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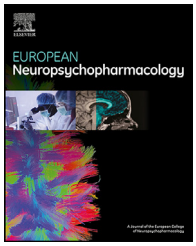

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Intranasal administration of olanzapine has beneficial outcome in a rat activity-based anorexia model

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Abstract
The atypical antipsychotic drug olanzapine is prescribed despite clinical studies on olanzapine treatment showing mixed results on treatment efficacy in anorexia nervosa. We investigated the effect of systemic and intranasal administration of olanzapine in the activity-based anorexia (ABA) model. Rats were habituated to a running wheel and exposed to the ABA model while treated with olanzapine. During ABA rats had 1.5 h of daily access to food and ad libitum access to a running wheel for seven consecutive days. Olanzapine was administered via an osmotic minipump (1, 2.75, and 7.5 mg/kg) or intranasally 2 h before dark onset (1 and 2.75 mg/kg). We monitored body weight, food intake, wheel revolutions, body temperature, and adipose tissue.
We found 2.75 and 7.5 mg/kg systemic olanzapine decreased wheel revolutions during ABA. Relative adipose tissue mass was increased in the 7.5 mg/kg olanzapine-treated group while body weight, food intake, and body temperature were unaltered by the systemic olanzapine. 1 and 2.75 mg/kg intranasal olanzapine diminished wheel revolutions and body temperature

Abbreviations: ABA, Activity based anorexia; AN, Anorexia nervosa; BW, Body weight; Cum, Cumulative; FAA, Food anticipatory activity; FI, Food intake; Rot, Rotation; RWA, Running wheel activity; WAT, White adipose tissue.

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during the first 2 h after administration. The intranasal olanzapine-treated rats had a higher body weight at the end of ABA.
We find that olanzapine has beneficial outcomes in the ABA via two administration routes by acting mainly on running wheel activity. Intranasal olanzapine showed a rapid effect in the first hours after administration in reducing locomotor activity. We recommend further exploring intranasal administration of olanzapine in anorectic patients to assist them in coping with restlessness.

1. Introduction

Anorexia nervosa (AN) is a severe mental disorder that most often affects adolescent women and has the highest mortality rate among psychiatric disorders (Arcelus et al., 2011). AN is characterized by hypophagia, fear of weight gain, body image disturbance, and often high levels of activity. Typically only half of the patients fully recover (Steinhausen, 2002). There is no approved pharmacological treatment for AN, but antipsychotics like olanzapine are prescribed off-label in the clinical setting to reduce irrational fears around food intake and gaining weight as well as diminish hyperactivity.

The efficacy of olanzapine in increasing body weight and improving eating disorder symptoms is debated. Most randomized control trials reported positive outcomes when olanzapine was used as pharmacological treatment (Attia et al., 2011, 2019; Bissada et al., 2008; Brambilla et al., 2014), while others do not (Brambilla et al., 2007; Kafantaris et al., 2011). Interestingly, two recent meta-analyses on the existing randomized controlled trials and case-control studies concluded beneficial (Çöpür and Çöpür, 2020) and non-beneficial (Cassioli et al., 2020) effectiveness of olanzapine on BMI or AN psychopathology. These inconclusive results can be caused by the heterogeneity between patients and studies using several doses and durations of olanzapine treatment and combination with other treatments. Animal models allow to unravel the effects of olanzapine in a controlled manner.

In an earlier study, a high dose of olanzapine gave advantageous outcomes in an animal model for anorexia, the activity-based anorexia (ABA) model. In the ABA model rodents have access to a running wheel in their cage. After being habituated to the wheel, the animals get food only for a restricted time each day. They then paradoxically increase running wheel activity, in particular during the inactive light phase. This hyperactivity combined with hypophagia results in a dramatic reduction in body weight (Epling et al., 1983; Routtenberg and Kuznesof, 1967) mimicking a subset of the AN symptomology. Since then, the ABA model has been the most used model for anorexia nervosa (Scharner and Stengel, 2021), with the applications reviewed in several articles (Foldi et al., 2017; Méquinion et al., 2015; J. Zhang and Dulawa, 2021). Throughout ABA exposure body temperature is lowered, likely to save energy. Hillebrand and colleagues found that daily 7.5 mg/kg systemic olanzapine reduced running wheel activity and increased body temperature and fat mass (Hillebrand et al., 2005). Similarly, 12 mg/kg/day increases survival in ABA by reducing food anticipatory activity (Klenotich et al., 2012). These high doses of olanzapine can result in immobility due to the dose-dependent sedative effect (Ahnaou et al., 2003). Outside of an ABA context, not only high (Evers et al., 2010) but also a lower dose of systemic olanzapine suppressed locomotor activity and increases motivation for a food reward in ad-lib-fed rats (van der Zwaal et al., 2010, 2012). These studies warrant testing dose dependency of systemic olanzapine in the ABA model, particularly given the inconclusive patient studies using different doses.

Recently, intranasal drug administration has gained interest when targeting the central nervous system (Erdő et al., 2018). We reasoned that intranasal administration might be more acceptable than oral administration in AN patients. Delivery of a drug in the vascular-rich nasal cavity improves the overall bioavailability of drugs compared to oral administration and may bypass the blood-brain barrier via the trigeminal and olfactory nerves (Dufes et al., 2003; Gartziandia et al., 2016). A phase one clinical trial to reduce agitation showed that intranasal application of olanzapine resulted in rapid absorption and pharmacodynamic effects (Shrewsbury et al., 2020). These fast pharmacodynamic kinetics allow acute application of olanzapine and potentially help AN patients around the fearful moments of eating and drive for activity. This would minimize systemic exposure and limit related side effects such as constipation and sleepiness. Additionally, the nasal route is a none invasive delivery method that does not require swallowing. This might help AN patients that fear swallowing food and medication called oropharyngeal dysphagia (Holmes et al., 2016). To controllably test its potential, intranasal administration should be tested in animals first.

Our goal was to test the efficacy of lower doses of systemic (via minipumps) and intranasal administration olanzapine on the development of ABA. This controlled pre-clinical study administered olanzapine via osmotic minipumps to have a continuous drug concentration as oral administration has a rapid half-life in rats. Exploration of intranasal administration could have potential advantages in AN patients.

2. Experimental procedures

2.1. Subjects

Female outbred WU rats (Crl:WU, $n = 96$, Charles River, Germany) weighing between 150 and 175 g ($\pm$ 6 to 7 weeks old) were socially

housed (conventionally in euro standard type IV cages, Tecniplast, Italy) upon arrival. The room was temperature- (21 °C ± 2 °C) and humidity-controlled (55% ± 10%) under a shifted light-dark cycle (1 pm lights off). The central committee for animal experiments (CCD) and the University of Utrecht ethical committee for animal experiments approved all described procedures (AVD1150020198686 & AVD1150020016799). All experiments were performed according to ARRIVE guidelines. Total discomfort for the animals was considered to be moderate, based on surgery and food restriction. The humane endpoint was reached when one of the following three criteria was met: a body weight loss of more than 20% after the eating period, food intake below 5 gs for 2 consecutive days, or when the body temperature was below 33 °C. No subject reached one of these endpoints.

The rats underwent the following phases: acclimatization (5 days), transmitter surgery and recovery (2 weeks), habituation running wheel cages (10 days), minipump surgery (only chronic infusion experiment, 1 day), ABA (5 or 7 days). The five days protocol was used because of a permit restriction (AVD1150020016799), while the experiments later were permitted seven days (AVD1150020198686).

2.2. Surgical procedures

On the fifth day after arrival, the rats underwent surgery to implant transmitters (TA10TA-F40 Datasciences International, St. Paul, Minnesota) in the abdominal cavity. The rats received analgesia by carprofen (Carporal, 50 mg/ml) via drinking water (0.054 mg/ml) one day before surgery and via subcutaneous injection (5 mg/kg) at least 30 min before surgery. The rats were anesthetized with isoflurane (Zoetis, 100% w/w) (5% induction, 2% maintenance flow rate 0.5 l/min). First hairs on the abdominal cavity were shaved whereafter the rats were placed on a heating pad of 37 °C to maintain body temperature. A 2 cm insertion in the abdominal cavity was made to place the transmitter and 1 ml saline, whereafter it was closed by intramuscular and intradermal continuous suture. The instruments were sterilized with a beat sterilizer after every subject. After surgery, the rats had access to carprofen water for 5 days. Rats were weighed daily to monitor recovery.

An additional surgery was performed for the chronic olanzapine experiments on ABA day 0. Osmotic minipumps (Alzet MINI-OSMOTIC PUMP MODEL 2001,Cupertino, CA) were filled with 8.32, 22.92, or, 62.5 mg/ml olanzapine or vehicle. These pumps were placed subcutaneously in the dorsal flank under a similar isoflurane and carprofen protocol 3–6 h before the start of the food restriction phase.

2.3. Experimental set-up

The systemic experiment was executed in two separate batches and the intranasal experiment in four due to the limited number of cages available. After two weeks of recovery from surgery, the rats were individually housed in a 42.2 × 42.2 × 47.5 cm cage including a running wheel (1-meter perimeter). During the first ten days chow (CRM(E), special diet service, UK), water, and the running wheel were available ad libitum. After that, food access was restricted to the first 1.5 h during the active dark phase. The food restriction in combination with ad libitum wheel access, the activity-based anorexia (ABA) period, lasted for five to seven days due to different permissions on both ethical licenses. Body weight was measured daily half an hour before dark onset. The running wheel activity was continuously recorded by Cage Registration Program (CageRag version 5.0 Dep. Biomedical Engineering, UMC Utrecht, The Netherlands), and temperature and locomotion in the cage by Acquisition software (Dataquest A.R.T. 4.1, Datasciences).

2.4. Drugs

We administered 1, 2.75 and 7.5 mg/kg/day olanzapine to our rats, similar as earlier research in our lab (van der Zwaal et al., 2008, 2010). These doses are higher than those given to anorexia patients (2.5 to 20 mg/day) (RCTs reviewed in Çöpür and Çöpür, 2020) due to the faster metabolism of olanzapine in rodents, resulting in a half-life of 2.5 h in rodents and 21–54 h in humans (Aravagiri et al., 1999; Callaghan et al., 1999; Kapur et al., 2003). To establish constant plasma levels in the chronic experiment, we used osmotic minipumps. Rats were pseudo-randomly assigned (by hand for the systemic experiment, via RandoMice (Van Eenige et al., 2020) for the intranasal experiment) to one of the olanzapine doses (1, 2.75, or 7.5 mg/kg) or vehicle, based on baseline running wheel activity (d-3 till d0), body weight (BW) (gain since arrival and absolute BW D-3 till D-1), food intake (D-3 till D-1) and distribution in and over the room(s). Olanzapine (LY170053, Cayman Chemical Company, 100 mg) was dissolved in the original vial in 400 μl glacial acetic acid (Merck) (GAA) and diluted to the desired concentrations with saline. The pH was set to 5 in steps of 5 μl 5 M NaOH while vortexing between steps to prevent fluid precipitation. The osmotic minipumps were activated by overnight incubation in a water bath at 37 °C.

Intranasal olanzapine, dissolved in 5% polypropylene glycol, 100% GAA, and a pH of 5 set with 5 M NaOH (in 8 steps of 5 μl) or vehicle were administered (10 μl / 100 gr) daily 2 h before dark onset during the ABA restriction phase. Awake rats were fixated and small drops of 3–6 μl were applied with a Gilson pipette in front of the nasal cavity and inhaled by the rats. The nasal openings were alternated. Rats were trained on this procedure two times before the experiment. The systemic minipump experiment was performed blinded to the experimenter. The intranasal experiment could not be blinded due to substance color differences.

2.5. Dissection

During the light phase of the 5th or 7th day of food restriction, the rats were sedated in a CO_2 gas chamber (+−3 min), followed by decapitation. The corpus was dissected for visceral fat (epididymal and perirenal) and subcutaneous white adipose tissue, whereafter the tissue was weighted (Mettler, AG245, 0.01 mg accuracy).

2.6. Data analysis

Body weight, food intake, and fat mass data were processed in Microsoft Excel (version 2016) and visualized in Graphpad prism (version 9.2.0). The running wheel activity and the temperature were processed and visualized with custom-made Python scripts (version 3.7.4). The statistical analysis was performed in SPSS (version 27). There were no batch differences for each parameter, so the data were pooled. A mixed model analysis of variances (ANOVA) tested whether the relative body weight, food intake, running wheel activity, and temperature differed over time (within-subject variable), between the conditions (between-subject variable), and whether they interacted. If the data was not spherically distributed, the data were corrected by the Greenhouse Geisser (GG) correction. Dissection data and cumulative data were analysed with an ANOVA, Kruskal-Wallis, or Welch's test based on the data distribution. The post hocs control vs. olanzapine dose were analysed with an independent *t*-test or Mann-Whitney U test and corrected for multiple comparisons by a Dunnet's D.

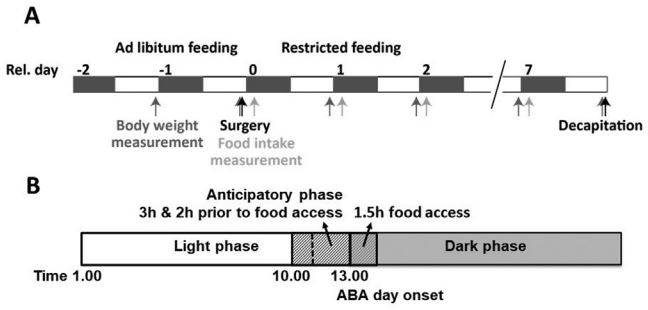

Fig. 1 Experimental overview. A schematic of the experimental design showing the procedures around the restriction phase (A). A schematic of one ABA day including the light, dark and anticipatory phase (B). The anticipatory phase was defined as 3 h in the minipump experiment and 2 h for the intranasal experiment due to the administration of the olanzapine.

3. Results

3.1. Olanzapine in the aba model via minipump administration

Before the start of the ABA phase, all groups had similar body weights ($F(3,41) = 0.4$, $p = 0.743$). When the rats were restricted to only 1.5 h chow access in the ABA period, all groups decreased the relative body weight (BW) as expected (Fig. 2A) (BW time $F_{(2.1,\ 85.4)} = 345.0$, $p < 0.001$). Over days the rats increased their food intake (FI) as they learned to eat during the time-restricted feeding period (Fig. 2B) (FI time $F_{(3.4,140.2)} = 287.2$, $p < 0.001$). Olanzapine altered FI, specifically 1 mg/kg olanzapine-treated rats ate more than control rats (FI condition $F_{(3,41)} = 4.9$, $p = 0.005$, ctr vs. 1 mg/kg $p = 0.041$). Yet, the systemic olanzapine administration did not impact on relative BW (Fig. 2A) (BW condition $F(3,41) = 0.3$, $p = 0.818$). After the ABA period fat mass was investigated. The 7.5 mg/kg treated group had significantly increased white adipose tissue (WAT) mass at the end of the 7 days experiment (Fig. 2C) (ANOVA $F_{(3,20)} = 5.9$, $p = 0.005$; ctr vs. 7.5 mg/kg $p = 0.004$). When analyzing the fat mass separately, we find an increase in subcutaneous WAT (sup Fig. 1A) (sWAT condition $F_{(3,8.3)} = 11.1$, $p = 0.003$; ctr vs. 1 mg/kg $p = 0.965$; ctr vs. 2.75 mg/kg $p = 0.016$; ctr vs. 7.5 mg/kg $p = 0.014$), but not in the visceral WAT (sup Fig. 1B, C) (epididymal $F(3,20) = 1.4$, $p = 0.272$; perirenal $(3,1.174) = 1.8$, $p = 0.204$). Thus, olanzapine treatment during ABA did not affect BW, although 1 mg/kg olanzapine increased FI during ABA while 2.75 and 7.5 mg/kg olanzapine elevated subcutaneous fat mass.

Another outcome measure was physical activity. The general locomotor activity (as assessed by telemetry and reflecting home cage activity) in the cage was unaltered by olanzapine treatment during the ABA phase (sup Table 1) ($H(3) = 2.9$, $p = 0.409$). However, total running wheel activity (RWA) during ABA (day 0–5) was decreased by 2.75 mg/kg and 7.5 mg/kg olanzapine (Fig. 3B) (RWA condition $F_{(3,\ 22.3)} = 25.3$, $p < 0.001$; ctr vs. 2.75 mg/kg $p = 0.010$; ctr vs. 7.5 mg/kg $p < 0.001$). When considering the active dark phases during ABA, olanzapine reduced running wheel revolutions and had a significant time x condition interaction effect (Fig. 3D) (condition $F_{(1,3)} = 8.5$, $p < 0.001$; time x condition $F_{(10.4,142.7)} = 2.2$, $p = 0.019$). Specifically the 2.75 mg/kg and the 7.5 mg/kg reduced dark phase rotations relative to controls (ctr vs. 2.75 mg/kg $p = 0.002$; ctr vs. 7.5 mg/kg $p < 0.001$).

One hallmark of the ABA paradigm is that animals start to show running before the feeding period. This gradual increase of food anticipatory activity (FAA) over ABA days was also seen in our experiment (Fig. 3A & 3C) (time $F_{(2.4,\ 98.5)} = 19.9$, $p < 0.001$). Overall, olanzapine impacts FAA (condition $F_{(1,3)} = 3.7$, $p = 0.020$), however, post hoc comparisons did not reveal differences between the control and the olanzapine-treated groups. During the entire light phase, RWA increased over time but was not significantly different when treated with olanzapine (time $F_{(1.8,\ 75.0)} = 16.0$, $p < 0.001$; condition $F_{(1,3)} = 2.8$, $p = 0.053$). In summary, 2.75 & 7.5 mg/kg systemic olanzapine decreased RWA during the active dark phase.

Lastly, we continuously measured body temperature during ABA. During ABA, body temperature decreased over ABA days during the light and dark phase as expected (Sup Fig. 2A) (light time $F_{(2.5,\ 91.8)} = 44.9$, $p < 0.001$; dark time $F_{(1.4,\ 51.5)} = 4.8$, $p = 0.032$). Olanzapine treatment did not alter the body temperature (light condition $F_{(1,3)} = 0.5$, $p = 0.693$; dark condition $F_{(1,3)} = 0.1$, $p = 0.983$).

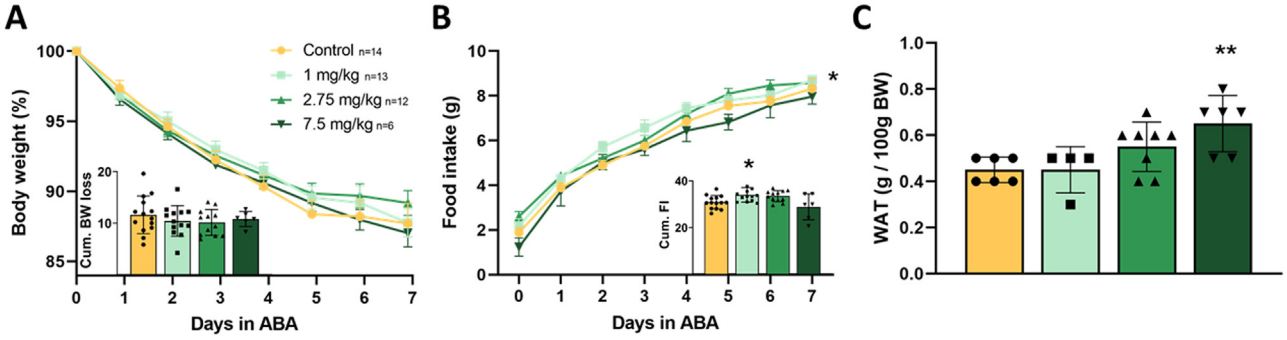

Fig. 2 Effects of minipump-administered olanzapine on body weight (BW), food intake (FI), and fat mass when exposed to the activity-based anorexia (ABA) model. Relative BW (A) and FI (B) per day and cumulative (Cum.) during the ABA period. White adipose tissue (WAT) (C) at the end of the 7-day ABA exposure. Results expressed as group means +- SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control.

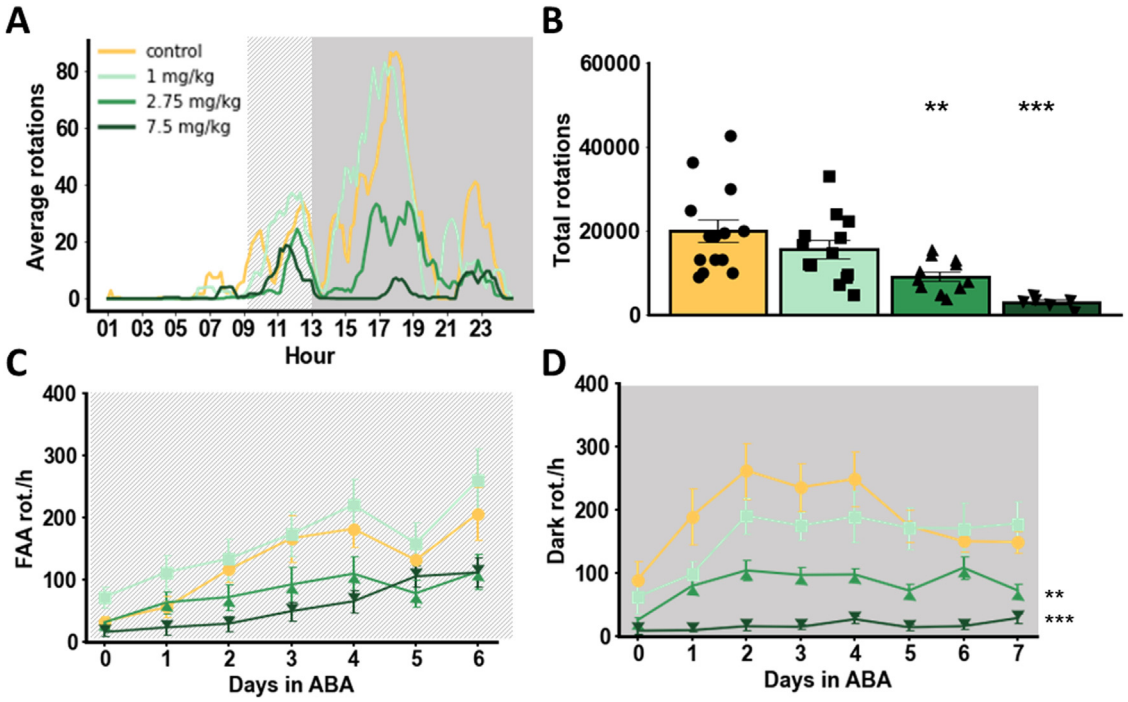

Fig. 3 Running wheel activity (RWA) upon minipump olanzapine during the activity-based anorexia (ABA) model. The smoothed average RWA on ABA day 4 (A). Cumulative revolutions during the ABA period (B). Average rotations per hour (rot/hr) per day during the 3 h anticipatory period (C) and the dark period (D). Results expressed as group means +- SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control.

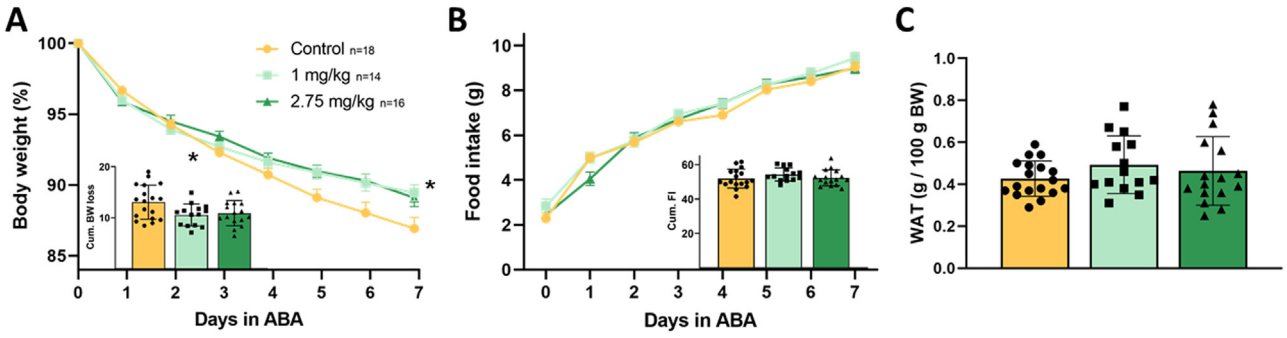

Fig. 4 Body weight (BW), food intake (FI), and fat mass during the activity-based anorexia (ABA) model after intranasal administration of olanzapine. Relative BW (A) and FI (B) per day and cumulative (Cum.) during the ABA period with daily intranasal olanzapine administration. The white adipose tissue (WAT) mass (C) at the end of the 7-day ABA exposure. Results expressed as group means +- SEM. $^{*}p<0.05$ vs. control.

3.2. Olanzapine in the aba model via intranasal administration

During this experiment, two doses of olanzapine (1 & 2.75 mg/kg) were administered intranasally 2 h prior to the feeding period on each ABA day. Before ABA, all treatment groups had comparable body weights ($F_{(2,45)} = 0.2$, $p = 0.785$). The olanzapine intervention resulted in a different BW development during the ABA phase (Fig. 4A) (time $F_{(2.0, 89.7)} = 457.4$, $p < 0.001$; time x condition $F_{(4.0, 89.7)} = 5.0$, $p = 0.001$). The relative BW loss at the end of the ABA period was significantly decreased for the 1 mg/kg group and had a trend for the 2.75 mg/kg condition (groups $F_{(2,45)} = 4.08$, $p = 0.024$; ctr vs. 1 mg/kg $p = 0.026$; ctr vs. 2.75 mg/kg $p = 0.051$). Interestingly, FI increased during the ABA period but was not affected by olanzapine delivery (Fig. 4B) (time $F_{(4.3, 489.5)} = 350.2$, $p < 0.001$; condition $F_{(1,2)} = 1.1$, $p = 0.330$). Moreover, WAT (Fig. 4C) mass was not different between treatment groups at the end of the 7 days ABA experiment (WAT $H_{(2)} = 0.8$, $p = 0.674$). However, when analyzing the fat mass separately, the 1 mg/kg group had increased perirenal WAT (sup Fig. 1E) (pWAT condition $H(2) = 6.5$, $p = 0.038$; ctr vs. 1 mg/kg $p = 0.022$; ctr vs. 2.75 mg/kg $p = 0.210$), while epididymal and subcutaneous WAT were unaltered (sup Fig. 1D, F) (epididymal $H(2) = 1.2$, $p = 0.558$; subcutaneous

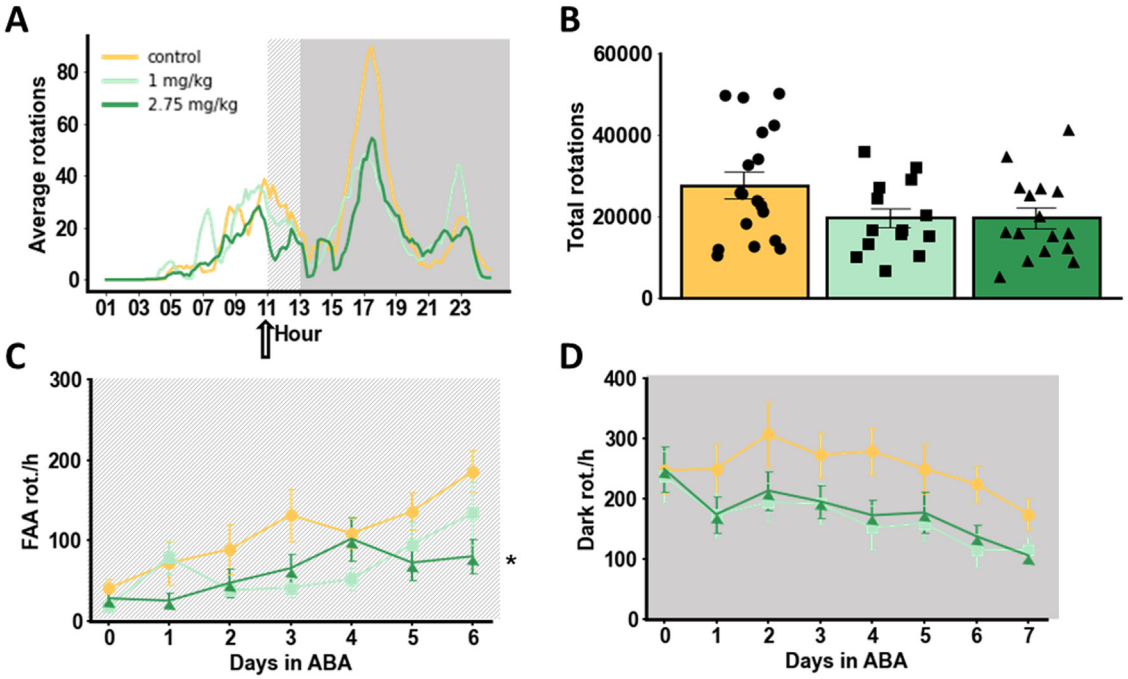

Fig. 5 Effects of intranasal olanzapine administration on running wheel activity (RWA) during the activity-based anorexia (ABA) model. Average running wheel activity (RWA) on ABA day 6 of the intranasal experiment (A). Cumulative revolutions during ABA (B). Average rotations per hour (rot/h) per day during the 2 h anticipatory period (C) and the dark period (C). Results expressed as group means +- SEM. *$P < 0.05$ vs. control.

$F_{(2,25.7)} = 1.2$, $p = 0.307$). Together this shows that 1 mg/kg intranasal olanzapine decreased BW loss and increased perirenal WAT mass without affecting food intake.

Perhaps the difference in body weight can be explained by altered activity. Intranasal olanzapine did not modify the total wheel revolutions during ABA (day 0–7) significantly (groups $F_{(2,45)} = 2.8$, $p = 0.072$) (Fig. 5B). However, when extracting the FAA 2 h after olanzapine administration, RWA was decreased in the 2.75 mg/kg treated group but not in the 1 mg/kg group (Fig. 5D) (condition $F_{(1,2)} = 3.2$, $p = 0.049$; ctr vs. 2.75 mg/kg $p = 0.048$; ctr vs. 1 mg/kg $p = 0.097$). During the dark phase, RWA increased over the ABA days and had a trend towards an olanzapine-dependent reduction (Fig. 5C) (time $F_{(4.7,\ 209.9)} = 7.4$, $p < 0.001$; condition $F_{(1,2)} = 3.2$, $p = 0.051$). General locomotor activity in the cage was neither altered by olanzapine during ABA ($H(2) = 0.2$, $p = 0.896$), nor during the first 2 h after administration (sup Table 1) ($H(2) = 1.5$, $p = 0.472$). Thus, intranasal olanzapine slightly reduces RWA, mainly shortly after administration.

Intranasal olanzapine administration acutely lowered the BT on day 0 (Fig. 6A) which remained lower during the anticipatory phases for all ABA days (Fig. 6C) (condition $F_{(1,2)} = 25.3$, $p < 0.001$; ctr vs. 1 mg/kg $p < 0.01$; ctr vs. 2.75 mg/kg $p < 0.001$). This temperature reduction by olanzapine appeared temporal as BT was not significantly different from controls during the dark phase (Fig. 6D) (condition $F_{(1,2)} = 3.1$, $p = 0.056$). The opposite direction was observed during the light phase before the administration, where the decrease in BT during ABA was less pronounced in the treated groups (Sup Fig. 2B) (light time $F_{(3.8,\ 164.5)} = 97.6$, $p < 0.001$; group $F_{(1,2)} = 4.4$, $p = 0.018$; time x group $F_{(7.6,\ 164.5)} = 3.2$, $p = 0.002$). Specifically, the 2.75 mg/kg group had a higher BT (ctr vs. 2.75 mg/kg $p = 0.012$). Overall body temperature was not changed (Fig. 6B) ($H_{(2)} = 1.4$, $p = 0.485$). So, intranasal olanzapine administration acutely lowered BT but increased the temperature right before re-administration.

4. Discussion

In this study, we investigated systemic and intranasal administration of various doses olanzapine in the activity-based anorexia model. Administering olanzapine via both routes reduced running wheel activity in the ABA model. Systemic olanzapine decreased the revolutions with 2.75 mg/kg as the lowest effective dose during the active periods and 7.5 mg/kg as more effective dose. When administered intranasally, both doses decreased running wheel activity during the first 2 h after administration and also lowered body temperature. The intranasal olanzapine-treated rats had a higher body weight at the end of ABA without difference in food intake, fat and total revolutions. Systemic olanzapine did not affect body weight but preserved fat mass significantly for the 2.75 and 7.5 mg/kg doses while the lowest dose modestly increased food intake. Overall, these data suggest that systemic as well as intranasal application of olanzapine reduced running wheel activity. Low-dose intranasal olanzapine had a beneficial outcome by increasing body weight and reducing food anticipatory running activity in the ABA model.

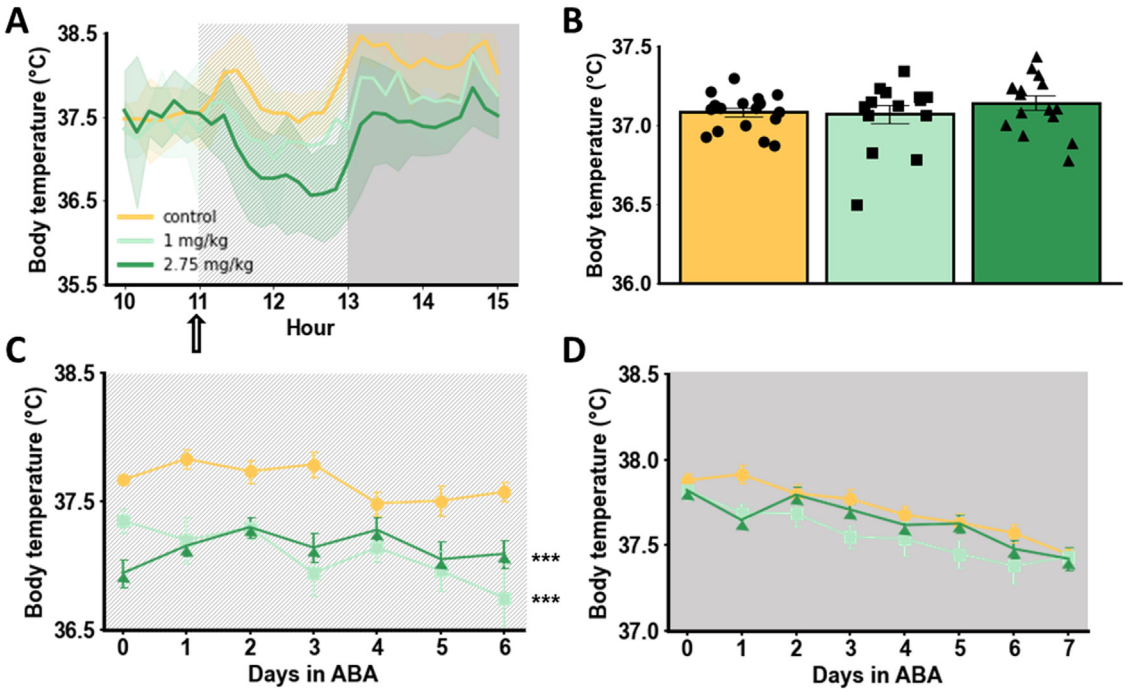

Fig. 6 Effects of intranasal olanzapine after administration on body temperature (BT). BT on the first day of intranasal administration of olanzapine (A). Average BT during the entire ABA period (B), and specifically during the anticipatory (C) and dark phases (D) in the ABA period. Results expressed as group means +- SEM, $^{***}P < 0.001$ vs. control.

Our results replicate our earlier study that investigated 7.5 mg/kg systemic administered olanzapine in the ABA model, which dampened running wheel activity while body weight and food intake remained unchanged and fat mass was preserved (Hillebrand et al., 2005). Additionally, we find that the lower dose of 2.75 mg/kg also decreased the wheel revolutions but the activity after 1 mg/kg remained unaffected. The reduced activity is likely due to known sedative actions of olanzapine (Ahnaou et al., 2003). Interestingly in a non-ABA context, olanzapine-treated rats decreased locomotor activity even with a 1 mg/kg dose (van der Zwaal et al., 2010). The potential higher internal drive for activity might be a reason why we found this reduction only at higher doses. Although the rats with the higher olanzapine doses ran less and ate similarly, this did not result in altered body weight. Likely the 2.75 and 7.5 mg/kg treated groups lost muscle mass while preserving fat mass resulting in similar body weight. Body weight and food intake changes are also modest in randomized controlled trials of olanzapine treatment in AN (Cassioli et al., 2020).

Intranasal olanzapine administration was effective in suppressing FAA and body temperature already at a low dose. This demonstrates the rapid absorption and pharmacodynamic effect, similar to intranasal olanzapine administration in human subjects (Shrewsbury et al., 2020). This strong effect with a low dose can be explained by the high peak concentration right after administration which is likely higher than with systemic administration where the drug is delivered during the day. Hypothermia is a known effect of olanzapine in rats (Evers et al., 2010; Oerther and Ahlenius, 2000) and humans (Docx et al., 2012). The strong hypothermia with both doses of the intranasal experiments suggests maximal effectiveness already with 1 mg/kg. In future studies, a lower dose should be taken into account. Even though the reduction of activity and body temperature was only significantly different in the first 2 h after administration, the treated rats had increased body weight at the end of the ABA phase. From our data, we cannot explain why the short-lived olanzapine effect was more successful in preventing body weight loss compared to the constant minipump delivery. These results suggest that the acute and transient effects of intranasal olanzapine treatment provide an interesting alternative to continuous treatment.

In human subjects, olanzapine increases appetite, but most olanzapine-treated rats in the ABA model did not show increased food intake. This might be due to the limited chow availability of 1.5 h and strong drive to eat. A subtle yet significant increased food intake was found in minipump-delivered 1 mg/kg group. Previous studies have reported both increased (van der Zwaal et al., 2010; Zhang et al., 2014) or absent (Evers et al., 2010; Lee and Clifton, 2002) food intake changes under olanzapine treatment in rodents. These mixed results in our data and literature hint towards different effects of the drug take place. When measuring olanzapine's effect on food intake the feeding context (ABA model or ad libitum), dose, and manner and time of administration likely impact on its effect.

Intranasal administration of olanzapine provides a promising alternative to oral administration, and is worth exploring further. In AN patients (the drive for) hyperactivity might be seen as a response to agitation. Previous studies found that orally administered olanzapine reduced hyperactivity and agitation in AN, psychotic and bipo-

lar patients. For example an open-label study found that orally administered olanzapine decreased agitation by 60% in AN patients (Spettigue et al., 2008). Additionally, olanzapine reduced physical activity in hyperactive AN patients (Hillebrand et al., 2005). Olanzapine decreased agitation scores of the Positive and Negative Syndrome Scale in psychotic and bipolar patients (Breier et al., 2002; Hsu et al., 2010; Kinon et al., 2004; Wright et al., 2001). However, this was statistically insignificant in a systematic review (Dundar et al., 2016). The rapid absorption of intranasal administration may immediately reduce hyperactivity, making it worthwhile for further examination in a clinical setting.

A limitation of these experiments is the use of only one control group that received both food restriction and wheel access in the ABA protocol. Further study is needed to determine if olanzapine also affects either of these components. Additionally, we observed beneficial effects of olanzapine in this study, exposing rats to ABA for one week. As a next step, it would be valuable to evaluate olanzapine treatment in more chronic models of AN (Frintrop et al., 2018; Méquinion et al., 2015).

In conclusion, we demonstrate that olanzapine via two administration routes is beneficial in the ABA model by reducing running wheel activity. The novel intranasal administration of olanzapine shows acute and strong effects. We recommend that intranasal olanzapine administration is explored further as potential treatment option for anorexia nervosa.

Availability of data and materials

Available upon request.

Author contributions

RA, ML and KK designed the experimental approach. ML and LD executed the experiment and prepossessed the data. KK processed and analysed the data and wrote the manuscript. RA guided the writing process. GP came up with the intranasal approach. AE gave advise on clinical impact. All authors read and approved the final manuscript.

Conflict of Interest

None of the authors has a conflict of interest regarding publishing this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2023.03.008.

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