DEEP BRAIN STIMULATION ALTERS LIGHT PHASE FOOD INTAKE MICROSTRUCTURE IN RATS

1Charite Center for Internal Medicine and Dermatology, Department for Psychosomatic Medicine; Charite - Universitaetsmedizin Berlin, corporate member of Freie Universitaet Berlin, Humboldt - Universitaet zu Berlin, and Berlin Institute of Health, Berlin, Germany; 2Department of Internal Medicine, Helios Clinic, Zerbst, Germany; 3Clinic for Neurology, Epilepsy Center Berlin-Brandenburg, Beelitz, Germany; 4Clinic for Neurosurgery, Charite - Universitaetsmedizin Berlin, corporate member of Freie Universitaet Berlin, Humboldt-Univesitaet zu Berlin, and Berlin Institute of Health, Berlin, Germany; 5Cognitive Neurobiology, Berlin Mouse Clinic for Neurology and Psychiatry, Humboldt University, Berlin, Germany; 6Department of Quantitative Health Sciences, Medical School University of Massachusetts, Worcester, Massachusetts, USA; 7Department of Psychosomatic Medicine and Psychotherapy, University Hospital Tuebingen, Tuebingen, Germany

Treatment of eating disorders like obesity or anorexia is challenging. Options are limited and new approaches desired. An interesting approach is the application of deep brain stimulation (DBS). The nucleus accumbens (NAcc) is part of the food reward system. A pilot study reported that DBS of the NAcc shell modulates food intake and body weight in rats. Underlying mechanisms such as the food intake microstructure are unknown so far. Normal weight female Sprague-Dawley rats were equipped with a custom-made DBS electrode placed unilaterally in the NAcc shell. Biphasic stimulation was performed for seven days. Body weight and food intake including the microstructure were assessed over the experimental period. Behavior was monitored manually. DBS tended to increase body weight gain (28.1 ± 5.4 g) compared to sham-stimulated controls (16.7 ± 3.4, P = 0.05) without affecting daily food intake (P > 0.05). Further analyses showed that light phase food intake was stimulated, whereas dark phase food intake was decreased in the DBS group (P < 0.05). During the light phase bout frequency (+50%), bout duration (+64%), meal duration (+71%) and overall time spent in meals (+92%) were increased in DBS rats (P < 0.05), while during the dark phase no alterations were observed (P > 0.05). Behavior did not show differences regarding overall eating and drinking behavior (including food/water approach), grooming or locomotion (P > 0.05). Summarized, although overall food intake was not changed by DBS, light phase food intake was stimulated likely via a reduction of satiation.

Key words: anorexia, deep brain stimulation, food intake, obesity, satiation, satiety, body weight, light phase, dark phase

INTRODUCTION

Since eating disorders are a common health burden, new approaches for the treatment of obesity or anorexia nervosa are needed to modulate food intake and body weight. One of the most promising and interesting recent approaches is the application of deep brain stimulation (DBS) (1). Originally, DBS was used in the treatment of neurological and psychiatric diseases (2) resulting in a substantial improvement of well-being in patients with Parkinson’s disease (3). Especially the subthalamic nucleus is an interesting target for DBS in the treatment of Parkinson’s disease resulting in an improvement of motor symptoms (4) and gait function (5). DBS is considered a potentially reversible method that influences the neuronal network and is currently also tested in the treatment of patients with Tourette’s syndrome (6, 7), epilepsy (8, 9), depression (10, 11) or eating disorders (12-14).

Animal studies have shown an increased food intake in normal weight rats following DBS of the medial nucleus accumbens shell (NAcc shell) (15) and a decrease in food intake in diet-induced obese (DIO) rats (16). These findings - although contradictory - might be promising for the prospective application of DBS for both, stimulation or inhibition in obesity or anorexia nervosa, respectively. The mechanisms by which DBS influences the neuronal network are far from being understood. However, recent years have witnessed an increasing understanding of these mechanisms (17) encompassing a modulation of transmitter release such as dopamine and gamma-aminobutyric acid (GABA) (16, 17) but also an alteration of neurogenesis (17, 18).

The NAcc is divided into core and shell and is - along with the ventral tegmental area (VTA), hypothalamic nuclei, amygdala, hippocampus and different areas of the cortex (the orbitofrontal cortex, the anterior cingulate cortex and the dorsolateral prefrontal cortex) - part of the reward circuitry (19). Peripheral metabolic signals like leptin, insulin or ghrelin do not only affect the arcuate nucleus of the hypothalamus but also the VTA to regulate food intake (20). The medial NAcc shell receives dopaminergic input from the VTA, a pathway modulated by well-known food intake-regulatory hormones.
such as insulin or leptin (21). The dopaminergic activation of the NAcc leads to reward-seeking behavior (e.g. for food). Moreover, the NAcc itself influences the lateral hypothalamus (LH) via GABA release which is involved in the regulation of food intake in the arcuate nucleus. Additionally, the LH affects the VTA via orexin signaling (Fig. 1) (22).

Previous studies confirmed the role of the medial NAcc shell in the regulation of feeding since microinjection of orexin-A into the NAcc shell increased food intake in rats (23). Microinjection of muscimol (a GABAa receptor agonist) decreased food ingestion when injected into the lateral medial NAcc shell, whereas food intake increased after microinjection into the rostral medial NAcc shell, pointing towards distinct functions of different areas of this nucleus (24). Besides the involvement in unconditioned behaviors like food consumption (25) the medial NAcc shell is involved in the hedonic response to food, an effect exerted via an opioidergic pathway (26). Taken together, these results highlight the NAcc shell as an interesting target to modulate food intake and body weight by DBS which might hold therapeutic potential in the treatment of eating disorders.

Since conflicting results have been reported following stimulation of the NAcc shell in rats before (15, 16) the aim of the present study was to investigate stimulation effects in more detail. We assessed body weight changes, food and water intake for seven days during continuous stimulation using a completely implantable system previously established in rats (27). To investigate the microstructure underlying possible alterations of food intake, we employed an automated food intake-monitoring system recently validated for the use in rats (28, 29) and mice (30). Lastly, also behavior was monitored as a lesion of the NAcc using electrolysis was shown to induce hyperactivity (31, 32). Female rats were used since a stimulation of food intake was expected and therefore this method might hold potential for the use in patients with severe anorexia nervosa, a disease with much higher prevalence in women compared to men (33). This was also proposed in an internationally recognized human pilot study (34) and followed up recently (14).

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats (Harlan-Winkelmann Co., Borchen, Germany) weighing 180 – 200 g were first group hosed (four rats/group) under controlled illumination (06.00 – 18.00 h) and temperature (21 – 23°C). During this time rats were handled daily to become accustomed to the interaction with the investigators. Rats had ad libitum access to rat chow (ssniff Spezialdietaen GmbH, Soest, Germany) and tap water. Animal care, experimental procedures as well as euthanasia followed institutional ethics guidelines and were approved by the state authority for animal research (Landesamt fur Gesundheit und Soziales Berlin, LaGeSo Berlin).

Surgery

After a habituation period of five days to accustom rats to single housing in the food intake monitoring cages as described below, animals were anesthetized with an intraperitoneal (i.p.) injection of 100 mg/kg ketamine (Ketanest™, Curamed, Karlsruhe, Germany) and 10 mg/kg xylazine (Rompun™, 2%, Bayer, Leverkusen, Germany) as described before (29). Afterwards, rats were placed in a stereotactic apparatus to implant DBS electrodes (custom-made at the Berlin Mouse Clinic for Neurology and Psychiatry, Berlin, Germany) unilaterally into the
medial shell of the left nucleus accumbens as described before (16). The NAcc shell extends from bregma: 0.96 mm to 2.76 mm anterior. The site of stimulation was based on a previous publication (15) with the coordinates (from bregma: 1.44 mm anterior, 3.0 mm left lateral, and 7.3 mm ventral, angle 17°) based on the atlas of Paxinos and Watson (35) with bregma 1.44 mm anterior reflecting the mid-region of the NAcc shell. The angle of 17° at the coronal level towards the midline was chosen to avoid penetration of the lateral brain ventricle. The electrode consisted of a platinum/iridium wire with a diameter of 70 µm.

Briefly, the skull was opened using an electric microdrill, the DBS electrode inserted and fixed to the skull by dental cement anchored by four sterile screws (Plastic One Inc., Roanoke, VA, USA) before the skin was closed. The stimulator (size: 4.0 cm x 2.0 cm x 0.8 cm) was placed subcutaneously at the back of the rats, and stimulator and DBS electrode connected by an isolated cable tunneled subcutaneously as described before (36). Control animals underwent the same surgical procedure except that a non-functional stimulator was implanted.

After the surgery, rats were housed individually and allowed to recover for five days. During this time, daily handling of the rats was continued. After five days, DBS electrodes were activated as described before (36). The stimulation parameters (130 Hz, 100 µA, biphasic) followed a published protocol (15). DBS and sham-stimulated rats were monitored over a period of seven days. During this time, food and water intake were monitored continuously as described below, body weight was assessed daily and behavior was assessed manually as described below.

Verification of correct electrode placement and stimulation

After the stimulation period of seven days, on day eight the correct placement of the DBS electrode was verified histologically. Rats were deeply anesthetized followed by transcardial perfusion as described before (37). After snap-freezing in dry ice-cooled 2-methylbutane, brains were cut into coronal sections (25 µm) at the area of interest and stained with cresyl violet (Sigma-Aldrich, Taufkirchen, Germany). Afterwards, sections were washed with deionized water twice for 1 min, dehydrated through a series of ethanol, incubated in xylene and cover slipped in Entellan™ new (Merck, Darmstadt, Germany). Correct placement was assessed using a light microscope (Axiophot, Zeiss, Jena, Germany).

Additional tests were performed to ensure correct function of the stimulator. In vivo, current flow was assessed after placing probes of an oscilloscope (130 Hz) at the rat’s ear and hind paw. Moreover, to detect possible leakage of the connected DBS electrode and stimulator, the DBS stimulation system including all connecting parts was placed into 0.9% saline solution and current flow was measured using the oscilloscope.

Animals with incorrect placement or deficient stimulation were retrospectively excluded from the analysis. Three rats had to be excluded due to misplacement of the electrode, two due to infections and three due to stimulator malfunction. A final n = 6/group was examined for all further analyses (Fig. 2).

Automated food and water intake monitoring

The BioDAQ episodic food intake-monitoring system (BioDAQ, Research Diets Inc., New Brunswick, USA) was employed to continuously monitor food and water intake and assess the underlying food intake microstructure as recently established for the use in rats (28). Rats were habituated for five days to single housing (in regular housing cages with normal bedding and enrichment) and feeding from the hopper and as shown before quickly adapted to these conditions within two to three days as indicated by normal food intake and regular body weight gain (28). Food was provided in low spill food hoppers and water in special drinking bottles placed on a balance.

The BioDAQ food intake-monitoring system weighs the hopper (± 0.01 g) with food (or water, in the following part described for food only) every second and detects ‘not eating’ as weight stable and ‘eating’ as weight unstable. ‘Feeding bouts’, defined as change in stable weight before and after an event, are recorded as vectors with starting time, duration and amount of food consumed. The feeding bouts are separated by an inter-bout interval (IBI) and meals can consist of one or more bouts. Furthermore, meals are separated by inter-meal intervals (IMI), in rats defined by a duration of 15 min. The minimum meal size in rats was defined as 0.1 g and therefore food intake was considered as one meal when feeding bouts occurred within 15 min of the previous response and consumed food was equal to or greater than 0.1 g. If the interval between bouts was greater than 15 min, this was considered a new meal. Meal parameters assessed in this study encompassed bout size (g/bout), meal size (g/meal), bout frequency (number/period), meal frequency (number/period), inter-meal interval (min), bout duration (sec/bout), meal duration (min/meal), eating rate (mg/min), time spent in meals (%) as well as the safety ratio (min/g food eaten) calculated using the parameters described above. These parameters were extracted from the software and visualized using the Data Viewer (BioDAQ Monitoring Software 2.3.07, Research Diets Inc.). Data analysis was performed in Excel (Microsoft). For assessment of the food intake microstructure, days two and three were selected as representative days during the stimulation period of seven days.

Manual monitoring of behavior

On days two and three of the stimulation period (selected as representative days), behavior was assessed manually as described before (28). Briefly, before the monitoring started the amount of bedding was reduced and a paper grid dividing the cage into six squares was placed underneath the cage. One hour before the start of the dark phase, behavior was monitored manually by assessing eating behavior (eating as well as food approach consisting of sniffing and licking food), drinking behavior (including drinking and water approach), grooming (including washing, licking, and scratching) and locomotion (defined as at least one rat paw crossing the boundary of one square). When the behavior lasted more than 5 seconds it was considered as a new behavior. For locomotion, the total number of squares crossed was counted. Two investigators monitored three rats per person for 1 hour. Pilot experiments showed an inter-investigator variability of < 5%.

Statistical analysis

Distribution of the data was determined by using the Kolmogorov-Smirnov test. Data are expressed as mean ± S.E.M. and were analyzed by t-tests or Mann-Whitney Rank Sum Test depending on the distribution of the data or two-way analysis of variance (ANOVA). Differences between groups were considered significant when P < 0.05 (SigmaStat 3.1, Systat Software, San Jose, CA, USA).

RESULTS

Deep brain stimulation tended to increase body weight in rats without affecting daily food and water intake

Body weight (g) was monitored daily over the stimulation period of seven days (Fig. 3A). Two-way ANOVA showed a significant impact of treatment (F1,195) = 4.4; P < 0.05) and time
indicated a significant influence of treatment ($F_{1,95} = 12.0; P < 0.05$) and time ($F_{7,95} = 17.3; P < 0.001$), while no interaction of treatment and time was observed ($F_{7,95} = 0.9; P > 0.05$). Food and water intake were monitored continuously using an automated monitoring system. When assessing the very first

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*Fig. 2.* Placement of stimulation electrode. Placement of the electrode in the NAcc shell was assessed after the experiments, stimulator function was verified *in vivo* and *ex vivo*. Three rats had to be retrospectively excluded due to misplacement of the electrode, three due to infections and three due to stimulator malfunction (●). A final $n = 6/\text{group}$ was included for the sham (▲) and the deep brain stimulation (DBS) group (■). These pictures are shown with permission of Elsevier, Inc.
hour, DBS rats showed a higher food intake compared to sham-treated controls (1.31 ± 0.52 versus 0.01 ± 0.01 g; P < 0.05). However, daily food intake did not differ between the DBS and sham-treated group (P > 0.05; Fig. 3B). Treatment did not alter food intake (C) or water intake (D). Food and water intake were expressed as g and ml, respectively. Data were normally distributed, expressed as mean ± S.E.M. and assessed using two-way ANOVA. Differences in body weight during the last day of stimulation were assessed using t-test.

Table 1. Deep brain stimulation did not alter behavior.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Deep brain stimulation</th>
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<tbody>
<tr>
<td>Eating behavior (number/1 h)</td>
<td>62.3 ± 13.3</td>
<td>69.8 ± 17.7</td>
</tr>
<tr>
<td>Drinking behavior (number/1 h)</td>
<td>24.0 ± 8.1</td>
<td>26.5 ± 7.0</td>
</tr>
<tr>
<td>Grooming (number/1 h)</td>
<td>70.5 ± 11.0</td>
<td>68.7 ± 17.8</td>
</tr>
<tr>
<td>Locomotion (number/1 h)</td>
<td>49.5 ± 8.2</td>
<td>51.6 ± 12.1</td>
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</table>

Data were normally distributed, expressed as mean ± S.E.M. and assessed using t-tests. n = 6/group. P > 0.05 versus sham.

Deep brain stimulation did not affect behavior

Behavior consisting of eating behavior (eating as well as food approach), drinking behavior (drinking as well as water approach), grooming (including washing, licking, and scratching) and locomotor activity was assessed manually in the pre-dark phase activity period. No differences were observed between DBS and sham-treated rats (P > 0.05; Table 1).

Deep brain stimulation increased food intake during the light phase, while dark phase food intake was reduced

When daily food intake was further analyzed using the automated food intake monitoring system, a stimulation of light phase food intake was detected (+57%; P < 0.01; Fig. 4A), whereas dark phase food intake was reduced (~10%, P < 0.05; Fig. 4B). The overall 24-h food intake did not differ between DBS and sham-stimulated rats (P > 0.05; Fig. 4C). Further investigation of the underlying food intake microstructure showed that during the light phase bout

Fig. 3. Deep brain-stimulated rats showed a trend towards higher body weight gain without differences in overall food and water intake. Rats were implanted with a stimulation electrode, and sham treatment or deep brain stimulation (DBS) of the NAcc shell was performed over a period of seven days. DBS-treated rats showed an increased body weight compared to the sham-treated group at the end of stimulation period, however, this did not reach statistical significance (P = 0.01; (A)). Body weight gain tended to be increased after seven days (P = 0.05; (B)). Treatment did not alter food intake (C) or water intake (D). Food and water intake were expressed as g and ml, respectively. Data were normally distributed, expressed as mean ± S.E.M. and assessed using two-way analysis of variance. Differences in body weight during the last day of stimulation were assessed using t-test.
frequency (+50%), bout duration (+64%), meal duration (+71%) and the time spent in meals (+92%) were increased in DBS compared to sham-treated animals (P < 0.05), whereas bout size, meal size, meal frequency and inter-meal interval were not significantly altered (P > 0.05; Table 2). Analysis of the eating rate showed a DBS-induced decrease during the dark phase (~14%, P < 0.05), while during the light phase no significant difference was observed compared to the sham group (P > 0.05; Table 3). The satiety ratio was neither affected during the light nor dark photoperiod (P > 0.05; Table 3).
Table 2. Deep brain stimulation did not alter food intake microstructure during the dark phase.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Deep brain stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bout size (g)</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Meal size (g)</td>
<td>2.15 ± 0.18</td>
<td>2.29 ± 0.22</td>
</tr>
<tr>
<td>Bout frequency (number)</td>
<td>89.42 ± 7.50</td>
<td>82.67 ± 17.77</td>
</tr>
<tr>
<td>Meal frequency (number)</td>
<td>7.33 ± 0.72</td>
<td>6.50 ± 0.86</td>
</tr>
<tr>
<td>Bout duration (s)</td>
<td>26.66 ± 1.73</td>
<td>31.89 ± 3.60</td>
</tr>
<tr>
<td>Meal duration (min)</td>
<td>25.37 ± 2.48</td>
<td>33.28 ± 16.97</td>
</tr>
<tr>
<td>Inter-meal interval (min)</td>
<td>82.25 ± 9.05</td>
<td>105.36 ± 12.83</td>
</tr>
<tr>
<td>Time spent in meals (%)</td>
<td>25.82 ± 3.01</td>
<td>27.17 ± 3.49</td>
</tr>
</tbody>
</table>

The food intake microstructure was assessed during the 12-h dark phase. Data were normally distributed (except for meal frequency), expressed as mean ± S.E.M. and assessed using t-tests or Mann-Whitney Rank Sum Test depending on the distribution of the data. n = 6/group. P > 0.05 versus sham.

Table 3. Deep brain stimulation reduced the eating rate during the dark phase, whereas the satiety ratio was not altered.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Deep brain stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light phase</td>
<td>Dark phase</td>
</tr>
<tr>
<td>Eating rate (mg/min)</td>
<td>10.50 ± 1.28</td>
<td>24.38 ± 1.22</td>
</tr>
<tr>
<td>Satiety ratio (min/g)</td>
<td>120.74 ± 14.25</td>
<td>106.90 ± 12.00</td>
</tr>
<tr>
<td>Eating rate (mg/min)</td>
<td>24.38 ± 1.22</td>
<td>20.96 ± 1.07*</td>
</tr>
<tr>
<td>Satiety ratio (min/g)</td>
<td>37.78 ± 2.55</td>
<td>45.12 ± 3.45</td>
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</tbody>
</table>

Data were normally distributed, expressed as mean ± S.E.M. and assessed using t-tests. n = 6/group. *P < 0.05 versus sham.

DISCUSSION

In the present study, we investigated the effects of DBS of the NAcc shell in female rats. DBS stimulated food intake during the first hour of stimulation compared to sham-treated controls. Therefore, the present study confirms previous results regarding acute (over 1 hour) effects on food intake using identical stimulation parameters (100 µA, biphasic) (15). However, when investigating food intake over a longer period (daily over a stimulation period of seven days) no effect on food intake has been observed, a difference that may be due to sex differences (female versus male), strain differences (Sprague-Dawley versus Wistar), stimulation site(s) (unilateral versus bilateral) or the duration of stimulation (subacute for 1 week versus acute for 1 hour). It is to note that estrous cycle was not controlled in the present study. Therefore, although previous studies reported that rats living together show estrous cycle synchrony (38), a finding also described in women housed together in a college dormitory (39), ovarectomized rats should be investigated as well.

Similar to food intake, no alteration of daily water intake was observed in DBS compared to sham-treated rats. A previous study investigated water intake under conditions of acute bilateral stimulation of the NAcc shell with a current ranging from 0 to 150 µA in rats and showed a slight reduction of water intake in stimulated animals at 30 min that did not reach statistical significance (40). Therefore, current data argue against a modulation of water intake by DBS of the NAcc shell, although the present stimulation period was not long enough in order to determine possible alterations following chronic stimulation.

To further analyze the effects of DBS on feeding behavior we assessed the underlying food intake microstructure which encompasses different parameters of feeding like eating rate, meal frequency, meal size, meal duration or inter-meal interval. These parameters can be used to describe two major components of food intake: satiation (mechanism causing meal termination) and satiety (mechanism causing a later onset of the next meal after the previous meal is completed) (41, 42). Interestingly, despite the fact that overall food intake was not altered, DBS rats showed an increase in light phase food intake, whereas dark phase food intake was reduced compared to sham-treated rats. The reduction of dark phase food intake likely represents a compensatory action to prevent overeating.

Subsequent analyses of the food intake microstructure during the light phase showed an increase in bout frequency, bout duration, meal duration and the time spent in meals. Since DBS did not affect meal frequency and inter-meal intervals, satiety is not altered. Although meal size was not significantly altered either, DBS is likely to reduce satiation based on the observed increase in the time spent in meals, especially since the prolonged meal duration is not due to a reduced eating rate. Interestingly, although dark phase food intake was reduced in DBS compared to sham-treated rats, the underlying food intake microstructure - except for a slower eating rate - was not altered. This is likely due to, although significant, the small percental reduction (~10%) of dark phase food intake compared to the control group. Whether a recruitment of orexigenic ghrelin (43) or cannabinoid (44) signaling during the light phase reported to specifically influence satiation (45, 46) underlies the DBS-induced stimulation of light phase food intake will have to be further investigated.

Since alterations of food intake are often accompanied by behavioral changes (47) we also tested the effect of DBS on behavior. When assessed manually as in our previous studies (28) we could not detect any differences in eating or drinking behavior (including drinking and food/water approach), grooming or locomotion during the pre-dark phase period, an
activity period of the rats (48). It has been shown before that an N-methyl-D-aspartate (NMDA)-induced lesion of the NAcc shell did not affect locomotor activity compared to the sham group, while a lesion of the core resulted in hyperactivity (49). The present data do not indicate reduced activity as underlying/contributing factor to the observed DBS-induced trend towards an increase in body weight gain. However, this should be assessed in more detail over the whole experimental period using continuous monitoring and a longer stimulation period.

Despite the fact that overall food intake was not altered in DBS compared to sham-treated rats, DBS tended to increase body weight gain observed during the 7-day stimulation period. This effect was only modest (13 versus 9% body weight gain) and missed significance, likely due to the short duration of the stimulation period. Whether a stimulation of cannabinoid signaling reported before to affect body weight gain (50, 51) plays a role in the present body weight changes will have to be further investigated. It is to note that a stimulation longer than seven days was not feasible due to limited battery capacity of the internal stimulation device. Therefore, the stimulation period has to be considered as limitation of the present study and subsequent studies over a longer period are necessary to further elucidate a possible application in eating disorders.

In addition, also the mechanisms underlying the trend towards stimulated body weight gain warrant further investigation. Although overall food intake was unchanged, DBS selectively stimulated light phase food intake. It has been reported before that the percentage of daytime food consumption positively correlates with total body weight without differences in total 24-h food intake in male mice (52). Furthermore, mice fed a high fat diet during the 12-h light phase gained significantly more body weight compared to mice fed the same diet during the 12-h dark phase (53). Whether a time shift of food intake-stimulatory hormones such as ghrelin shown before to display a pronounced circadian rhythm (54) contributes to these changes will have to be further addressed.

It also might be speculated that the lesion of the NAcc shell by the electrode itself affects body weight. In line with this finding, a previous study using the exotoxin NMDA reported a higher body weight gain in NAcc shell-lesioned compared to sham-treated rats at 10 days after the lesion (49). However, this effect is unlikely to underlie the differences observed in the current study as both groups of rats, DBS and sham, received an electrode placed in the NAcc shell. Moreover, the electrode only accounted for one tenth of the diameter of the NAcc shell. It is to note that another study did not observe any differences in body weight gain between a NAcc shell DBS-stimulated and a sham-treated group of rats fed regular rodent chow over a period of 14 days (16). Whether sex (female vs. male) or stimulation (100 µA versus 500 µA) differences contribute to these discordant results needs further investigation. It is important to note that currents of 100 µA applied to the anterior thalamic nucleus decreased spontaneous seizures, while at 500 µA an increase of recurrent seizures in chronically epileptic rats was observed (55), possibly pointing towards an overstimulation at this current.

Taken together, in the present study we show that subacute DBS of the NAcc shell tends to promote body weight gain in rats compared to sham-treated controls during a stimulation period of seven days. As overall food intake was not altered, possible changes in body weight might be associated with the shift towards higher food consumption during the light phase and reduced satiation during this photoperiod. However, a stimulation period of 7 d only allows to investigate subacute changes, whereas chronic alterations (stimulation for several weeks or months) might be missed. It is to note that the duration of continuous stimulation using the present internal device was limited by battery capacity, whereas other studies mainly use external devices (15, 16, 56-58) that might affect the behavior of the rats. Therefore, future studies should be performed using improved internal devices for longer stimulation periods preferably in a larger cohort of animals. Lastly, to better mimic the stimulation regimen applied in humans also bilateral stimulation should be performed.

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Conflicts of interests: None declared.

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Author’s address: Dr. Andreas Stengel, Charite Center for Internal Medicine and Dermatology, Department for Psychosomatic Medicine, Charite - Universitaetsmedizin Berlin, Chariteplatz 1, 10117 Berlin, Germany.  
E-mail: andreas.stengel@charite.de