The effects of electrical stimulation or an electrolytic lesion in the mediodorsal thalamus of the rat on survival, body weight, food intake and running activity in the activity-based anorexia model

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1. ABSTRACT

The glucose metabolism in the mediodorsal thalamus (MD) is increased in rats in the activity-based anorexia (ABA) model. In patients, electrical stimulation in hyperactive brain regions reduced symptoms in e.g. major depressive disorder and cluster headache. In two blinded randomised controlled experiments, we therefore examined the effects of high-frequency electrical stimulation and an electrolytic lesion in the MD in a validated rat model for anorexia nervosa. The ABA model was successfully replicated in all our experiments, with a reduction in body weight, food intake, and survival time and an increase in running activity.

In a first experiment, we evaluated the effect of electrical stimulation or a curative lesion in the MD on survival, body weight, food intake and locomotor activity in ABA rats. Electrical MD stimulation or an electrolytic MD lesion did not improve the symptoms of rats in the ABA model, compared to control groups.

In a second experiment, we investigated the effect of a preventive electrolytic lesion in the MD on rats in the ABA model. Although there was no significant improvement of survival, body weight and food intake, locomotor activity was significantly reduced in the lesion group compared to the control group. Apart from this positive effect on running activity, we found no convincing evidence for the suitability of the MD as a neuromodulation target for anorexia nervosa patients.

2. INTRODUCTION

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by a refusal to maintain body weight at or above a minimally normal weight for age and height, an intense fear of gaining weight, and a disturbance in the way one’s body weight and shape are experienced. In postmenarcheal females, there is an absence of at least three consecutive menstrual cycles [1]. In addition, the lifetime occurrence of excessive exercise is between 75 and 84% [6].
Despite intensive treatment programs combining nutritional therapy with psychotherapy and/or pharmacotherapy, around 20% of the patients continues to suffer chronically. In accordance, mortality rates are high [41], with physical complications accompanying extreme starvation and suicide as the most common reported causes of death. Therefore, developing new treatment options is of utmost importance [31].

The ABA model is one of the best characterized animal models for AN and has been used for investigating potential new AN treatments (e.g. [9]). In this model, food restriction to 1.5h daily in the presence of a running wheel leads to the development of hyperactivity and a spontaneous restriction of food intake, leading to severe emaciation and often death [29]. This behaviour models some of the core symptoms in AN patients, e.g. dieting, loss of body weight and excessive exercising [12].

On the other hand, AN is characterized by many psychological symptoms. Although many hold expectations that all features of AN including psychological, physiological, and psychosocial symptoms should be modelled, an animal model will never provide enough psychological complexity to explain truly the associated human psychopathology [28]. However, this observation does not alter the fact that an animal model can be very useful to study certain aspects of the human disease. Moreover, the ABA model has been used extensively and it has been argued to be the best animal model available of AN [8].

Functional neuroimaging and neuroendocrinological studies provide substantial evidence for the involvement of the central nervous system in the mediation and maintenance of the symptoms in AN [30]. We therefore speculate that therapeutic interventions directed at the central nervous system, like electrical brain stimulation, may alleviate symptoms in patients with treatment-resistant AN. Likewise, electrical brain stimulation and lesioning have proven to be successful in patients suffering from treatment-resistant obsessive-compulsive disorder and major depressive disorder, two psychiatric disorders related to AN [21, 24, 26]. However, in AN it is unknown in which brain targets neuromodulation may relieve symptoms.

Previous results from a functional neuroimaging study [36] in which the cerebral $^{18}$F-fluorodeoxyglucose (FDG) uptake of rats in the ABA model was compared to normal rats, revealed certain hyperactive regions, among which the mediodorsal thalamus (MD). Based upon these findings and since this target selection strategy has been successful in major depressive disorder [21], we chose the MD as a neuromodulation target in the present study.

We hypothesize that an electrolytic lesion and/or electrical brain stimulation in the mediodorsal thalamus increases survival, body weight and food intake, and reduces locomotor activity.
3. MATERIALS AND METHODS

3.1. Experiment 1

3.1.1. Subjects and housing
Experiments were conducted on 48 female Wistar rats weighing 200–250 g at arrival. They were housed individually with food and water ad libitum available. A 12:12 h light/dark cycle was imposed (lights on at 7:00 a.m.). The temperature in the testing room was kept constant at ±20°C. All experiments were carried out in accordance with protocols approved by the local university animal ethics committee and in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

3.1.2. Experimental groups
Rats were divided into 5 groups. The first group (stim MD+ABA) was stimulated in the MD, the second one (no stim MD+ABA; negative control) was not stimulated, the third one (stim MD–ABA; ABA-specificity control) was stimulated in the MD, but had 24h access to food, the fourth one (stim random+ABA; MD-specificity control) was stimulated in a random nucleus and the fifth group (lesion MD+ABA) received a lesion in the MD.

3.1.3. Surgery
Monopolar platinum-iridium stimulation electrodes (200 µm diameter) with a bare transversally cut tip were stereotactically implanted under general anaesthesia (0.5g/kg chloral hydrate, s.c.; Sigma C-8383, Steinheim, Germany) bilaterally in the MD (stim MD+ABA, no stim MD+ABA and stim MD–ABA groups; 2.9 mm posterior to bregma, 2.2 mm lateral to the midline, 5.3 mm ventral to the dural surface, 15° angle to the sagittal plane [27]) or in a region elsewhere in the brain not activated on PET [36] (stim random+ABA group) (caudate putamen, cingulate area 2, septofimbrial nucleus, CA1 hippocampus, peduncular part of lateral hypothalamus, external globus pallidus, zona incerta and substantia nigra reticular part, one rat for each region). For the production of electrolytic lesions (lesion MD+ABA group), insulated stainless steel electrodes (200 µm diameter) with a bare tip of 0.5 mm were implanted bilaterally in the MD (coordinates, see above). In order to keep the observer blind for group division during subsequent testing, two connectors were mounted on the skull of all animals. Those connectors conducted the current in case of electrical stimulation or lesioning. The surgery and introduction in the ABA cage were on diestrus 2 of the oestrus cycle as determined by light microscopy evaluation (x10 and x20 magnification; Leica DM LA; Leica Microsystems, Wetzlar, Germany) of vaginal smears [19]. The oestrus cycle may indeed affect food intake and motor activity [4].

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3.1.4. ABA model

All experiments were conducted in custom-made ABA cages (0.36x0.36x0.36 m) with a running wheel (Ø 0.35 m) (Campden Instruments, Loughborough, UK). An electro-magnetic rotary encoder (TWK-Elektronik GmbH, Düsseldorf, Germany) was mounted on the shaft of the running wheel for continuous registration of the position of the running wheel with a digital I/O card (National Instruments, Austin, Texas, USA) and LabVIEW 7.0 (National Instruments).

Eight days after surgery, rats were introduced in the ABA cages with 1.5 h access to 50 g of food daily starting at 9.30 a.m., except for the stim MD–ABA group which had 24 h access to food. In all groups, daily food intake (g) and body weight (g) were measured at 11 a.m. Water was ad libitum available during the whole day. The intervention started when body weight decreased to less than 85% compared to body weight at introduction in the ABA cage. From the intervention moment on, rats in the stim MD+ABA, stim random+ABA and stim MD–ABA groups were bilaterally stimulated with biphasic rectangular pulses of 60 µs per phase at 100 Hz, with for each rat the highest amplitude that caused no visible disturbance of normal behaviour during a 3-minute observation period. Rats from the stim MD–ABA group did not develop activity-based anorexia, because they were not food-restricted. These rats never reached the intervention criterion (< 85% body weight), so they were assigned a “sister” in the stim MD+ABA group. For each rat of the stim MD–ABA group, stimulation was started at the same moment as for its “sister”. In rats of the lesion MD+ABA group, bilateral electrolytic lesions were produced at the intervention moment, through the already implanted electrodes with the same lesioning parameters as in experiment 2 (see below). Rats that were not stimulated (lesion MD+ABA group and no stim+ABA group) were also connected with cables to the stimulator in order to keep the observer blind for group division during subsequent testing.

3.1.5. Euthanasia and histology

At the end of the experiment (i.e. when the body weight was decreased to ≤ 70% of the initial body weight or after 10 days of intervention), animals were euthanized with an overdose of Nembutal (3 ml, i.p.; CEVA Santé Animale, Brussels, Belgium) and brains were processed for paraffin coronal sectioning (5 µm). Brain slices were stained with cresyl violet (Merck KGaA, Darmstadt, Germany) and examined under a light microscope. Lesion volumes were estimated for both lesions in each rat of the lesion MD+ABA group (experiment 1) and the lesion group (experiment 2). The antero-posterior depth of the lesion was calculated as the distance between the first and the last histological slices which showed a section of the lesion. In addition, the maximal medio-lateral width and dorso-ventral height of the lesions on the histological slice with the largest lesion area were determined. The approximate lesion volume (mm³) was calculated using the ellipsoid formula (V = DWHπ/6, where V is volume, D is depth, W is width, and H is height) [2]. For each group the mean and standard deviation of lesion volumes were calculated. Bilateral lesion volume was correlated with survival.
(Pearson correlation) to exclude an effect of lesion size on survival. Rats were included in statistical analyses when the tips of the electrodes or the lesions were bilaterally located in the target region.

3.2. Experiment 2

Experiment 2 was performed in the same way as experiment 1, with minor differences:

Experiments were conducted on 16 male Wistar rats weighing 300–350 g at arrival. Rats were divided into 2 groups: lesion and control.

MD lesions were made during surgery (lesion group; 0.8 mm posterior to bregma, 2.2 mm lateral to the midline, 4.8 mm ventral to the dural surface) with an anodal direct current pulse of 0.5 mA during 15 seconds. After lesioning, the electrodes were removed. In the control group, the skin was incised and burr holes were made but no electrodes were inserted. At the end of surgery, the wound was sutured.

The experiment ended when body weight was decreased to ≤ 70% of the initial body weight or when the rats developed a stable body weight (body weight on day x+4 > body weight on day x [34]). Cryostat sections (25µm) were made instead of paraffin sections.

3.3. Statistical analysis

The average % body weight (body weight on day x / body weight on the day of introduction in the ABA cage), % food intake (food intake on day x / body weight on day x) and running activity (the number of wheel revolutions during the 24 hours preceding the measurements on day x) observed after start of the intervention were compared between groups using multivariate regression models, with group as a factor and a heterogeneous autoregressive covariance structure to account for the within-rat...
correlation over time. P-values for multiple comparisons between groups were adapted using Tukey’s procedure. A comparison of average time trends was not considered. A natural logarithm (log) was used as transformation for the number of wheel revolutions to obtain a symmetric distribution of the residuals in the model. The analyses were performed using the SAS procedure PROC MIXED, which can cope with a varying number of observations between rats. Missedness at random was assumed. Details on this statistical approach can be found in Verbeke and Molenberghs [37]. Reported p-values are two-sided and were considered significant if $P < 0.05$.

The survival time (defined as time to the day that body weight decreased to $\leq 70\%$ of initial body weight) was compared between groups using an exact log-rank test (using the package StatXact 3.0). Survival times were censored in experiment 1 if rats did not reach the 70% body weight criterion during the intervention period of maximally 10 days ($n = 9$) or in case of premature termination of the experiment ($n = 3$). Survival times were censored in experiment 2 if rats developed a stable body weight (body weight on day $x+4 >$ body weight on day $x$) and thus considered to survive the experimental period ($n = 10$) or in case of premature termination of the experiment ($n = 1$). Bonferroni correction was applied for multiple testing, P-values smaller than 0.005 are considered statistically significant.

4. RESULTS

4.1. Experiment 1

The average stimulation amplitudes (the highest amplitudes that caused no visible disturbance of normal behaviour during a 3-minute observation period) are 1272μA (range: 470 μA – 2700 μA) in the stim group.

![Fig. 2: Estimates of the mean (± standard error) percentage body weight (A), percentage food intake (B) and log rotations (C) in all groups.](image)

*Significantly different from all other groups at $P < 0.01$. #Significantly different from the stim MD+ABA group at $P = 0.0165$.

Stim: electrical stimulation; +ABA: 24h running wheel and 1.5h food availability daily; -ABA: 24h running wheel and 24h food availability daily.
MD+ABA group, 1650 µA (range: 700 µA – 2500 µA) in the stim MD–ABA group, and 1158 µA (range: 200 µA – 2400 µA) in the stim random+ABA group. These amplitudes are not significantly different between the 3 groups (Kruskal-Wallis, P = 0.35). Visible disturbances of normal behaviour induced by stimulation with maximal stimulation amplitudes during the 3-min observation period included extreme salivation, facial and oral activity, head bobbing, chasing its own tail, forelimb clonus, narrowing the eyes and rearing accompanied by spastic head movements. The localization of the electrode tips targeted to the MD is shown on Fig. 1.

In the lesion MD+ABA group, the unilateral lesion volume (mean ± standard deviation) was 1.01 mm³ ± 1.19 mm³. There was no significant correlation of bilateral lesion extent with survival (r = 0.45, P > .05).

In the ‘+ABA’ groups, body weight decreased to less than 85% after 7 ± 2.3 days (mean ± standard deviation) in the ABA cage. The stim MD–ABA rats never reached this weight loss criterion, since they were not food-restricted.

Percentage body weight (Fig. 2A) is significantly higher (P < 0.01) in the stim MD–ABA group compared to all other groups and in the no stim MD+ABA group compared to the stim MD+ABA group (P = 0.0165). Percentage food intake (Fig. 2B) is significantly higher (P < 0.01) in the stim MD–ABA group compared to all other groups. Locomotor activity is (Fig. 2C) significantly lower (P < 0.01) in the stim MD–ABA group compared to all other groups. The distances run per day were 4.2 km (± 1.4 km) in the stim MD+ABA group, 4.7 km (± 1.6 km) in the no stim MD+ABA group, 0.9 km (± 0.2 km) in the stim MD–ABA group, 3.4 km (± 2.5 km) in the stim random+ABA group and 3.8 km (± 1.8 km) in the lesion MD+ABA group (distances are means (± standard deviations) per group, calculated from raw data averaged per rat).

Log-rank analysis indicates no significantly increased survival of the stim MD+ABA group compared to the no stim MD+ABA group, stim random+ABA group or lesion MD+ABA group (Fig. 3). On the other hand, the survival probability is
significantly higher in the stim MD–ABA group compared to the stim MD+ABA group (P = 0.0022) and the no stim MD+ABA group (P = 0.0001).

4.2. Experiment 2

In the lesion group, the unilateral lesion volume (mean ± standard deviation) was 0.93 mm³ ± 0.82 mm³. There was no significant correlation of bilateral lesion extent with survival (r = -0.08, P > .05). Fig. 4 shows a representative lesion.

In the lesion and control groups, body weight decreased to 85% after 11 ± 4.1 days and 7 ± 1.8 days respectively (mean ± standard deviation) in the ABA cage. Percentage body weight (Fig. 5A) and percentage food intake (Fig. 5B) in the lesion group are not significantly different (P = 0.73, P = 0.27 respectively) from the control group. However, locomotor activity (Fig. 5C) is significantly lower (P = 0.037) in the lesion group compared to the control group. The distances run per day were 2.0 km (± 1.2 km) in the control group and 1.2 km (± 1.1 km) in the lesion group (distances are means (± standard deviations) per group, calculated from raw data averaged per rat).

Log-rank survival analysis indicates no significantly increased survival of the lesioned animals compared to controls (P = 0.598) (Fig. 6).

5. DISCUSSION

In the current study, we were able to prevent excessive running activity in the ABA model with electrolytic MD lesions. However, a curative lesion or electrical stimulation in the MD of rats with fully developed activity anorexia, had no effect on this hyperactivity. We observed no increase in survival, body weight and food intake, neither with preventive or curative MD lesions, nor with electrical stimulation in the MD. As we were only able to modulate the hyperactivity, and not the other principal parameters in the model, the MD might not be the optimal target for therapeutic neuromodulation in anorexia nervosa. However, other core symptoms of anorexia nervosa, like the disturbance of body weight and shape perception, could not be investigated in the animal model.
5.1. Selection of target and stimulation parameters

The strategy of choosing a target for stimulation based on functional neuroimaging has previously been proven successful in humans. Based upon the observation that the subgenual cingulate region was metabolically overactive in patients with treatment-resistant major depressive disorder compared to controls, six patients were electrically stimulated in the white matter tracts adjacent to the subgenual cingulate gyrus. Chronic stimulation induced a striking and sustained remission of depression in four of them [21]. According to the same line of reasoning, the posterior hypothalamus was chosen as a stimulation target in a patient with severe intractable chronic cluster headache [5]. Electrical stimulation produced complete and long-term pain relief without major side effects.

In the present study, the MD was selected because of its increased metabolism in a preceding PET study in rats in the ABA model compared to healthy controls [35]. This PET study presented valuable insights into the metabolic changes due to the activity anorexia, but the results of the present study do not convincingly support the hypothesized efficacy of lesioning or high-frequency stimulation of the hyperactive MD. One plausible explanation for the lack of effects of an electrolytic lesion or electrical stimulation may be that FDG-PET scanning is performed under general anesthetic instead of in the awake state. It is possible that FDG-PET activity measured under general anesthetic does not optimally represent brain activity related to the neurocircuitry in the model. In addition, pentobarbital anaesthesia, although administered in both ABA and control groups, might produce region-specific metabolic changes, thereby concealing the true activity in the neural circuitry of AN [11, 20].

Electrical stimulation was performed with a frequency of 100 Hz. Although higher frequencies are used for motor disorders (e.g. 130 to 180 Hz in Parkinson’s disease), stimulation with 100 Hz induced symptom-relief in patients suffering from treatment-resistant obsessive-compulsive disorder, a psychiatric disorder that is highly co-morbid in patients with AN [25]. Moreover, it is observed in other brain areas (e.g. ventral tegmental area and lateral hypothalamus) that electrical stimulation-induced increases in food intake level off with frequencies of 50 to 100 Hz [18, 38].
5.2. Relation MD with AN

The MD is part of a cortico-striato-thalamo-cortical circuitry: In this circuitry, which has previously been linked to neuropsychiatric disorders like obsessive-compulsive disorder, the cingulate cortex projects to the ventral striatum (including the nucleus accumbens), which in turn projects via the ventral pallidum to the MD, which closes the loop by sending projections to the cingulate cortex [22]. The cingulate cortex and the ventral striatum are also involved in the ABA model [36]. Neuromodulation in brain areas of this circuitry in patients with obsessive-compulsive disorder may relieve symptoms [24]. Furthermore, a bilateral stereotactic thalamotomy induced weight gain in 2 patients suffering from extremely severe, chronic and refractory AN [40].

5.3. Relation MD with motor activity

An electrolytic MD lesion, applied before introduction in the ABA model, decreased locomotor activity in rats in the ABA model in experiment 2.

The MD appears to be an important component of the neural circuitry involved in regulating spontaneous and psychostimulant-induced motor activity [23]. The MD receives input from structures closely related to the basal ganglia [7, 14] and has robust reciprocal connections with the prelimbic prefrontal cortex, which, in turn, innervates nucleus accumbens and the ventral tegmental area [7]. Both prefrontal cortex and nucleus accumbens receive dopaminergic input, and axon terminals from MD and ventral tegmental area converge on the same pyramidal cells in deep layers of the rat prefrontal cortex [16], suggesting that the MD is in a strong position to regulate prefrontal cortex dopamine function and motor activity.

Indeed, pharmacological blockade of glutamatergic projections from MD to prefrontal cortex has been shown to decrease prefrontal dopamine transmission, increase dopamine transmission in the nucleus accumbens and enhance locomotion [13, 14]. Also injections in the MD of a mu-opioid receptor agonist elicited a dose dependent increase in motor activity [15].

However, MD lesioning studies have yielded conflicting data on motor activity. For instance, excitotoxic MD lesions in rats increased spontaneous motor activity [10, 39]. In contrast, ibotenic MD lesions did not change the motor activity in rats in response to mild stress and amphetamine. [17]. Also electrolytic MD lesions in rats had no effect on baseline locomotion, however, they did diminish
the locomotor activation produced by intracerebral injection of the gamma-aminobutyric acid antagonist picrotoxin into the ventral pallidum [33]. Bilateral MD lesions also reduce the locomotor response observed following stimulation of dopamine receptors in the nucleus accumbens [32].

5.4. Outcome differences between experiment 1 and 2
Outcome differences between experiment 1 and 2 could be attributed to different experimental designs. In experiment 2 we used male instead of female rats. Doerries found that male rats were more susceptible to the ABA model than were female rats since they reached the weight loss criterion more rapidly and ate less [3]. In addition, using male rats excluded potential effects of the strong behavioural rhythms that female rats display during the oestrus cycle [4]. Although the female rats were synchronized in experiment 1, the fluctuating oestrus cycle still might have influenced food intake and motor activity. Also the time of intervention i.e. the start of stimulation or making the lesion (at 85% of initial body weight for experiment 1 and at surgery for experiment 2) could be a factor of variation. In experiment 2, the lesion could already have an effect during ABA development, which was not the case in the first experiment. Due to the experimental design, the follow-up was much longer in experiment 2 (16 to 35 days) compared to experiment 1 (2 to 10 days).

6. CONCLUSION
In the current study, neither an electrolytic lesion nor high-frequency electrical stimulation affected behaviour in the activity-based anorexia model. Future research should further validate the novel approach of identifying treatment targets based on functional neuroimaging in animal models. Either other stimulation parameters could be tested in the MD or other brain areas within the circuitry revealed by functional neuroimaging could be investigated.

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8. REFERENCES


