – by ablation – of the NAcc shell would lead to increased caloric intake, and consequently, the subject’s weight. Also, if the procedure would affect their behavior.

Twenty-four female Wistar rats, weighting between 230-370g, and ages varying from 6 to 8 months at the start of the experiment, were housed individually before and after the surgery, under rigid control of temperature (25±2°C) and lighting. They were exposed to light for a daily period of 12 hours (7pm-7am). They had ad libitum access to commercial ration and water during the experiment. The whole trial was...
carried out in accordance with protocols approved by the Ethics Committee on Animal Use (CEUA) of the Faculty of Medical and Health Sciences, Pontifical Catholic University (FCMS-PUC/SP), under protocol number 2018/96.

The experiment design consists in the random division of the animals in two groups: a. experimental group: a) experimental group consisted in 20 animals that would endure the surgery, and b) control group consisted in 4 animals that would not endure the surgery.

For 9 days, at a predefined time, the animals of both groups had their weight and caloric intake measured. To evaluate the caloric intake, the amount of food (in grams) put into the animal’s feeding bowl the day before was subtracted by the amount of food left into the animal’s feeding bowl on the day the measures were taken.

The surgical procedure took place at the Surgical Technique Laboratory at FCMS-PUC/SP. First, the rats were placed under general anesthesia with ketamine (90mg/kg, i.m. Ketalar®, Cristália, São Paulo, Brazil) and xylazine (5mg/kg, i.m. Coopazine®, Coopers Brasil Ltda, São Paulo, Brazil), and under local anaesthesia with lidocaine 0.4ml sc; Anestesico bravet® 2% 20mg/ml; BRAVET, São Paulo, Brazil). This combination provides approximately 1 hour of sedative effect, enough to perform the surgery.

The rat’s head was shaved and disinfected with 70% isopropyl alcohol. Then, they were placed in the stereotactic frame (EFF-331 INSIGHT EQUIPAMENTOS Ltda*), the head was set in a position that allowed bregma and lambda to be in the horizontal plane (Figure 1).

After the preparation, the skin was incised, and a single trepanation hole was drilled using a cooled saline dental drill after the visualization of the bregma. Next, the radiofrequency needle (Figures 2 and 3) with 1mm of diameter was positioned in the NAcc medial shell, bilaterally, using the following coordinates: 1.44 mm before Bregma; 3.00 mm bilateral to the midline; 7.30 mm ventral to the dural surface, removed from stereotactic atlas for rats2, with the following ablations parameters: temperature set as 90°C for 60 minutes. All the materials used were sterilized in an autoclave system.

After the procedure, the rats were housed individually in cages, having 3 days for recuperation of the surgery. In the fourth day, our ‘day zero’, we started our measures.
Behavioral analysis

The behavioral analysis was conducted empirically; the animals were observed for 10 minutes every day, before being handled for the measurements (weight, caloric intake), comparing them according to the following parameters: excessive grooming, aggressive behavior, sniffing, hiding, searching/exploring, avoidance/escape. Since this was an observational analysis, the behavioral variables had their normality standard considering the one shown by the control groups. Since this was an observational analysis, the behavior variables had their normality pattern considering the ones presented by the control groups. Thus, the rats in the experimental group were analyzed considering that pattern, and if they deviated from normality, the variations in standard behavior would be qualified in terms of their intensity. That is, the extent to which they deviated from the pattern.

Data analysis

The data gathered (weight and caloric intake) were analyzed using two-way ANOVA with repeated measures in the SPSS 26.0 software. The significance level was determined as p<0.05.

RESULTS

Of the 20 experimental rats, 9 were removed from the statistical analysis because they did not resist surgery or did not survive significant post-surgical time.

In Figure 4, the graph represents a description of the weight variation over time for the control and experimental groups. Both curves represent the average weight gain of each group and its development over time (discriminated by days). The red curve, representing the experimental group, shows a significant weight gain throughout the days after the procedure, which is more substantial between days 1 and 6. After that, the average weight grows slightly less. The blue curve, representing the average weight of the control group, shows no growth and a rather linear slight descendancy. Therefore, the overall growth indicated by the experimental curve suggests a weight gain relevant to the group in general. Compared to the mostly constant curve representing the control group, the ascendancy of the experimental is more significant.
the ascendancy of the experimental group is more relevant.

The two-way ANOVA with repeated measures was used to compare the possible interaction of time, weight and caloric intake. It revealed that there was no significant main effect of time and weight $F(1482, 19261) = 1337, p = 0.27$. However, here was significant main effect to time and caloric intake $F(8,104) = 4388, p = 0.001$.

Comparing the interaction of time to the groups, there was significant main effect in the interaction, in both weight $F(1482,19261) = 4076, p = 0.044$, and caloric intake related to the groups $F(8, 104) = 4.333, p = 0.001$. This reflects a statistical difference occurring in relation of weight and caloric intake between the control and experimental groups.

Table 1 presents the variation in both the weight and the caloric intake of the animals, discriminated according to the groups, experimental and control, on the first and last day of the experiment. Weight and caloric intake variation were higher, in general, in the experimental group. The highest variation of the weight was in rat 2, reaching a value of 56.25%, and the highest variation of caloric intake was in rat 8, reaching a value of 926.85%.

| Animals - Group | Weight Variation (percentage) | Caloric Intake Variation (percentage) |
|-----------------|------------------------------|--------------------------------------|
| Experimental    |                              |                                      |
| 1               | 8.05%                        | 1.59%                                |
| 2               | 56.25%                       | 59.72%                               |
| 3               | 29.77%                       | 201.74%                              |
| 4               | 26.01%                       | 158.07%                              |
| 5               | 21.91%                       | 368.94%                              |
| 6               | 9.65%                        | 107.23%                              |
| 7               | 1.48%                        | 107.23%                              |
| 8               | -2.52%                       | 926.85%                              |
| 9               | 8.55%                        | 38.24%                               |
| 10              | 13.39%                       | 399.80%                              |
| 11              | 11.68%                       | 106.17%                              |
| Control         |                              |                                      |
| 1               | -2.42%                       | -32.93%                              |
| 2               | -2.51%                       | 2.78%                                |
| 3               | -1.69%                       | -6.23%                               |
| 4               | 2.16%                        | -31.20%                              |

Table 1. Variation of the weight and caloric intake at the beginning and end of the experiment.
Analysis of behavioral findings

In Table 2, it was observed that excessive grooming, sniffing and searching/exploration were present in all subjects of the experimental group. But hiding was present only in subject number four, and avoidance/escape were absent in only in subject number 8, the same one that an intensive aggressive behavior was present.

### Table 2. Behaviour patterns

| Animals - Group | Excessive grooming | Aggressive behaviour | Sniffing | Hiding | Searching/Exploring | Avoidance/Escape |
|-----------------|--------------------|----------------------|----------|--------|---------------------|------------------|
| Experimental    | ++                 | -                    | ++       | -      | ++                  | +                |
| 1               | ++                 | -                    | ++       | -      | +                   | +                |
| 2               | ++                 | -                    | ++       | -      | ++                  | +                |
| 3               | ++                 | -                    | +        | -      | +                   | +                |
| 4               | ++                 | -                    | ++++     | +      | ++                  | ++++             |
| 5               | +++                | -                    | ++       | -      | +                   | +                |
| 6               | +                  | -                    | ++       | -      | +                   | +                |
| 7               | ++                 | -                    | ++++     | -      | +                   | +                |
| 8               | ++++               | ++++                 | ++++     | -      | ++++                | -                |
| 9               | +                  | -                    | +        | -      | ++                  | +                |
| 10              | ++                 | -                    | ++       | -      | +                   | +                |
| 11              | +++                | -                    | ++       | -      | ++                  | +                |

Note: The rats were considered positive (+) for each pattern when the behaviour was present regularly during the observations. The more intense manifestations were quantified in “++”, in comparison with the other rats. All analysis of the experimental group was based on the normal behaviour of the control group.

DISCUSSION

The inquiry to be addressed by the study is whether bilateral ablation of the NAcc shell in healthy rats would result in an increase in the animal’s caloric intake and consequently in their weight.

The results of the study demonstrated a significant result in the interaction between the weight and caloric intake of the rats, comparing the values obtained by the control and experimental rats (p<0.001). This means that the participation of the reward system, represented by the Nucleus Accumbens, in the pathophysiology of anorexia may be correct, since the inhibition of this brain structure causes an alteration in the feeding behavior of the animals. This is supported by the literature\textsuperscript{23,24}. However, the exact way the alterations occur in the Nucleus Accumbens and its interference in the brain connections are still to be elucidated.
Comparing our results with those obtained by other studies that have performed experimental surgery on animals, Prinz et al.2, implanted a unilateral DBS in the left NAcc medial shell of healthy rats and conducted biphasic stimulation for seven days. The group found a statistically significant result comparing the experimental group to the control group, finding no changes in behavior. Van der Plasse et al.3 implanted electrodes in three different anatomic location: NAcc core, NAcc lateral shell and NAcc medial shell and compared the results among them. The group concluded that the DBS of the NAcc core did not show statistical results, as well the DBS of the NAcc lateral shell, whereas the DBS of the NAcc medial shell increased caloric intake, and it did not alter grooming and locomotor activity. Even comparing different techniques, the results obtained by this study and the mentioned ones were similar regarding calorie intake and weight gain.

In terms of the outcomes obtained in human interventions, Wang et al. implanted bilateral electrodes in the NAcc core – DBS – in two patients with AN and six patients with AN underwent surgery to be done a bilateral ablation of the same anatomic location. The group's results showed an increase in the body mass index (BMI) of the patients, comparing the pre-surgery weight with the post-surgery weight for one year. Sun et al. was cited by Wu et al. and implanted bilateral electrodes in the NAcc medial shell in four patients with AN, the group's results after a median range of thirty eight months of follow up, were an improvement in the BMI. Comparing the results of the research in humans with those of the present study, even though they are in different species, there is a consistency in the outcomes, suggesting a similar physiology in the operation of the reward system – Nucleus Accumbens – and, therefore, there is a reinforcement of the hypothesis of the interventions (DBS or ablation) in that brain structure for the treatment of psychiatric disorders.

Concerning the behavioral changes observed in this research, they were not present in the researches of Prinz et al.2 and van der Plasse et al.3. However, van Kuyck et al.25, described the study of Hano et al. who after stimulation of the NAcc septi of rats, found an increase in activity, sniffing and aggression. Reinforcing the theory that the Nucleus Accumbens participates in the fight-or-flight response system by modulating the amygdala response to external stimuli26.

In our findings of behavioral changes, the growth in activity shown by Hano et al. can be correlated with the increase of grooming and searching/exploring, while the growth in the aggressiveness could be related with the higher avoidance/escape and aggressive changes. The hiding behavior was not described by that group of researchers. However, it can be understood by the fact that the Nucleus Accumbens is part of the fight-and-flight system, contributing to the enhancement of the escape response of the rat.

The performance of an ablation or DBS in the Nucleus Accumbens, promotes an inhibition – yet not so well understood – of its modulation in the reward system, affecting the neuronal connections that pass through this system. The consequences resulting from this interference, weight gain, increased caloric intake, and behavioral changes need to be better elucidated and fully comprehended for that intervention to be eligible as a therapeutic tool.

Therefore, more investigations are needed to determine its benefits and risks. In the case of anorexia nervosa, since there is an imbalance of neurotransmitters with a greater activation of the Nucleus Accumbens region, its inhibition seems to bring positive effects. Again, more studies are needed to determine the extent of this progress, especially since there are gaps in the understanding of both NAcc functioning28, the pathophysiology of anorexia nervosa, and the functioning of ablation and DBS.

**CONCLUSION**

In this study we investigated whether even in healthy rats, performing an inhibition/ablation of the Nucleus Accumbens would cause an increase in caloric intake and consequently in the weight of the rats, and if the procedure would cause changes in their behavior. From the results obtained, we concluded that this assumption is valid. Further studies, meanwhile, are needed to explore this causal relationship in depth, either with a larger number of variables and/or assessing other data, to conclude how accurate is the relationship.

**Acknowledgments**

We would like to thank Micromar® for lending us the
radiofrequency generator, used in the experiment, and for having manufactured the radiofrequency needle, exclusively for this research.

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CORRESPONDING AUTHOR

Carolina Simão Martini
Department of Surgical Technique and
Division of Experimental Surgery
Pontifical Catholic University of São Paulo
Sorocaba, Brazil

Conflicts of interest: nothing to disclose.