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Food restriction and hyperactivity induce changes in corticolimbic brain dopamine and serotonin levels in female rats

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ABSTRACT

Compelling data support altered dopamine (DA) and serotonin (5-HT) signaling in anorexia nervosa (AN). However, their exact role in the etiopathogenesis of AN has yet to be elucidated. Here, we evaluated the corticolimbic brain levels of DA and 5-HT in the induction and recovery phases of the activity-based anorexia (ABA) model of AN. We exposed female rats to the ABA paradigm and measured the levels of DA, 5-HT, the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and the dopaminergic type 2 (D2) receptors density in feeding- and reward-implicated brain regions (i.e., cerebral cortex, Cx; prefrontal cortex, PFC; caudate putamen, CPu; nucleus accumbens, NAcc; amygdala, Amy; hypothalamus, Hyp; hippocampus, Hipp). DA levels were significantly increased in the Cx, PFC and NAcc, while 5-HT was significantly enhanced in the NAcc and Hipp of ABA rats. Following recovery, DA was still elevated in the NAcc, while 5-HT was increased in the Hyp of recovered ABA rats. DA and 5-HT turnover were impaired at both ABA induction and recovery. D2 receptors density was increased in the NAcc shell. These results provide further proof of the impairment of the dopaminergic and serotoninergic systems in the brain of ABA rats and support the knowledge of the involvement of these two important neurotransmitter systems in the development and progression of AN. Thus, providing new insights on the corticolimbic regions involved in the monoamine dysregulations in the ABA model of AN.

1. Introduction

Anorexia nervosa (AN) is a serious and potentially life-threatening psychiatric pathology mostly diagnosed during adolescence [1,2]. People suffering from this eating disorder typically present a wearing fixation to reach a thin figure by extreme control over consumption of low-calorie diet and obsessive physical exercise which results in abnormally low body weight and physical hyperactivity. This dramatic condition is associated to severe medical complications and leads very often to death [3,4].

Dopamine (DA) and serotonin (5-HT) are known to be key regulators of feeding-related processes and their alteration in the brain, together with the alteration of their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), respectively, has been linked to disturbances in appetite and mood in patients affected by eating disorders [5–7]. Pre-clinical and clinical evidence have reported impairments of the dopaminergic and serotoninergic signaling in AN that may represent key pathogenic features influencing motivation-related dysfunctions, altered satiety and mood disruptions [5,8].

Indeed, levels of HVA, the main metabolite of DA, were found markedly decreased in the cerebral spinal fluid (CSF) of acutely ill and recovered AN patients [9–11]. Similarly, significantly reduced concentration of 5-HT and its metabolite 5-HIAA were reported in the CSF in ill

Abbreviations: ABA, activity-based anorexia; AN, anorexia nervosa; ANOVA, analysis of variance; CSF, cerebral spinal fluid; D2, dopaminergic type 2 receptor; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; HPLC, high performance liquid chromatography; HVA, homovanillic acid; PET, positron emission tomography.

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or recovered AN patients [12,13]. Moreover, positron emission tomography (PET) imaging studies have revealed increased expression of the DA receptors type 2 and 3 (D2/D3) in the anteroventral striatum of women recovered from AN [14], as well as enhanced density and activity of the 5-HT1A serotonin receptor in several cortical areas in AN women [14,15]. Interestingly, such alterations were found to be positively correlated with psychometric tests scores for perfectionism and interpersonal distrust in recovered anorexic patients [16]. It is still unclear, however, whether these impairments rise because of the disorder or if these are preexisting conditions.

Research over the last decades has moved important steps in the discovery of key neurological targets driving AN onset and influencing its development and animal paradigms have been helpful in the search for a possible biological drive or changes in physiological parameters that trigger food restriction and hyperactivity. Among these, the activity-based anorexia (ABA) model currently represents the most well-characterized and validated rodent model of AN. As well documented, the simultaneous combination of time-restricted feeding and free access to exercise in a running wheel let rodents to progressively develop typical anorexic-like traits comparable to the most representative aspects of the human pathological condition [17]. Accordingly, reduced food intake, significant weight loss, extreme exercise, and neuroendocrine alterations are well modeled in ABA rodents [18].

Comparable to human studies, rodents exposed to the ABA model of AN showed increased expression of D2 receptors in the dorsal striatum and chronic administration of a D2 antagonist was found to ameliorate ABA features [19,20]. Moreover, altered striatal dopaminergic neurotransmission was suggested to contribute to the anorexic phenotype in the anx/anx genetic mouse model [21]. On the other hand, exposing rats to restricted feeding for one week was found to reduce 5-HT synthesis and metabolism [22]. Also, altered 5-HT turnover was observed in the hypothalamus of mice showing inflammation-induced anorexia [23].

Disfunctions of reward-related brain areas have been highlighted both in AN patients and animal models of AN. When stimulated, the reward pathway, including the ventral tegmental area and the nucleus accumbens (NAcc) in ABA rats, causes an increase in food intake and food anticipatory activity which results in the attenuation of weight loss [24]. A reduction in spines density and alteration of glutamate synapse in the medial prefrontal cortex (mPFC), as well as an impairment in recency discrimination in the temporal order object recognition test, was also found in ABA rats. Interestingly, this condition was mitigated after 7 days of recovery [25]. Compensatory behaviors, such as food restriction and intense physical exercise, seem to stimulate the activity of particular areas like PFC and NAcc, especially after presentation of food stimuli following a period of food deprivation [26,27]. Imaging studies in AN patients have provided different evidence of the impaired signal in brain areas (e.g., PFC, ventral striatum, thalamus) taking part to reward-related circuits implicated in the pathology [28,29].

To date, no direct measurement of brain tissue levels of DA and 5-HT have been reported in ABA rats. Therefore, in this study, levels of DA and 5-HT, as well as of the corresponding metabolites DOPAC, HVA, and 5-HIAA were quantitatively analyzed in limbic-related regions, including cerebral cortex (Cx), prefrontal cortex (PFC), nucleus accumbens (NAcc), caudate putamen (CPu), amygdala (Amy), hypothalamus (Hyp), and hippocampus (Hipp), which control a multitude of functions strongly correlated to AN pathophysiology. The density of D2 receptors was investigated in key areas of the striatum (i.e., NAcc and CPu) and the DA and 5-HT turnover in the brain of ABA rats were explored.

2. Materials and methods

2.1. Animals

A total of 50 Sprague-Dawley female rats (Envigo, Italy) weighing 125–150 g (PND ~50) were used as experimental subjects. Animals were housed in a climate-controlled animal room (21 ± 2 °C; 60% humidity) under a reverse 12 h/12 h light/dark cycle (lights on at 12:00 a.m.) and fed with standard rat chow (rats chow pellets: 3% kcal from fat, 61% kcal from carbohydrate, 16% kcal from protein, 0% moisture, containing 2.9 kcal/g, Safe, France) and water available throughout the entire duration of the study. According to the experimental group, housing chambers consisted of standard polycarbonate cages [48 (h) x 32 (w) x 47 (d) cm] equipped (or not) with a running wheel (35 cm in diameter, 11 cm in width) connected to a digital magnetic LCD revolution counter (1-wheel revolution equals 1.1 m) (Ugo Basile, Italy). All procedures and experiments were carried out in an animal facility according to Italian (D.L. 26/2014) and European Council directives (63/2010) and in compliance with the approved animal policies by the Ethical Committee for Animal Experiments at the University of Cagliari (Sardinia, Italy) and the Italian Department of Health (286/2016).

2.2. The activity-based anorexia protocol

The experimental protocol used in this study was designed as previously described [18,30,31]. Briefly, after 7 days of acclimation to the animal facility, animals were randomly divided into two experimental groups named as the control group (Control) or the ABA group (ABA) with no statistically significant differences in terms of basal body weight and food intake. Control animals were individually housed in standard cages, while ABA animals were housed in activity cages equipped with a running wheel. During the adaptation phase food was provided ad libitum to both groups and ABA animals were allowed to access the running wheel. Running wheel activity (RWA) was monitored daily for ABA rats to achieve a stable activity and the daily average of wheel rotations during this 7-day phase was used as the basal activity. On the last day of the adaptation phase (day 7), food was removed from the cages of the ABA group only and the ABA induction phase started. For the following 6 days, the ABA group had access to food only for 1.5 h per day, during which wheels were maintained locked to avoid competition between food and running. Access to wheel was allowed for the remaining 22.5 h a day. For ethical reasons, body weight loss during the ABA induction phase cannot exceed 25% of the initial body weight. On day 6 of the ABA induction phase, animals acceded to the following recovery phase during which food was provided ad libitum to both experimental groups for 7 days. During this phase ABA animals were allowed to access the wheel 24 h per day. Measures of body weight, food intake and physical activity (RWA) were monitored daily during each experimental phase.

2.3. Tissue collection and preparation

Half of the animals for each group were sacrificed by decapitation at the end of the 12 h light phase on the last day of the ABA induction phase, while the other half at the end of the 12 h light phase on the last day of the recovery phase. Immediately after decapitation brains were rapidly collected and processed according to next analysis. For high performance liquid chromatography (HPLC) analysis the areas of interest were obtained by regional dissection using a pre-cooled rat brain slicer, frozen on an aluminum plate over dry-ice and stored at − 80 °C until subsequent tissue extraction. Brain regions selected for analysis, according to the Paxinos Atlas [32] included the Cx (AP: +3.20), PFC (AP: +2.80), CPu and NAcc (AP: +1.60), Amy (AP: −2.12 to −3.14), Hyp (AP: −2.14), and Hipp (AP: from −2.12 to −3.14). For autoradiographic binding the whole brains were frozen in 2-methylbutane (Sigma-Aldrich) and then stored at − 80 °C before being sliced in a cryostat. Coronal sections (12 μm thick) were prepared with a Leica cryostat at − 20 °C, thaw-mounted onto Superfrost Plus slides (Clini-Lab s.r.l., Conselve, Italy) and stored at − 20 °C until use.

2.4. Tissue extraction and HPLC analysis

DA and 5-HT, as well as corresponding metabolites DOPAC, HVA and

5-HIAA were extracted from the brain areas of interest (i.e., Cx, PFC, CPu, NAcc, Amy, Hyp, Hipp) and subsequently quantified as previously described [33]. Briefly, tissue samples were weighted and homogenized in ice-cold 0.1 M perchloric acid (1:30, wt/vol), sonicated and then centrifuged for 20 mins (18600 x g, 4 °C). After centrifugation, the supernatants were filtered through a 0.22 μm Spin-X Centrifuge Tube Filter (Costar, Corning Incorporated, Corning, NY) and finally injected (two injections of 20 μl each per sample) into a HPLC system with a C18 column (LC18 DB Supelco, 5 μm, 4.6 × 150 mm) and the Coulochem III detector (ESA Inc., Chelmsford, MA, USA). The first electrode of the detector analytical cell was set at + 20 mV and the second at + 320 mV; column temperature was set at 26 °C. The mobile phase consisted of 90% 50 mM sodium acetate, 35 mM citric acid, 105 mg/L octane sulfonic acid, 48 mg/L sodium EDTA solution, and 10% methanol at pH 4.3. The flow rate was set at 1 ml/min. Data were collected and analyzed using the EZchrom SI 3.2 software. The values obtained are reported in Supplemental materia and are expressed as ng/mg tissue wet weight.

2.5. D2 receptor binding

Brain regions selected for analysis included the CPu, and the NAcc core and shell (AP: +1.60). [^{3}H]YM-09151–2 binding autoradiography protocol was adapted [34]. Briefly, sections were first incubated at room temperature for 1 hr in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA, 1 nM di [^{3}H]YM-09151–2 (specific activity: 83.1 Ci/mmol; Perkin Elmer, Boston, MA, USA). Non-specific binding was determined in the presence of 30 μM S(-)-sulpiride. After incubation, slides were rinsed (2 times for 5 min) in ice-cold Tris-HCl buffer, dipped in ice-cold deionised water, and air dried. Dried tissue sections and slide-mounted [^{3}H]micro-scales standards (RPA 501 and 505; Amersham, USA) for [^{3}H]YM-09151–2 autoradiography were placed in a Fujifilm BAS cassette with a BAS-5000 imaging plate. The resulting images were analyzed with a Fujifilm-BAS 5000 imaging system (Automatic Image Data Analyzer, Ray test, Wilmington, NC, USA), and optical densities were transformed into levels of bound radioactivity (fmol/mg protein) with gray values generated by co-exposed [^{3}H].

2.6. Statistical analysis

Data were analyzed with GraphPad Prism® 9 for Windows (Graph Pad software, USA). Between or within group differences were analysed using the one-way analysis of variance (ANOVA) with group as a between-subjects factor or unpaired student's t-test, wherever appropriate. Data are expressed as mean ± SEM. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. ABA induction and recovery

As previously reported, diet restriction combined with intense physical activity lead ABA animals to dramatic body weight loss and hyperactivity [18,30]. One-way ANOVA revealed a main significant effect of group on body weight ($F_{(3,44)}$= 494.2, $p < 0.0001$). During the ABA induction phase the body weight of ABA rats dramatically decreased and on the last day, ABA animals lost a total of − 22% of the initial weight (Unpaired t-test: t(30) = 29.20, p = 0.0008; Fig. 1A). After recovery, ABA animals partially restored their body weight, but this was still significantly lower as compared to the control group (Unpaired t-test: t(14) = 4.248, $p < 0.0001$; Fig. 1A). Physical activity in ABA rats was significantly affected by the ABA paradigm (One-way ANOVA: $F_{(2, 37)}$= 19.55, $p < 0.0001$). During the ABA induction phase, the running activity was significantly enhanced as compared to the basal activity (Unpaired t-test: t(30) = 5.634, $p < 0.000$; Fig. 1B), while during recovery, when free access to food was provided, the average running activity significantly dropped (Unpaired t-test: t(22) = 2.205, p = 0.0382, − 35%; Fig. 1B). Concomitantly, the ABA group during induction phase showed an evident reduction in food consumption (Unpaired t-test: t(30) = 18.89, $p < 0.0001$; Fig. 1C), that was completely reverted during the following recovery, when ABA animals consumed significantly more food than Control rats (Unpaired t-test: t(14) = 8.044, $p < 0.0001$; Fig. 1C). One-way ANOVA revealed a main significant effect of group on food intake ($F_{(3,44)}$= 255.7, $p < 0.0001$).

3.2. DA

The effect of induction and recovery phases on the levels of DA and its metabolites DOPAC and HVA are presented in Fig. 2. At the ABA induction phase, levels of DA were significantly increased in the Cx (unpaired t-test: t(14) = 2.645, p = 0.0192, + 23%), PFC (unpaired t-test: t(14) = 2.513, p = 0.0248, +30%), and NAcc (unpaired t-test: t(14) = 4.329, p = 0.0007, + 37%) of ABA rats as compared to Control group levels. No statistical significant change was found in the Amy even if a 20% of increased was noted compared to Control (Unpaired t-test: t(14) = 1.245, p = 0.2336).

At the recovery phase, DA returned to basal control levels in the Cx (Unpaired t-test: t(14) = 0.7723, p = 0.4528), PFC (Unpaired t-test: t(14) = 0.03653, p = 0.9714) and Amy (Unpaired t-test: t(14) = 0.2613, p = 0.7977), while persisted significantly increased in the NAcc (Unpaired t-test: t(14) = 2.718, p = 0.0167, + 20%) of recovered ABA rats as compared to the Control group. No statistically relevant variations were found in the CPu (Unpaired t-test: t(14) = 0.1697, p = 0.8677),

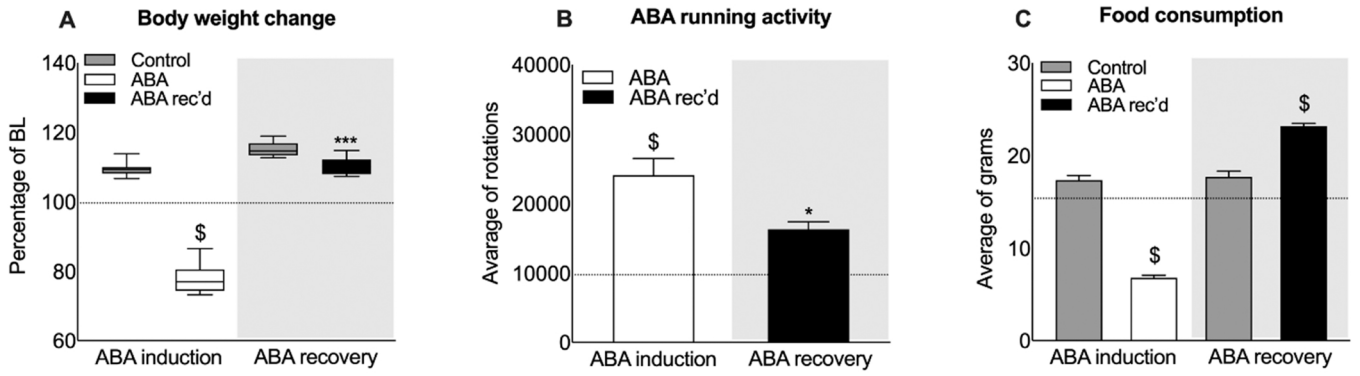

Fig. 1. Behavioral parameters in Control, ABA and ABA recovered (ABA rec'd) groups during the ABA induction and recovery phases. (A) Body weight change expressed as percentage of baseline (BL, 100% dashed line; $ $p < 0.0001$ ABA vs Control, n = 16 per group; *** $p < 0.001$ ABA rec'd vs Control, n = 8 per group); (B) ABA running activity expressed as the average of rotations of the ABA group (dashed line representing basal running activity; $ $p < 0.0001$ ABA vs BL n = 16 per group; * p = 0.0382 ABA rec'd vs ABA, n = 8 per group); (C) Food consumption expressed as the average of grams of chow consumed (dashed line representing basal food consumption; $ $p < 0.0001$ ABA vs Control, n = 16 per group; $ $p < 0.0001$ ABA rec'd vs Control, n = 8 per group). Data are presented as the mean ± SEM.

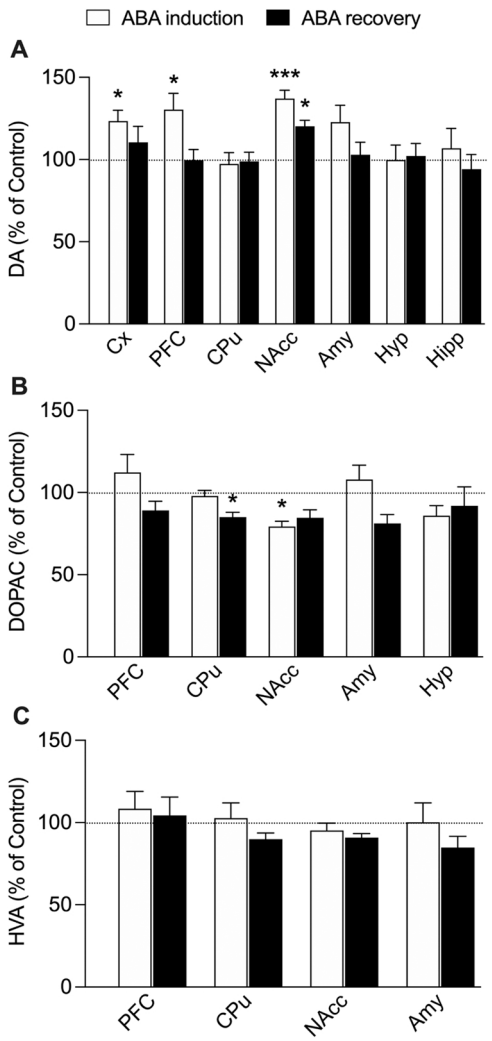

Fig. 2. Levels of (A) dopamine (DA), (B) dihydroxyphenylacetic acid (DOPAC) and (C) homovanillic acid (HVA) in the cerebral cortex (Cx), prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAcc), amygdala (Amy), hypothalamus (Hyp) and hippocampus (Hipp) of ABA rats at the end of the ABA induction (white bars) and ABA recovery (black bars) phases (n = 8 per group). Data are presented as the mean ± SEM of percentage (%) of the corresponding Control group (100% dashed line) (* $p < 0.05$, *** $p < 0.001$ vs corresponding Control group, n = 8 per group).

Hyp (Unpaired t-test: $t(14) = 0.2014$, $p = 0.8432$) and Hipp (Unpaired t-test: $t(14) = 0.4485$, $p = 0.6607$) at the end of the recovery phase.

3.3. DOPAC and HVA

At the induction phase, levels of DOPAC were significantly decreased in the NAcc of ABA rats compared to Control rats (Unpaired t-test: $t(14) = 2.458$, $p = 0.0276$), −20%; Fig. 2B). A trend of increase (+12%) was observed in the PFC (Unpaired t-test: $t(14) = 1.257$, $p = 0.2294$) of ABA rats, while no changes were observed in the CPu (Unpaired t-test: $t(14) = 0.4195$, $p = 0.6812$), Amy (Unpaired t-test: $t(14) = 0.4933$, $p = 0.6295$) and Hyp (Unpaired t-test: $t(14) = 1.015$, $p = 0.3274$). After recovery, levels of DOPAC were significantly reduced in the CPu (Unpaired t-test: $t(14) = 2.290$, $p = 0.0321$, − 15%; Fig. 2B) of recovered ABA rats. Moreover, even though statistical analysis did not reveal a significant result, DOPAC was still reduced in the NAcc (Unpaired t-test $t(14) = 1.925$, $p = 0.0747$, −15%; Fig. 2B). No changes were observed in the PFC (Unpaired t-test: $t(14) = 1.257$, $p = 0.2294$), Amy (Unpaired t-test: $t(14) = 2.011$, $p = 0.0640$) and Hyp (Unpaired t-test: $t(14) = 0.5102$, $p = 0.6179$) at the end of recovery.

The brain levels of the DA metabolite HVA did not significantly change across the ABA induction (Unpaired t-test: PFC $t(14) = 0.5490$, $p = 0.5917$; CPu $t(14) = 0.2744$, $p = 0.7878$; NAcc $t(14) = 0.9734$, $p = 0.3469$; Amy $t(14) = 0.01714$, $p = 0.9866$) and recovery (Unpaired t-test: PFC $t(14) = 0.3215$, $p = 0.7526$; CPu $t(14) = 1.904$, $p = 0.0776$; NAcc $t(14) = 1.185$, $p = 0.2556$; Amy $t(14) = 1.029$, $p = 0.3211$) phases (Fig. 2 C). However, a trend of reduction was observed in the CPu (−13%), NAcc (−10%), and Amy (−15%) in recovered ABA rats at the end of the ABA recovery phase.

3.4. 5-HT

Levels of 5-HT were significantly increased in the NAcc (Unpaired t-test: $t(14) = 2.463$, $p = 0.0273$, + 24%; Fig. 3A) and in the Hipp (Unpaired t-test: $t(14) = 2.287$, $p = 0.0383$, + 44%; Fig. 3A) of ABA rats at the end of the ABA induction phase. No change has been found in the other areas (Unpaired t-test: Cx $t(14) = 0.1656$, $p = 0.8708$; PFC t $(14) = 0.5844$, $p = 0.5683$; CPu $t(14) = 0.5052$, $p = 0.6213$; Amy t $(14) = 1.338$, $p = 0.2023$; Hyp $t(14) = 0.6304$, $p = 0.5386$). In recovery, the increase of 5-HT levels in the NAcc persisted (Unpaired t-test: t $(14) = 1.439$, $p = 0.1723$, + 20%; Fig. 3A), while it normalized to basal levels in the Hipp (Unpaired t-test: $t(14) = 1.348$, $p = 0.1991$). On the other hand, in the Hyp, levels of 5-HT were found significantly elevated in recovered ABA rats (Unpaired t-test: $t(14) = 2.493$, $p = 0.0258$, + 23%; Fig. 3 A). No changes were found in the remaining areas in the recovery phase (Unpaired t-test: Cx $t(14) = 1.848$, $p = 0.0718$; PFC t $(14) = 1.096$, $p = 0.2918$; CPu $t(14) = 0.2303$, $p = 0.8212$; Amy t $(14) = 1.379$, $p = 0.1895$).

3.5. 5-HIAA

ABA animals at the end of the induction phase showed a 18%

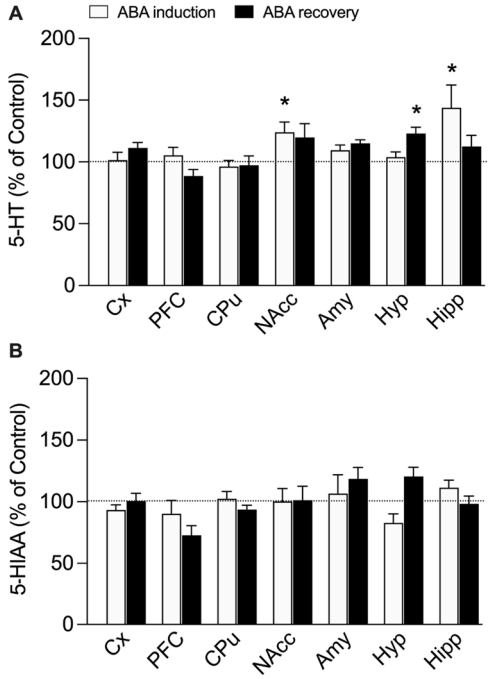

Fig. 3. Levels of (A) serotonin (5-HT) and (B) 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex (Cx), prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAcc), amygdala (Amy), hypothalamus (Hyp) and hippocampus (Hipp) of ABA rats at the end of the ABA induction (white bars) and ABA recovery (black bars) phases (n = 8 per group). Data are presented as the mean ± SEM of percentage (%) of the corresponding Control group (100% dashed line) (* $p < 0.05$ vs corresponding Control group, n = 8 per group).

reduction in the levels of the 5-HT metabolite, 5-HIAA, in the Hyp (Unpaired t-test: $t(14) = 1.498$, $p = 0.1563$) (Fig. 3B). Interestingly, even though such reduction did not result statistically significant, levels of 5-HIAA increased by 20% at the following recovery phase in the same area (Unpaired t-test: $t(14) = 1.734$, $p = 0.1049$). Moreover, a 28% reduction of 5-HIAA levels were also observed in the PFC (Unpaired t-test $t(14) = 0.6308$, $p = 0.5383$) of recovered ABA rats. No significant variations were observed in the other regions at the induction (Unpaired t-test: Cx $t(14) = 0.6313$, $p = 0.5380$; PFC $t(14) = 0.6308$, $p = 0.5383$; CPu $t(14) = 0.3071$, $p = 0.7633$; NAcc $t(14) = 0.01982$, $p = 0.9845$; Amy $t(14) = 0.3381$, $p = 0.7403$; Hipp $t(14) = 1.498$, $p = 0.1563$) and recovery (Unpaired t-test: Cx $t(14) = 0.08976$, $p = 0.9298$; PFC $t(14) = 1.905$, $p = 0.0775$; CPu $t(14) = 1.142$, $p = 0.2726$; NAcc $t(14) = 0.03993$, $p = 0.9687$; Amy $t(14) = 1.240$, $p = 0.2352$; Hipp $t(14) = 1.734$, $p = 0.1049$).

3.6. Monoamines metabolism

ABA animals at both induction and recovery phases displayed significantly decreased DA turnover in the NAcc (ABA induction: DOPAC/DA $p = 0.0016$, HVA/DA $p < 0.0001$; ABA recovery: DOPAC/DA $p = 0.0198$, HVA/DA $p = 0.0002$; Fig. 4A-B) and Amy (ABA induction: DOPAC/DA $p = 0.0435$; ABA recovery: DOPAC/DA $p = 0.0012$; Fig. 4A-B) relative to the Control group. One-way ANOVA revealed a main significant effect of group on NAcc and Amy DA turnover [NAcc: $F_{(2,29)} = 8.282$, $p = 0.0014$; Amy: $F_{(2,29)} = 8.156$, $p = 0.005$]. Moreover, at the end of the ABA induction phase, a significant reduction of 5-HT turnover was observed in the Hyp (ABA induction: 5-HIAA/5-HT $p = 0.0054$; Fig. 5) and Hipp (ABA induction: 5-HIAA/5-HT $p = 0.0359$; Fig. 5) of ABA rats as compared to the Control group. One-way ANOVA revealed a main significant effect of group on Hyp and Hipp 5-HT turnover [Hyp: $F_{(2,29)} = 6.025$, $p = 0.0065$; Hipp: $F_{(2,29)} = 3.317$, $p = 0.0504$].

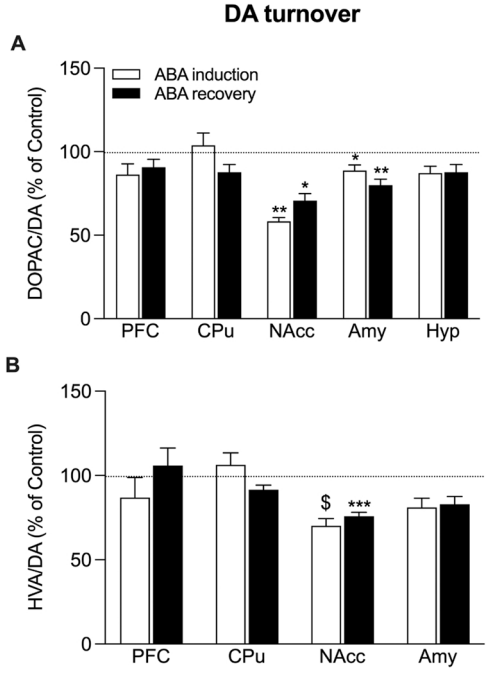

Fig. 4. Dopamine (DA) turnover represented as the ratio of the DA metabolites DOPAC (**A**) and HVA (**B**) to DA in the prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAcc), amygdala (Amy) and hypothalamus (Hyp) in ABA rats at the end of the ABA induction (white bars) and ABA recovery (black bars) phases. Data are presented as the mean ± SEM of percentage (%) of the corresponding Control group (100% dashed line) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $ $p < 0.0001$ vs corresponding Control group, $n = 8$ per group).

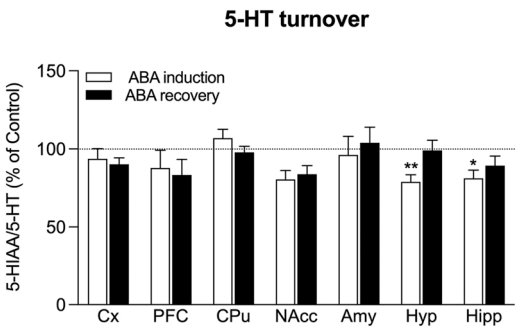

Fig. 5. Serotonin (5-HT) turnover represented as the ratio of the 5-HT metabolite 5-HIAA to 5-HT in the cerebral cortex (Cx), prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAcc), amygdala (Amy), hypothalamus (Hyp) and hippocampus (Hipp) in ABA rats at the end of the ABA induction (white bars) and ABA recovery (black bars) phases. Data are presented as the mean ± SEM of the percentage (%) of the corresponding Control group (100% dashed line) (* $p < 0.05$, ** $p < 0.01$ vs corresponding Control group, $n = 8$ per group).

3.7. D2 receptors density

Brain regions selected for analysis included the CPu and the NAcc core and shell (Fig. 6A). Receptor binding analysis revealed a significantly enhanced density of D2 receptors in the NAcc shell of ABA animals at the end of the induction phase with an increase of +70% with respect to control levels (Unpaired t-test: $t(7) = 4.286$, $p = 0.0036$; Fig. 6B). Following body weight recovery, the enhanced D2 receptor density normalized to control levels in the NAcc shell (Unpaired t-test: $t(7) = 0.5058$, $p = 0.6285$). No significant variations were observed in the CPu (Unpaired t-test: induction $t(7) = 0.1146$, $p = 0.9120$; recovery $t(7) = 0.8850$, $p = 0.4055$).

4. Discussion

In the present study, we explored the central alteration of the dopaminergic and serotoninergic systems in the ABA model of AN. First, we confirmed that rats exhibit typical anorexic-like behavioral phenotype when exposed to the well-established ABA model of AN, including rapid decline in body weight and increased physical activity. Under these experimental conditions, we found that ABA induction and ABA recovery phases were associated to significant changes in the corticolimbic content of DA and 5-HT, as well as of the corresponding metabolites (i.e., DOPAC, HVA, and 5-HIAA, respectively), and of the D2 receptor expression levels. Importantly, such changes affected key corticolimbic brain areas, including Cx, PFC, CPu, NAcc, Amy, Hyp and Hipp, involved in feeding behavior, as well as in mood and reward-related pathological conditions linked to AN [35,36].

In detail, post-mortem quantitative HPLC analysis revealed a significant increase in the content of DA in the Cx, PFC and NAcc. Of note, increased levels in the NAcc were not completely reverted by the recovery. Moreover, in our experimental animals, the levels of the DA metabolite DOPAC were significantly decreased only in the NAcc of ABA rats and in the CPu of recovered animals, while the levels of HVA did not significantly change in the regions examined. Similarly, Verhagen and collaborators showed an in vivo increased release of extracellular DA in the NAcc of ABA rats and no changes of DOPAC and HVA during food-anticipatory activity of the ABA paradigm [37]. As stated in the introduction, the implication of DA impairments in the etiology of AN has been demonstrated by several evidence in human and animal studies [19,38,39]. Accordingly, DA antagonism showed its effective results by improving AN condition in human patients [40], and by reducing activity and body weight loss and increasing food consumption in ABA rats [37]. NAcc has an important role in food-anticipatory activity and selective lesions of this region or local administration of dopamine

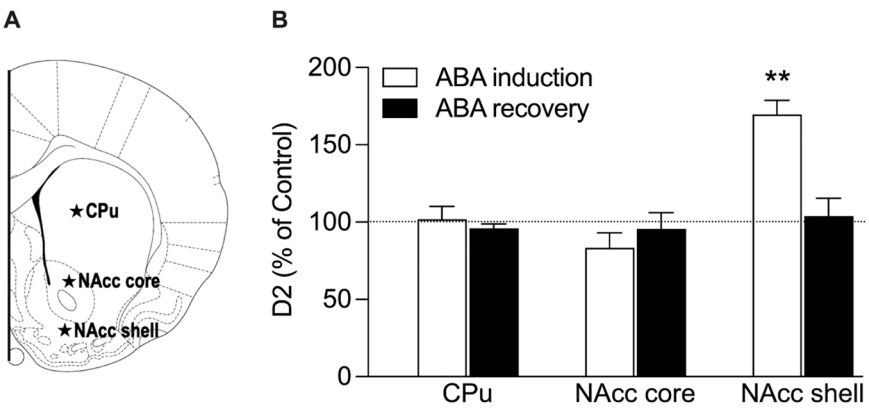

Fig. 6. (A) Schematic redrawing of the brain areas studied showing coronal sections of the caudate putamen (CPu) and nucleus accumbens (NAcc) core and shell. Highlighted points indicate the approximate site of the regions analyzed. (B) Levels of dopamine type 2 (D2) receptors in the CPu, NAcc core and shell of ABA rats at the end of the ABA induction (white bars) and ABA recovery (black bars) phases (n = 6 per group). Data are presented as the mean ± SEM of percentage (%) of the corresponding Control group (100% dashed line) (** $p < 0.01$ vs corresponding Control group, Control group n = 3, ABA group n = 6).

antagonists markedly decrease locomotor behavior [41,42]. This is in accordance with our results in which DA is strongly increased in NAcc in ABA animals showing hyperactivity. Moreover, enhanced DA in hyper-dopaminergic genetically modified mice was shown to promote ABA vulnerability by increasing restriction-derived hyperactivity [43].

Regarding 5-HT, the main brain changes occurred in the NAcc and Hipp of ABA rats, as well as in the Hyp of recovered ABA animals that showed increased concentrations as compared to control animals. Like DA, 5-HT increased levels in the NAcc were not overturned by the ABA recovery phase. Inflammation-induced anorexia was associated with increased hypothalamic 5-HT release in mice [23]. Also, impaired Hyp 5-HT signaling was correlated with altered food intake behavior in a mice tumor model with associated severe cachectic condition [44]. Fenfluramine, a 5-HT releaser and 5-HT reuptake inhibitor, enhanced weight loss rate in female ABA rats, suggesting 5-HT involvement in the ABA derived body weight loss in rats [45]. In contrast to our findings, decreased release of 5-HT and of its metabolite 5-HIAA were previously reported in the NAcc of ABA rats [37].

5-HT together with DA are implicated in food reward and feeding regulation and the administration of 5-HT1B agonist increases activity levels in rats and reduces food intake, while the stimulation of the 5-HT4 receptor in the NAcc reduces food intake [46,47]. In our model we observed an increase of 5-HT in NAcc which is implicated in motivation and in the Hyp, known to be a key center for feeding regulation.

Starvation-induced hyperactivity was shown to modulate dopaminergic neurons activity in the reward circuits [48]. Wheel running itself is rewarding in rats and activates the mesolimbic dopaminergic system which exert significant control on motivation and motor activity [49]. Moreover, increased density of dopaminergic and serotonergic cells was recently observed in key areas of the reward system (i.e., ventral tegmental area, substantia nigra pars compacta, dorsal raphe nucleus) in the ABA model in conjunction with a chronic stress induced by maternal separation, which might lead to changes in the reward system that could be linked to the altered behavioral phenotype observed [50].

Aberrant motivation and responses to rewarding stimuli, which seems to lead to pathological feeding and compensatory behaviors in AN, have been linked with altered functioning of corticolimbic networks involving both ventral (NAcc) and dorsal (CPu) striatal neural circuits [51–53]. Therefore, we decided to deeply explore these two regions to search for possible effects of the ABA paradigm conditions on the expression of D2 receptors. Interestingly, our findings point out a significant increase in the levels of D2 receptors in the NAcc of ABA rats. In particular, the increased density of D2 receptors was localized in the shell of the NAcc, suggesting this as a possible key sub-region in the impaired dopaminergic tone displayed by ABA rats. More recently, Welch and collaborators were able to show a correlation between the overexpression of the D2 receptor found in the NAcc and the marked body weight loss displayed by female mice exposed to the ABA protocol [54]. Supporting this finding, increased D2 and D3 receptors binding was also revealed in the anteroventral striatum of recovered AN patients measured by the [^{11}C] raclopride binding PET [14]. Also, different concentrations and dynamic DA signaling were found in the NAcc core and shell of freely moving rats [55]. Other findings indicate a different enhanced activation of D2/D3 receptors in the core and shell sub-regions of the NAcc in the regulation of hyperactivity and impulsivity in rats [56]. NAcc D1 and D2 blockade alters motor behavior, amount and duration of feeding [57].

Our ABA animals displayed decreased DA turnover in the NAcc and Amy at the end of both ABA induction and recovery, and a decreased 5-HT turnover in the Hyp and Hipp at the end of the ABA induction phase. These results confirm that both acute induction of ABA and recovery from it can alter the DA and 5-HT neurochemistry in the brain of ABA rats. Our data highlight that the combination of diet restriction with physical activity in ABA animals can modulate this altered process. It is possible that the consequent dramatic body weight loss and hyperactivity leads to decreases in DA and 5-HT turnover in response to their repeated release within the corticolimbic regions examined involved in feeding-, reward- and mood-related behaviors.

Overall, the results presented here support previous pre-clinical and clinical data on the involvement of impaired DA and 5-HT systems in AN and during the development and maintenance of the ABA paradigm. Furthermore, our findings expand this knowledge by providing additional information of the brain region-specific alteration of DA and 5-HT in ABA rats. Additional studies are needed to investigate the involvement of these alterations in key brain regions playing a role in the onset and progression of anorectic behaviors and to expand the knowledge on the possibility to target these two neurotransmitter systems for more effective therapeutic strategies.

CRediT authorship contribution statement

Elisa Giunti: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. Roberto Collu: Investigation, Formal analysis. **Simona Dedoni:** Investigation, Formal analysis. **M. Paola Castelli:** Investigation, Formal analysis. **Maria Scherma:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Paola Fadda:** Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114374.

References

[1] S. Zipfel, K.E. Giel, C.M. Bulik, P. Hay, U. Schmidt, Anorexia nervosa: aetiology, assessment, and treatment, Lancet Psychiatry 2 (2015) 1099–1111, https://doi.org/10.1016/S2215-0366(15)00356-9.
[2] J. Neale, L.D. Hudson, Anorexia nervosa in adolescents, Br. J. Hosp. Med 81 (2020), https://doi.org/10.12968/hmed.2020.0099.
[3] American Psychiatric Association, DSM-5 Update October 2018: Supplement to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, APA. (2018).
[4] N. Achamrah, M. Coëffier, P. Déchelotte, Physical activity in patients with anorexia nervosa, Nutr. Rev. 74 (2016), https://doi.org/10.1093/nutrit/nuw001.
[5] N.M. Avena, M.E. Bocarsly, Dysregulation of brain reward systems in eating disorders: Neurochemical information from animal models of binge eating, bulimia nervosa, and anorexia nervosa, Neuropharmacology 63 (2012), https://doi.org/10.1016/j.neuropharm.2011.11.010.
[6] M. Ericsson, W.S.C. Poston, J.P. Foreyt, Common biological pathways in eating disorders and obesity, Addict. Behav. 21 (1996), https://doi.org/10.1016/0306-4603(96)00032-9.
[7] M.M. Meguid, S.O. Fetissov, M. Varma, T. Sato, L. Zhang, A. Laviano, F. Rossi-Fanelli, Hypothalamic dopamine and serotonin in the regulation of food intake, : Nutr. (2000), https://doi.org/10.1016/S0899-9007(00)00449-4.
[8] W. Kaye, Neurobiology of anorexia and bulimia nervosa, Physiol. Behav. 94 (2008) 121–135, https://doi.org/10.1016/j.physbeh.2007.11.037.
[9] N.C. Barbarich, W.H. Kaye, D. Jimerson, Neurotransmitter and imaging studies in anorexia nervosa: new targets for treatment, Curr. Drug Targets CNS Neurol. Disord. 2 (2003) 61–72, https://doi.org/10.2174/1568007033338779.
[10] W.H. Kaye, M.H. Ebert, M. Raleigh, R. Lake, Abnormalities in CNS monoamine metabolism in anorexia nervosa, Arch. Gen. Psychiatry 41 (1984) 350–355, https://doi.org/10.1001/archpsyc.1984.01790150040007.
[11] W.H. Kaye, G.K. Frank, C. McConaha, Altered dopamine activity after recovery from restricting-type anorexia nervosa, Neuropsychopharmacology 21 (1999) 503–506, https://doi.org/10.1016/S0893-133X(99)00053-6.
[12] U.F. Bailer, G.K. Frank, S.E. Henry, J.C. Price, C.C. Meltzer, L. Weissfeld, C. A. Mathis, W.C. Drevets, A. Wagner, J. Hoge, S.K. Ziolko, C.W. McConaha, W. H. Kaye, Altered brain serotonin 5-HT1A receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [carbonyl11C] WAY-100635, Arch. Gen. Psychiatry 62 (2005) 1032–1041, https://doi.org/10.1001/archpsyc.62.9.1032.
[13] W.H. Kaye, M.H. Ebert, M. Raleigh, R. Lake, Abnormalities in CNS monoamine metabolism in anorexia nervosa, Arch. Gen. Psychiatry 41 (1984) 350–355, https://doi.org/10.1001/archpsyc.1984.01790150040007.
[14] G.K. Frank, U.F. Bailer, S.E. Henry, W. Drevets, C.C. Meltzer, J.C. Price, C. A. Mathis, A. Wagner, J. Hoge, S. Ziolko, N. Barbarich-Marsteller, L. Weissfeld, W. H. Kaye, Increased dopamine D2/D3 receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [11c]raclopride, Biol. Psychiatry 58 (2005) 908–912, https://doi.org/10.1016/j.biopsych.2005.05.003.
[15] U.F. Bailer, G.K. Frank, S.E. Henry, J.C. Price, C.C. Meltzer, C. Becker, S.K. Ziolko, C.A. Mathis, A. Wagner, N.C. Barbarich-Marsteller, K. Putnam, W.H. Kaye, Serotonin transporter binding after recovery from eating disorders, Psychopharmacol. (Berl.) 195 (2007), https://doi.org/10.1007/s00213-007-0896-7.
[16] B. Galusca, N. Costes, N.G. Zito, R. Peyron, C. Bossu, F. Lang, D. le Bars, B. Estour, Organic background of restrictive-type anorexia nervosa suggested by increased Serotonin1A receptor binding in right frontotemporal cortex of both lean and recovered patients: [18F]MPPF PET Scan Study, Biol. Psychiatry 64 (2008), https://doi.org/10.1016/j.biopsych.2008.06.006.
[17] S. Spadini, M. Ferro, J. Lamanna, A. Malgaroli, Activity-based anorexia animal model: a review of the main neurobiological findings, J. Eat. Disord. 9 (2021), https://doi.org/10.1186/s40337-021-00481-x.
[18] M. Scherma, V. Satta, R. Collu, M.F. Boi, P. Usai, W. Fratta, P. Fadda, Cannabinoid CB1/CB2 receptor agonists attenuate hyperactivity and body weight loss in a rat model of activity-based anorexia, Br. J. Pharm. 174 (2017), https://doi.org/10.1111/bph.13892.
[19] C. Gelegen, J. van den Heuvel, D.A. Collier, I.C. Campbell, H. Oppelaar, E. Hessel, M.J. Kas, Dopaminergic and brain-derived neurotrophic factor signalling in inbred mice exposed to a restricted feeding schedule, Genes Brain Behav. 7 (2008) 552–559, https://doi.org/10.1111/j.1601-183X.2008.00394.x.
[20] S.J. Klenotich, E. v Ho, M.S. McMurray, C.H. Server, S.C. Dulawa, Dopamine D2/3 receptor antagonism reduces activity-based anorexia, Transl. Psychiatry 5 (2015), e613, https://doi.org/10.1038/tp.2015.109.
[21] J.E. Johansen, V.L. Teixeira, C. Johansson, P. Serrão, P.O. Berggren, P. Soares-Da-Silva, M. Schalling, A.M. Bertorello, Altered dopaminergic transmission in the anorexic anx/anx mouse striatum, Neuroreport 12 (2001), https://doi.org/10.1097/00001756-200108280-00029.
[22] D.J. Haleem, S. Haider, Food restriction decreases serotonin and its synthesis rate in the hypothalamus, Neuroreport 7 (1996) 1153–1156, https://doi.org/10.1097/00001756-199604260-00011.
[23] J.T. Dwarkasing, R.F. Witkamp, M. v Boekschoten, M.C. ter Laak, M.S. Heins, K. van Norren, Increased hypothalamic serotonin turnover in inflammation-induced anorexia, BMC Neurosci. 17 (2016), https://doi.org/10.1186/s12868-016-0260-0.
[24] C.J. Foldi, L.K. Milton, B.J. Oldfield, The role of mesolimbic reward neurocircuitry in prevention and rescue of the Activity-Based Anorexia (ABA) phenotype in rats, Neuropsychopharmacology 42 (2017) 2292–2300, https://doi.org/10.1038/npp.2017.63.
[25] F. Mottarlini, G. Targa, G. Bottan, B. Tarenzi, F. Fumagalli, L. Caffino, Cortical reorganization of the glutamate synapse in the activity-based anorexia rat model: Impact on cognition, J. Neurochem. 161 (2022), https://doi.org/10.1111/jnc.15605.
[26] M. Coletta, S. Platek, F.B. Mohamed, J.J. van Steenburgh, D. Green, M.R. Lowe, Brain Activation in Restrained and Unrestrained Eaters: an fMRI Study, J. Abnorm Psychol. 118 (2009), https://doi.org/10.1037/a0016201.
[27] K.E. Demos, W.M. Kelley, T.F. Heatherton, Dietary restraint violations influence reward responses in nucleus accumbens and amygdala, J. Cogn. Neurosci. 23 (2011), https://doi.org/10.1162/jocn.2010.21568.
[28] A.M. Monteleone, G. Castellini, U. Volpe, V. Ricca, L. Lelli, P. Monteleone, M. Maj, Neuroendocrinology and brain imaging of reward in eating disorders: a possible key to the treatment of anorexia nervosa and bulimia nervosa, Prog. Neuropsychopharmacol. Biol. Psychiatry 80 (2018), https://doi.org/10.1016/j.pnpbp.2017.02.020.
[29] D. Biezonski, J. Cha, J. Steinglass, J. Posner, Evidence for thalamocortical circuit abnormalities and associated cognitive dysfunctions in underweight individuals with anorexia nervosa, Neuropsychopharmacology 41 (2016), https://doi.org/10.1038/npp.2015.314.
[30] R. Collu, M. Scherma, F. Piscitelli, E. Giunti, V. Satta, M.P. Castelli, R. Verde, W. Fratta, T. Bisogno, P. Fadda, Impaired brain endocannabinoid tone in the activity-based model of anorexia nervosa, Int. J. Eat. Disord. 52 (2019) 1251–1262, https://doi.org/10.1002/eat.23157.
[31] R. Collu, J.M. Post, M. Scherma, E. Giunti, W. Fratta, B. Lutz, P. Fadda, L. Bindila, Altered brain levels of arachidonic acid-derived inflammatory eicosanoids in a rodent model of anorexia nervosa, Biochim Biophys. Acta Mol. Cell Biol. Lipids (2020) (1865), https://doi.org/10.1016/j.bbalip.2019.158578.
[32] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Seventh ed., Elsevier Academic Press, 2014.
[33] H.L. Martin, M. Santoro, S. Mustafa, G. Riedel, J. v Forrester, P. Teismann, Evidence for a role of adaptive immune response in the disease pathogenesis of the MPTP mouse model of Parkinson's disease, Glia 64 (2016), https://doi.org/10.1002/glia.22935.
[34] P. Fadda, M.C. Martellotta, M.G. de Montis, G.L. Gessa, W. Fratta, Dopamine D1 and opioid receptor binding changes in the limbic system of sleep deprived rats, Neurochem. Int. 20 (1992), https://doi.org/10.1016/0197-0186(92)90229-K.
[35] G.K.W. Frank, M.E. Shott, M.C. DeGuzman, The neurobiology of eating disorders, Child Adolesc. Psychiatr. Clin. N. Am. 28 (2019), https://doi.org/10.1016/j.chc.2019.05.007.
[36] D. Howard, P. Negraes, A.N. Voineskos, A.S. Kaplan, A.R. Muotri, V. Duvvuri, L. French, Molecular neuroanatomy of anorexia nervosa, Sci. Rep. 10 (2020), https://doi.org/10.1038/s41598-020-67692-1.
[37] L.A. Verhagen, M.C. Luijendijk, G.A. Korte-Bouws, S.M. Korte, R.A. Adan, Dopamine and serotonin release in the nucleus accumbens during starvation-induced hyperactivity, Eur. Neuropsychopharmacol. 19 (2009) 309–316, https://doi.org/10.1016/j.euroneuro.2008.12.008.
[38] U.F. Bailer, J.C. Price, C.C. Meltzer, A. Wagner, C.A. Mathis, A. Gamst, W.H. Kaye, Dopaminergic activity and altered reward modulation in anorexia nervosa—insight from multimodal imaging, Int. J. Eat. Disord. 50 (2017), https://doi.org/10.1002/eat.22638.
[39] S.J. Klenotich, E. v Ho, M.S. McMurray, C.H. Server, S.C. Dulawa, Dopamine D2/3 receptor antagonism reduces activity-based anorexia, Transl. Psychiatry 5 (2015), e613, https://doi.org/10.1038/tp.2015.109.
[40] F. Brambilla, C.S. Garcia, S. Fassino, G.A. Daga, A. Favaro, P. Santonastaso, C. Ramaciotti, E. Bondi, C. Mellado, R. Borriello, P. Monteleone, Olanzapine therapy in anorexia nervosa: Psychobiological effects, Int. Clin. Psychopharmacol. 22 (2007), https://doi.org/10.1097/YIC.0b013e328080ca31.
[41] J. Mendoza, M. Angeles-Castellanos, C. Escobar, Entrainment by a palatable meal induces food-anticipatory activity and c-Fos expression in reward-related areas of the brain, Neuroscience 133 (2005), https://doi.org/10.1016/j.neuroscience.2005.01.064.
[42] A.E. Kelley, B.A. Baldo, W.E. Pratt, M.J. Will, 2005. Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward, in: Physiol Behav, 2005. https://doi.org/10.1016/j.physbeh.2005.08.066.
[43] J.A. Beeler, N.S. Burghardt, The rise and fall of dopamine: a two-stage model of the development and entrenchment of anorexia nervosa, Front. Psychiatry 12 (2022), https://doi.org/10.3389/fpsyt.2021.799548.

[44] J.T. Dwarkasing, M. v Boekschoten, J.M. Argilès, M. van Dijk, S. Busquets, F. Penna, M. Toledo, A. Laviano, R.F. Witkamp, K. van Norren, Differences in food intake of tumour-bearing cachectic mice are associated with hypothalamic serotonin signalling, J. Cachex-.-. Sarcopenia Muscle (2015), https://doi.org/10.1002/jcsm.12008.

[45] D.P.D. Atchley, K.L. Weaver, L.A. Eckel, Taste responses to dilute sucrose solutions are modulated by stage of the estrous cycle and fenfluramine treatment in female rats, Physiol. Behav. 86 (2005), https://doi.org/10.1016/j.physbeh.2005.08.001.

[46] M.D. Lee, G.A. Kennett, C.T. Dourish, P.G. Clifton, 5-HT1B receptors modulate components of satiety in the rat: Behavioural and pharmacological analyses of the selective serotonin1B agonist CP-94,253, Psychopharmacology 164 (2002), https://doi.org/10.1007/s00213-002-1162-7.

[47] A. Jean, G. Conductier, C. Manrique, C. Bouras, P. Berta, R. Hen, Y. Charnay, J. Bockaert, V. Compan, Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens, Proc. Natl. Acad. Sci. USA 104 (2007), https://doi.org/10.1073/pnas.0701471104.

[48] V.R. Burden, B.D. White, R.G. Dean, R.J. Martin, Activity of the hypothalamic-pituitary-adrenal axis is elevated in rats with activity-based anorexia, J. Nutr. 123 (1993), https://doi.org/10.1093/jn/123.7.1217.

[49] R.A. Wise, Dopamine, learning and motivation, Nat. Rev. Neurosci. 5 (2004), https://doi.org/10.1038/nrn1406.

[50] D. Aspesi, A. Farinetti, M. Marraudino, G.S.K. Morgan, E. Marzola, G. Abbate-Daga, S. Gotti, Maternal separation alters the reward system of activity-based anorexia rats, Psychoneuroendocrinology 133 (2021), https://doi.org/10.1016/j.psyneuen.2021.105393.

[51] F.A. Cowdrey, R.J. Park, C.J. Harmer, C. McCabe, Increased neural processing of rewarding and aversive food stimuli in recovered anorexia nervosa, Biol. Psychiatry 70 (2011), https://doi.org/10.1016/j.biopsych.2011.05.028.

[52] L. Décarie-Spain, S. Sharma, C. Hryhorczuk, V. Issa-Garcia, P.A. Barker, N. Arbour, T. Alquier, S. Fulton, Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat, Mol. Metab. 10 (2018), https://doi.org/10.1016/j.molmet.2018.01.018.

[53] J.C. Felger, M.T. Treadway, Inflammation effects on motivation and motor activity: role of dopamine, Neuropsychopharmacology 42 (2017), https://doi.org/10.1038/npp.2016.143.

[54] A.C. Welch, J. Zhang, J. Lyu, M.S. McMurray, J.A. Javitch, C. Kellendonk, S. C. Dulawa, Dopamine D2 receptor overexpression in the nucleus accumbens core induces robust weight loss during scheduled fasting selectively in female mice, Mol. Psychiatry 26 (2021), https://doi.org/10.1038/s41380-019-0633-8.

[55] J.K. Dreyer, C.M. vander Weele, V. Lovic, B.J. Aragona, Functionally distinct dopamine signals in nucleus accumbens core and shell in the freely moving rat, J. Neurosci. 36 (2016), https://doi.org/10.1523/JNEUROSCI.2326-15.2016.

[56] M. Moreno, D. Economidou, A.C. Mar, C. López-Granero, D. Caprioli, D. E. Theobald, A. Fernando, A.H. Newman, T.W. Robbins, J.W. Dalley, Divergent effects of D2/3 receptor activation in the nucleus accumbens core and shell on impulsivity and locomotor activity in high and low impulsive rats, Psychopharmacology 228 (2013), https://doi.org/10.1007/s00213-013-3010-3.

[57] B.A. Baldo, K. Sadeghian, A.M. Basso, A.E. Kelley, Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity, Behav. Brain Res. 137 (2002), https://doi.org/10.1016/S0166-4328(02)00293-0.