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Research article

Deep brain stimulation of the nucleus accumbens shell induces anti-obesity effects in obese rats with alteration of dopamine neurotransmission

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HIGHLIGHTS

- NAc-sh DBS suppresses appetite and reduces weight gain in DIO rats.
- DA levels are increased in DIO rats subjected to DBS.
- DBS increases the gene expression of the D2 receptor in DIO rats.
- Normal chow-fed rats are not sensitive to NAc-sh DBS.
- Positive modulation of DA levels may be correlated with the anti-obesity effects of DBS.

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ABSTRACT

The aim of this study was to assess the anti-obesity effects of nucleus accumbens shell (NAc-sh) deep brain stimulation (DBS) in diet-induced obese (DIO) and chow-fed (chow) rats. The influence of DBS on dopamine (DA) signaling in the NAc-sh was also evaluated. DIO and chow rats were subjected to DBS for 14 consecutive days. Food intake and weight gain were measured daily. The gene expression of the dopamine D1 and D2 receptors was evaluated by qPCR. In addition, the extracellular levels of DA and its metabolite, dihydroxyphenylacetic acid (DOPAC), were determined by microdialysis. We observed that chronic DBS induced significant reductions in total energy intake (596.0 ± 65.0 kcal *vs.* 1161.6 ± 22.2 kcal, $p < 0.001$) and weight gain ($1.45 \pm 0.57\%$ *vs.* $9.64 \pm 0.38\%$, $p < 0.001$) in DIO rats compared to sham-DIO rats. Up-regulated D2 receptor gene expression (2.43 ± 0.12 *vs.* 0.64 ± 0.04, $p < 0.001$) and increased DA levels (2.73 ± 0.15 pmol/mL *vs.* 0.62 ± 0.05 pmol/mL, $p < 0.001$) were observed in DIO rats compared to sham-DIO rats. DBS had no influence on food intake, weight gain, or DA neurotransmission in chow rats. Our results support an association of the anorexigenic effects of NAc-sh DBS with mesolimbic DA signaling and indicate that the positive alteration of DA function in DIO rats may be responsible for the different effects of DBS in DIO and chow rats.

1. Introduction

The mesolimbic dopamine (DA) pathway plays an important role in palatable food intake by modulating the reward system, mainly through projections from the ventral tegmental area to the nucleus accumbens (NAc) [1]. Several studies have indicated hypofunction of DA signaling of the striatal regions in obesity [2,3]. These DA deficits contributes to the maintenance and development of obesity *via* the compulsive consumption of palatable food to compensate the reward hypofunction [1]. Hence, strategies aimed at improving DA function may facilitate the treatment of obesity.

As an innovative and neuromodulating treatment method, deep brain stimulation (DBS) is being used to treat movement disorders and neuropsychiatric diseases [4,5]. Clinical studies of patients suffering from obesity, alcoholism, smoking and drug abuse have indicated abstinence and reduced craving after NAc DBS [6–8]. However, the role of DA signaling regulation in the effects of NAc-

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sh DBS in obesity is unclear. Because DA function differs between normal and obese individuals, DBS may have different effects on chow-fed (chow) and diet-induced obese (DIO) animals. To address these questions, we performed chronic nucleus accumbens shell (NAc-sh) DBS on chow and DIO rats. Changes in weight gain, food intake, DA receptors gene expression, and DA and dihydroxyphenylacetic acid (DOPAC) levels were analyzed.

2. Materials and methods

2.1. Animals

Weight-matched six-week-old male Sprague-Dawley rats (200–205 g, Vital River Laboratories, Beijing, China) were housed in groups under a 12 h light/dark cycle. The ambient temperature was maintained at 20–23 °C. Rats were randomly divided in two groups. One group received a high-fat diet containing 45% fat by calories and an energy density of 4.58 kcal/g (Academy of Military Medical Sciences, Beijing, China) to induce the DIO phenotype. The other group remained on standard chow diet (standard chow contained 10% fat by calories, with an energy density of 3.28 kcal/g). All animal experiments were performed in accordance with the Guidance for Animal Experimentation of the Capital Medical University and the Beijing Guidelines for the Care and Use of Laboratory Animals.

2.2. Stereotactic surgery and DBS procedure

After habituation, the rats were anesthetized and placed in the stereotactic frame (Kopf1404, Germany). Electrodes (CBCRJ30, FHC, USA) were implanted into the left NAc shell according to the Paxinos and Watson rat brain atlas (1.2 mm anterior, 0.7 mm lateral, and 7.4 mm deep from the bregma). The DBS was delivered using a pulse stimulator (Master 8, AMPI). The stimulation parameters were 500 μA, 130 Hz, and 90 μs, approximating the DBS used in clinical practice.

2.3. Body weight and food intake measurements

After 10 weeks diet exposure, continuous DBS was provided to chow rats (sham, $n = 8$; DBS, $n = 8$) and DIO rats (sham, $n = 8$; DBS, $n = 8$) for 14 days. Body weight and 24-h food intake (standard chow or high-fat chow) were measured daily. After stimulation, the rats were sacrificed *via* decapitation. The brains were quickly removed, frozen and stored at −80 °C prior to PCR analysis.

2.4. Quantitative PCR (qPCR)

Samples of the NAc-sh were microdissected with a 0.75 mm^2 punch from 1 mm sections. RNA was extracted using the TRIzol method according to the manufacturer's instructions (Invitrogen, Sweden). Genomic DNA was removed by incubation with DNase I (Takara Biotechnology, China). cDNA was synthesized using sMMLV reverse transcriptase (Takara Biotechnology, China) and random hexamers as primers. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control transcript. Each qPCR contained 20 mM Tris/HCl, 50 mM KCl, 4 mM $MgCl_2$, 0.2 mM dNTPs, DMSO (1:20), SYBR Green (1:50,000), 0.02 U/μL Taq DNA polymerase and 2 pmol/L each primer. The protocol consisted of an initial denaturation step at 95 °C for 3 min, followed by 50 cycles of denaturing at 95 °C for 3 s, annealing at 51 °C for 15 s and extension at 72 °C for 30 s. Each sample was evaluated in triplicate.

The primer sequences were as follows: GAPDH: forward, ACATGGCCTCCA-AGGAGTAA, reverse, CCTCCTGTTGTTATGGGGTC; D1 receptor: forward, GCC-TTTGGGTCCCTTTTGTA, reverse, TCATACTGGAAAGGGCTGGA; D2 receptor: forward, ATGGCTG-TATCCCGAGAGAA, reverse, AATTTCCACTCACCCACCAC.

2.5. Microdialysis and high-performance liquid chromatography (HPLC)

In a separate experiment, electrodes were implanted as mentioned. After 7 days of recovery, microdialysis guide cannulas equipped with stylets (Bioanalytical Systems, USA) were implanted into the ipsilateral NAc-sh. The cannulas were obliquely implanted into the NAc-sh. After recovery, DBS was performed for 14 days. The guide stylets were then replaced by microdialysis probes (CMA12, Sweden), which were perfused with artificial cerebrospinal fluid at a flow rate of 2 μL/min. Dialysate samples were collected at 30-min intervals for 1.5 h. DA and DOPAC levels were analyzed by HPLC (Sykam GmbH, Germany) with electrochemical detection as described previously [9]. After the experiments, the rats were sacrificed and then perfused with 4% paraformaldehyde. Brains were removed and fixed.

2.6. Verification of the location of electrodes and microdialysis probes

For the samples from the qRCR experiment, coronal sections at a thickness of 10 μm were prepared with a cryostat microtome and processed by hematoxylin and eosin (H&E) staining. For the samples from the microdialysis experiment, sagittal sections were cut with a freezing microtome at a thickness of 10 μm and processed by H&E staining. Rats with accurate location of electrodes and microdialysis probes were included in analysis. Related experiments were re-conducted to ensure there were 8 samples in each group.

2.7. Data analysis

The results are presented as the means ± SEM. Body weight data are expressed as daily weight gain relative to the weight on the day before DBS. The body weight and food intake before stimulation were analyzed using Student's *T*-test. Two-way ANOVA with Bonferroni *post-hoc* test was used to detect differences in weight gain, food intake, DA and DOPAC levels, and DA receptors gene expression between groups during and after the DBS. GraphPad Prism V5.0 (GraphPad Software, San Diego, CA) was used for statistical analysis. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. Electrodes and probes localization

Histological analysis confirmed the placement of the electrodes in the qPCR experiment (Fig. 1A) and the placement of the electrodes and microdialysis probes in the microdialysis experiment (Fig. 1B and C). A total of 7 rats with 4 misplaced of electrodes and 3 misplaced of microdialysis probes were found (Fig. 1A–C).

3.2. DBS reduces body weight gain and food intake in DIO rats

Before the stimulation, DIO rats exhibited an increase in weight of more than 20% compared to chow rats (542 ± 6 g *vs.* 422 ± 4 g, $p < 0.001$). The total caloric intake of 10 weeks before the DBS of DIO rats was higher than that of chow rats (5815 ± 63 kcal *vs.* 4831 ± 104 kcal, $p < 0.001$). DBS–DIO and sham-DIO rats showed no differences of body weight (535 ± 8 g *vs.* 550 ± 10 g, $p = 0.24$) and total caloric intake (5811 ± 96 kcal *vs.* 5819 ± 89 kcal, $p = 0.95$). Similarly, the body weight (418 ± 5 g *vs.* 426 ± 7 g, $p = 0.40$) and total