Received: 20 November 2023 | Revised: 22 March 2024 | Accepted: 1 April 2024

DOI: 10.1002/eat.24216

ORIGINAL ARTICLE

International Journal of EATING DISORDERS WILEY

Repeated binge-like eating episodes in female rats alter adenosine A_{2A} and dopamine *D2* receptor genes regulation in the brain reward system

Francesca Mercante[1] | Emanuela Micioni Di Bonaventura PhD[2] | Mariangela Pucci PhD[1,3] | Luca Botticelli PhD[2] | Carlo Cifani PhD[2] | Claudio D'Addario PhD[1,4] | Maria Vittoria Micioni Di Bonaventura PhD[2]

[1]Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

[2]Pharmacology Unit, School of Pharmacy, University of Camerino, Camerino, Italy

[3]Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden

[4]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Correspondence
Claudio D'Addario, Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via Renato Balzarini 10, Teramo 64100, Italy.
Email: cdaddario@unite.it

Carlo Cifani, Pharmacology Unit, School of Pharmacy, University of Camerino, Via Madonna delle Carceri 9, Camerino 62032, Italy.
Email: carlo.cifani@unicam.it

Funding information
Italian Ministry of University and Research, Grant/Award Number: PRIN2015KP7T2Y to C.C.; European Union – Next Generation EU, Grant/Award Number: PRIN2022LHMF43 to M.V.M.D.B.; Under Italian Ministry of University and Research, European Union–Next Generation EU, Grant/Award Number: ECS00000041; Innovation, digitalization and sustainability for the diffused economy in Central Italy–VITALITY to C.D.

Action Editor: Britny A. Hildebrandt

Abstract

Objective: Binge-eating disorder is an eating disorder characterized by recurrent binge-eating episodes, during which individuals consume excessive amounts of highly palatable food (HPF) in a short time. This study investigates the intricate relationship between repeated binge-eating episode and the transcriptional regulation of two key genes, adenosine A_{2A} receptor ($A_{2A}AR$) and dopamine *D2* receptor (*D2R*), in selected brain regions of rats.

Method: Binge-like eating behavior on HPF was induced through the combination of food restrictions and frustration stress (15 min exposure to HPF without access to it) in female rats, compared to control rats subjected to only restriction or only stress or none of these two conditions. After chronic binge-eating episodes, nucleic acids were extracted from different brain regions, and gene expression levels were assessed through real-time quantitative PCR. The methylation pattern on genes' promoters was investigated using pyrosequencing.

Results: The analysis revealed $A_{2A}AR$ upregulation in the amygdala and in the ventral tegmental area (VTA), and *D2R* downregulation in the nucleus accumbens in binge-eating rats. Concurrently, site-specific DNA methylation alterations at gene promoters were identified in the VTA for $A_{2A}AR$ and in the amygdala and caudate putamen for *D2R*.

Discussion: The alterations on $A_{2A}AR$ and *D2R* genes regulation highlight the significance of epigenetic mechanisms in the etiology of binge-eating behavior, and underscore the potential for targeted therapeutic interventions, to prevent the development of this maladaptive feeding behavior. These findings provide valuable insights for future research in the field of eating disorders.

Francesca Mercante and Emanuela Micioni Di Bonaventura contributed equally to this study.

Public Significance: Using an animal model with face, construct, and predictive validity, in which cycles of food restriction and frustration stress evoke binge-eating behavior, we highlight the significance of epigenetic mechanisms on adenosine A_{2A} receptor ($A_{2A}AR$) and dopamine D2 receptor ($D2R$) genes regulation. They could represent new potential targets for the pharmacological management of eating disorders characterized by this maladaptive feeding behavior.

KEYWORDS
adenosine A_{2A} receptor ($A_{2A}AR$), animal model, binge eating, brain reward system, dopamine $D2$ receptor ($D2R$), epigenetics, female rats, rat's brain

1 | INTRODUCTION

Binge eating (BE) is a primary diagnostic symptom in binge-eating disorder (BED), as well as bulimia nervosa, and anorexia nervosa (binge/purge subtype), and it is characterized by quickly consuming large quantities of highly palatable food (HPF), together with a feeling of loss of control over eating (American Psychiatric Association, 2022). BED is characterized by recurrent, and brief (usually ≤2 h) BE episodes. It is often associated with various physical comorbidities, high impulsivity, and mood disorders (Giel et al., 2017; Keski-Rahkonen, 2021). The worldwide prevalence of BED in adults from 2018 to 2020 is estimated to be 0.6%–1.8% in women and 0.3%–0.7% in men (Giel et al., 2022). Understanding the neurobiological and epigenetic mechanisms involved in BED is crucial for advancing the knowledge of this psychiatric disorder and developing effective interventions. Currently, only one drug, lisdexamfetamine dimesylate, was approved by the Food and Drug Administration (FDA) for BED, although some adverse effects are present (Heo & Duggan, 2017; Schneider et al., 2021). Several factors contribute to the development of BED, including enhanced food craving (Yu et al., 2022), impaired decision making (Goldschmidt et al., 2018; Witt & Lowe, 2014), diminished executive function (Cury et al., 2020; Iceta et al., 2021), and impulsivity, a personality trait strongly associated with the loss of control during BE episodes (Dawe & Loxton, 2004; Kessler et al., 2016). Neurobiological research has recently addressed the involvement of several interconnected brain regions and neurocircuitries in the development of BED, influencing both impulsive and eating behaviors (Manfredi et al., 2021).

Dieting (Pankevich et al., 2010; Polivy et al., 1994) and stress (Micioni Di Bonaventura, Micioni Di Bonaventura, et al., 2020; Pecoraro et al., 2004; Teegarden & Bale, 2008) are common triggers for BED, which can be replicated in animal models (Micioni Di Bonaventura et al., 2021).

Consistently, cycles of food restriction and refeeding, with the addition of frustration stress (in which the rats can see and smell the HPF, locked in a metallic grid, and so inaccessible to them), were used to evoke BE behavior in Cifani et al. rat model (Cifani et al., 2009), and revealed the involvement of the transcriptional regulation of different genes (Micioni Di Bonaventura, Ubaldi, et al., 2017; Pucci et al., 2016, 2019). Although mild, this manipulation increased plasma levels of the stress hormone corticosterone, indicating that it is stressful to rats (Cifani et al., 2009, 2010; Micioni Di Bonaventura, Vitale, et al., 2012), and eliciting a robust behavioral activation (Cifani et al., 2020; Micioni Di Bonaventura, Lutz, et al., 2017) to try to consume the HPF (Cifani et al., 2013). In particular, in this BE model, we investigated the genes coding for the adenosine A_{2A} receptor ($A_{2A}AR$) and the dopamine $D2$ receptor ($D2R$) in the development of BE behavior (Micioni Di Bonaventura et al., 2019), being both implicated in reward-related processes. Their dysregulation could represent an interesting target for the management of this feeding disorder.

Central adenosine neurotransmission is known to regulate HPF intake, food and drug seeking behaviors, and altered adenosine' receptors expression was detected after chronic drug abuse (Kavanagh et al., 2015; Micioni Di Bonaventura, Cifani, et al., 2012; Mingote et al., 2008; Pritchett et al., 2010; Wydra et al., 2015). Adenosine is a neuromodulator in the central nervous system and, regulating especially dopamine neurotransmission, it is linked to reward-related behavior (Ballesteros-Yáñez et al., 2017; Nunes et al., 2013). These effects are primarily due to co-localization in several brain regions and molecular interactions between adenosine and dopamine receptors, which are able to form heteromeric receptor complexes (Ballesteros-Yáñez et al., 2017; Fuxe et al., 2010). The $A_{2A}AR$ activation is mostly responsible for the inhibitory role of adenosine (Ferré et al., 2018). Additionally, adenosine modulates dopaminergic activity through its receptors, affecting the reward pathway and the mesolimbic system (Ferré et al., 2008, 2018).

The mesolimbic pathway originates in the ventral tegmental area (VTA), which sends dopaminergic axons to the prefrontal cortex, striatum, amygdala, and other brain areas (Li & Jasanoff, 2020).

The dopamine circuitry is associated with increased susceptibility to BE and other addictive disorders (Avena, Bocarsly, et al., 2008; Johnson & Kenny, 2010; Kenny et al., 2013; Volkow et al., 2009). Dopamine greatly influences the reward value of food through the dopaminergic pathway primarily involved in the mesolimbic system (Lewis et al., 2021).

Interestingly, the interaction between the adenosinergic and dopaminergic systems has an impact on both appetite and effort-related aspects of food motivation (Font et al., 2008; Nunes et al., 2013; Randall et al., 2012; Salamone et al., 2018; Salamone & Correa, 2009), and enhanced frequency of the dopamine transporter and D2R polymorphisms are linked with BE disturbance (Bello & Hajnal, 2010; Davis et al., 2012).

In this context, it is crucial to study the regulation of the epigenetic mechanisms in gene transcription to explain how environmental triggers, particularly food restriction and stress, contribute to the development of BE behavior.

We investigated the selective epigenetic modulation at the promoters of the above mentioned genes, in particular brain areas implicated in the regulation of reward-related processes (Schultz, 2015), including the amygdaloid complex (Amy), for its role in emotional reactivity, food-related behavior and uncontrolled consumption of HPF (Blasio et al., 2013; Bohon & Stice, 2012; Galarce et al., 2010; Gallagher & Chiba, 1996; Holland & Gallagher, 2003; Pringle et al., 2011), the nucleus accumbens (NAc) and the caudate putamen (CP) that are part of the striatum, and the VTA. This latter brain area combines information from peripheral to hypothalamic, midbrain/hindbrain, limbic and cortical areas (Watabe-Uchida et al., 2012) to encode motivation for HPF and stress- or cue-induced feeding (Meye & Adan, 2014). Additionally, the NAc is involved in motivation-related processes associated with food-seeking behavior and stress (Avena, Rada, & Hoebel, 2008; Nunes et al., 2013; Wang et al., 2011). The CP, a key region of the mesolimbic network, is involved in food-related behaviors, and closely associated with signals of energy homeostasis and aberrant eating habits (Zhang et al., 2019).

In our previous works, the molecular changes on the same genes were evaluated immediately after only one episode of BE (acute exposure), when the BE phenotype was established. Herein instead, we extended the investigations to recurrent episodes of BE (chronic exposure), using the same animal model (Cifani et al., 2009), to better mimic human BED. Furthermore, repeated BE episodes may lead the reward system to become accustomed to receiving food, resulting in a lower level of responsiveness and requiring the consumption of increasing amounts of food to provoke the same response (Leenaerts et al., 2022).

In this animal model, once established, BE behavior remains stable over time (Piccoli et al., 2012; Pucci et al., 2022). Accordingly, female rats were subjected to intermittent periods of restriction and frustration stress, as described above, to induce several episodes of BE. The gene transcription regulation was analyzed after these episodes in the same brain regions as our previous studies (Micioni Di Bonaventura et al., 2019; Pucci et al., 2016) to compare exposure to a single or chronic BE episodes.

2 | MATERIALS AND METHODS

2.1 | Animal model

The BE protocol was performed as previously established (Cifani et al., 2009, 2020; Pucci et al., 2022). The BE episode is operationally defined as the large amount of HPF consumed by rats within 2 h. Brain samples of 24 adult female Sprague–Dawley rats (Charles River, Calco, Italy; 200–225 g, 9 weeks old at the beginning of the experiment) analyzed in this study were originally collected in a previous work (Pucci et al., 2022).

Briefly, the rats, individually housed rats under a 12 h light/dark cycle (lights on at 8:00 a.m.), after 1 week of adaptation followed the BE protocol (see Suppl. Methods for experimental details and Supplementary Figure 1) and were subdivided into 4 groups of 6 rats each: NR + NS: non-restricted and non-stressed rats; NR + S: non-restricted and stressed rats; R + NS: restricted and non-stressed rats; R + S: restricted and stressed rats. The BE phenotype was induced by restrictions of standard chow plus frustration stress, during which the animals can see and smell the known HPF but cannot consume it for 15 min. Only R + S rats showed BE behavior, consuming significantly more HPF during the 2 h feeding test compared to the other three groups.

The HPF, offered in a coffee cup, is a mix of Nutella chocolate cream (Ferrero, Alba, Torino, Italy, 5.33 kcal/g), ground chow pellets (4RF18, Mucedola, Settimo Milanese, Italy, 2.6 kcal/g), and water, in the following percentages (w/w): 52%, 33%, and 15%, respectively.

Following 4 episodes of BE, all rats were sacrificed in the next episode, immediately after stress manipulation, before eating HPF (Supplementary Figure 1).

The results of body weight and HPF intake measurements were already published in Pucci et al., 2022. During the chow restriction days, the restricted rats (R + NS and R + S) lose their body weight, which was immediately recovered in the following days of ad libitum chow access. Therefore, on test days, the mean body weights of all groups of rats were comparable. Thus, the significant increase in HPF intake in R + S rats (BE group) in each feeding test was not due to hunger or energy deficit.

2.2 | Tissue collection and nucleic acid extraction

Immediately after sacrifice, brains were collected. The Amy, NAc, CP, and VTA were dissected, frozen and stored at −80°C until processing (D'Addario et al., 2020). Nucleic acids were extracted following the Chomczynski and Sacchi method (Chomczynski & Sacchi, 2006). The quantity and purity were evaluated using NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA). We used total RNA to assess the relative abundance of $A_{2A}AR$ and *D2R* genes through real-time qPCR, and DNA to examine the methylation pattern in the promoter regions of the genes using pyrosequencing.

2.3 | Gene expression analysis

mRNA was transformed into complementary DNA (cDNA) using SensiFAST cDNA synthesis Kit (Bioline Reagents, London, UK), following the instructions provided by the manufacturer and the resulting cDNAs were diluted three times. The relative abundance of mRNA was evaluated through quantitative reverse transcription-polymerase chain reaction (RT-qPCR). The RT-qPCR analysis was carried out using the DNA Engine Opticon 2 Continuous Fluorescence Detection System (MJ Research) (Pucci et al., 2022). The amplification primers are reported in Table 1. The RT-qPCR analysis data were normalized using

the average CT (cycle threshold) value derived from two endogenous reference genes, specifically *β-actin* and *Gapdh*. To determine the fold change in gene expression, the Livak ($2^{-\Delta\Delta CT}$) method was used (Livak & Schmittgen, 2001). This approach calculates the rate of increased expression by comparing the CT values between the target gene and the reference genes after normalization.

2.4 | DNA methylation analysis

The CpG islands were predicted using MethPrimer software. MethPrimer is a widely used bioinformatics tool specifically designed for the prediction and analysis of CpG islands in DNA sequences. It utilizes a set of predefined criteria, including GC content, observed-to-expected CpG ratio, and length, to identify potential CpG islands within a given DNA sequence (Li & Dahiya, 2002). Methylation analysis was conducted on DNA that had undergone bisulfite conversion, extracted from different rat's brain regions (Amy, NAc, CP, and VTA) using pyrosequencing.

After DNA extraction, a quantity of 250 ng of DNA from each sample was subjected to bisulfite treatment using the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, CA, USA). This kit converts unmethylated cytosines to uracil while leaving methylated cytosines unchanged, enabling the identification of DNA methylation patterns.

The bisulfite-treated DNA was then amplified using PyroMark PCR Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The PCR conditions include an initial step at 95°C for 15 min, followed by 45 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min (Pucci et al., 2022). PCR products were verified by agarose electrophoresis. Methylation analysis was conducted using PyroMark Q48 Autoprep (Qiagen, Hilden, Germany) and the degree of methylation was assessed using PyroMark Q48 Software (Qiagen, Hilden, Germany). This software calculates the methylation percentage [mC/(mC + C)] for each CpG site, enabling quantitative comparisons (mC represents methylated cytosine, C denotes unmethylated cytosine) (Pucci et al., 2022). The primers sequences are reported in Table 2.

TABLE 1 List of primers used for quantitative real-time PCR.

Gene	Forward	Reverse
β-Actin	agatcaagatcattgctcctcct	acgcagctcagtaacagtcc
Gapdh	agacagccgcatcttcttgt	cttgccgtgggtagagtcat
$A_{2A}AR$	ttcgcctgttttgtcctggt	aagccattgtaccggagtgg
D2R	tacgtgcccttcatcgtcac	gtgggtacagttgcccttga

2.5 | Statistical analysis

The results were analyzed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). To assess statistical differences in gene expression a two-way ANOVA, (food restriction [no or yes] and frustration stress during testing [no or yes]), followed by multiple comparison with Bonferroni's post hoc tests was performed. DNA methylation level at each CpG site was analyzed using multiple-*t*-test and corrected for multiple comparison through Sidak-Bonferroni method. Correlations were performed by Spearman's coefficient and $p < 0.05$ was considered to be statistically significant.

3 | RESULTS

According to our previous studies with the same BE model (Cifani et al., 2009; Micioni Di Bonaventura et al., 2019; Romano et al., 2020), in each feeding test (total of four tests), the R + S rats showed the BE episode, significantly increasing the HPF intake (kcal/kg) compared to the other experimental groups (NR + NS, NR + S and R + NS), as reported in Pucci et al., 2022. More specifically, during the entire duration of each test (2 h), the R + S rats ate 59.4% (I EPISODE), 46.8% (II EPISODE), 63.1% (III EPISODE), and 51.5% (IV EPISODE) more than the other groups. Since no significant differences were observed in the HPF intake among the three control groups (Pucci et al., 2022), the percentage difference in HPF intake was calculated comparing the mean of HPF intake of R + S rats with the mean HPF intake of the other groups for each episode. For more details about each behavioral experiment, see Pucci et al., 2022.

To explore the influence of recurrent episodes of BE on the regulation of *$A_{2A}AR$* and *D2R* gene expression, we examined mRNA levels and potential epigenetic modifications in these brain regions: Amy, NAc, CP, and VTA.

TABLE 2 List of primers and sequences used during the methylation analysis.

Gene	Primers	Sequence to analyze	Genomic location (rat mRatBN7.2)
$A_{2A}AR$	Fwr (Biotin): ATTAGGGTGGGGGTGGGA Rev: AAACCCCCAACAAAACACCCTT Seq: AAACACCCTTCTCCC	…gagtgg**CG**ctggaggagg**CG**gtcagga**CGCG**tggacttgaag **CG**acca**CG**ttcc…	Chromosome 22: 24417879–24442357
D2R	Included in the assay PM00586096	…tc**CG**a**CG**ggcagattg**CG**cct**CG**gg**CG**t**CG**gaa…	Chromosome 8: 49708927–49772875

3.1 | Regulation of $A_{2A}AR$ and *D2R* in a model of BE

Significant differences in $A_{2A}AR$ and *D2R* gene expression and DNA methylation levels across the different brain regions were reported.

In the Amy, significant changes in gene expression were observed for $A_{2A}AR$ but not for *D2R* (as shown in Figure 1a, b). These changes in $A_{2A}AR$ mRNA levels were influenced by stress ($F_{(1,16)} = 25.85$; $p < 0.05$) but not by food restriction ($F_{(1,16)} = 0.73$; $p = 0.406$) neither by the interaction ($F_{(1,16)} = 4.30$; $p = 0.054$).

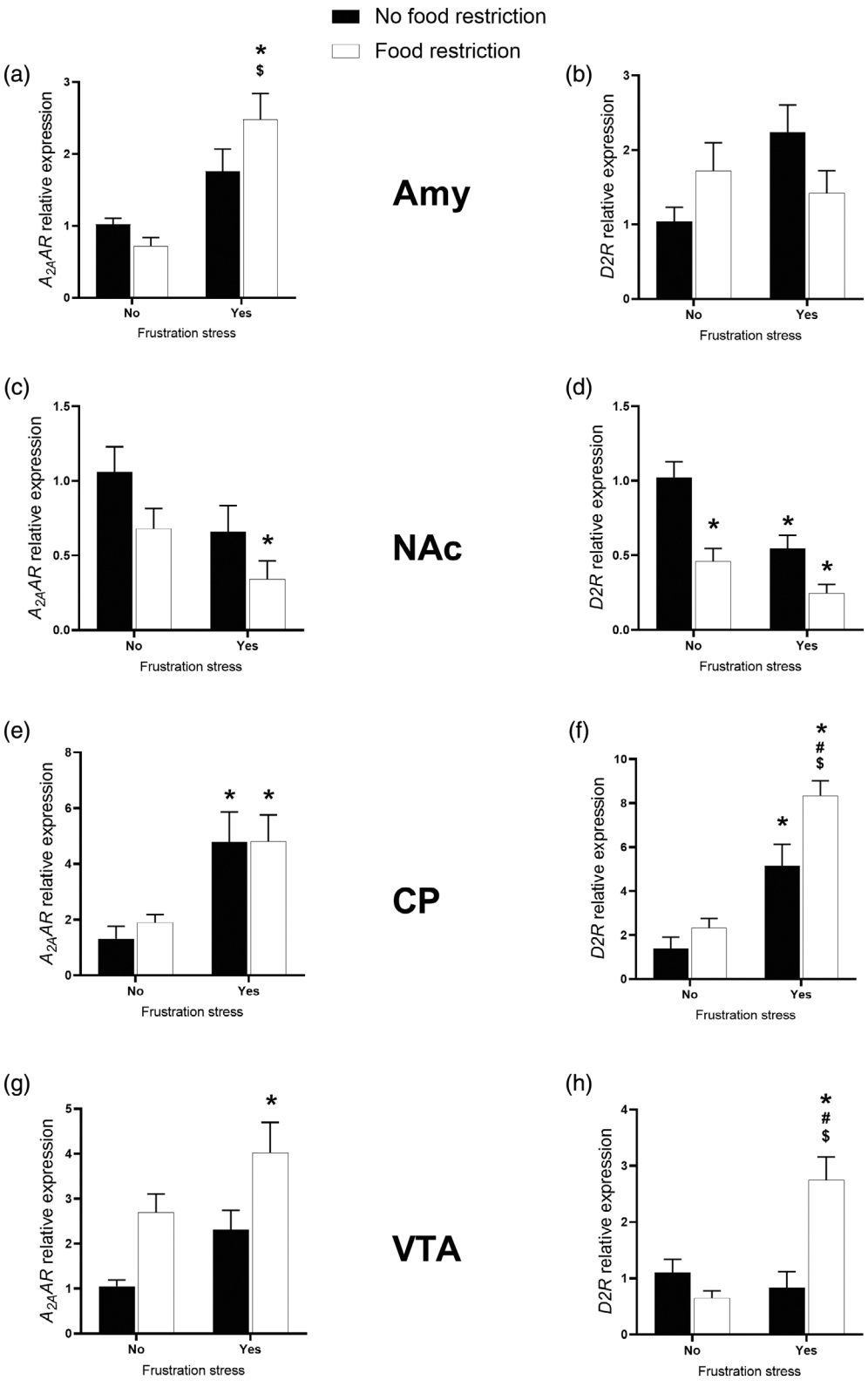

FIGURE 1 $A_{2A}AR$ and *D2R* relative gene expression in amygdaloid complex (Amy), nucleus accumbens (NAc), caudate putamen (CP), and ventral tegmental area (VTA) of rat exposed (or not) to restriction and stress (NR + NS: non-restricted and non-stressed rats; NR + S: non-restricted and stressed rats; R + NS: restricted and non-stressed rats; R + S restricted and stressed rats) reported as $2^{-\Delta\Delta Ct}$ values calculated by Delta–Delta Ct ($\Delta\Delta$Ct) method. Expression was normalized using an average Ct-value from both reference genes (*Gapdh* and *β-act*), and data are reported as mean ± SEM with a sample size of $n = 5$ per group. Relative expression of: Amy (a) $A_{2A}AR$ (adenosine 2A receptor); (b) *D2R* (dopamine receptor 2); NAc (c) $A_{2A}AR$ and (d) *D2R*; CP (e) $A_{2A}AR$ and (f) *D2R*; VTA (g) $A_{2A}AR$ and (h) *D2R*. Data were analyzed by two-way ANOVA in a 2 (frustration stress during testing: no, yes) × 2 (food restriction: no, yes) factorial design. Bonferroni's post hoc tests were used to follow up on significant interaction or main effects ($p < 0.05$). Significant differences are indicated: $^{*}p < 0.05$ versus NR + NS; $^{\#}p < 0.05$ versus NR + S; $^{\$}p < 0.05$ versus R + NS.

Multiple comparisons test further revealed a significant increase between the R + S group and both NR + NS ($p = 0.004$) and R + NS ($p = 0.0007$) (Figure 1a).

In the NAc, significant changes in both $A_{2A}AR$ and *D2R* mRNA levels were found in R + S rats. The alterations in $A_{2A}AR$ were influenced by both stress ($F_{(1,16)} = 5.88$; $p < 0.05$) and food restriction ($F_{(1,16)} = 5.26$;

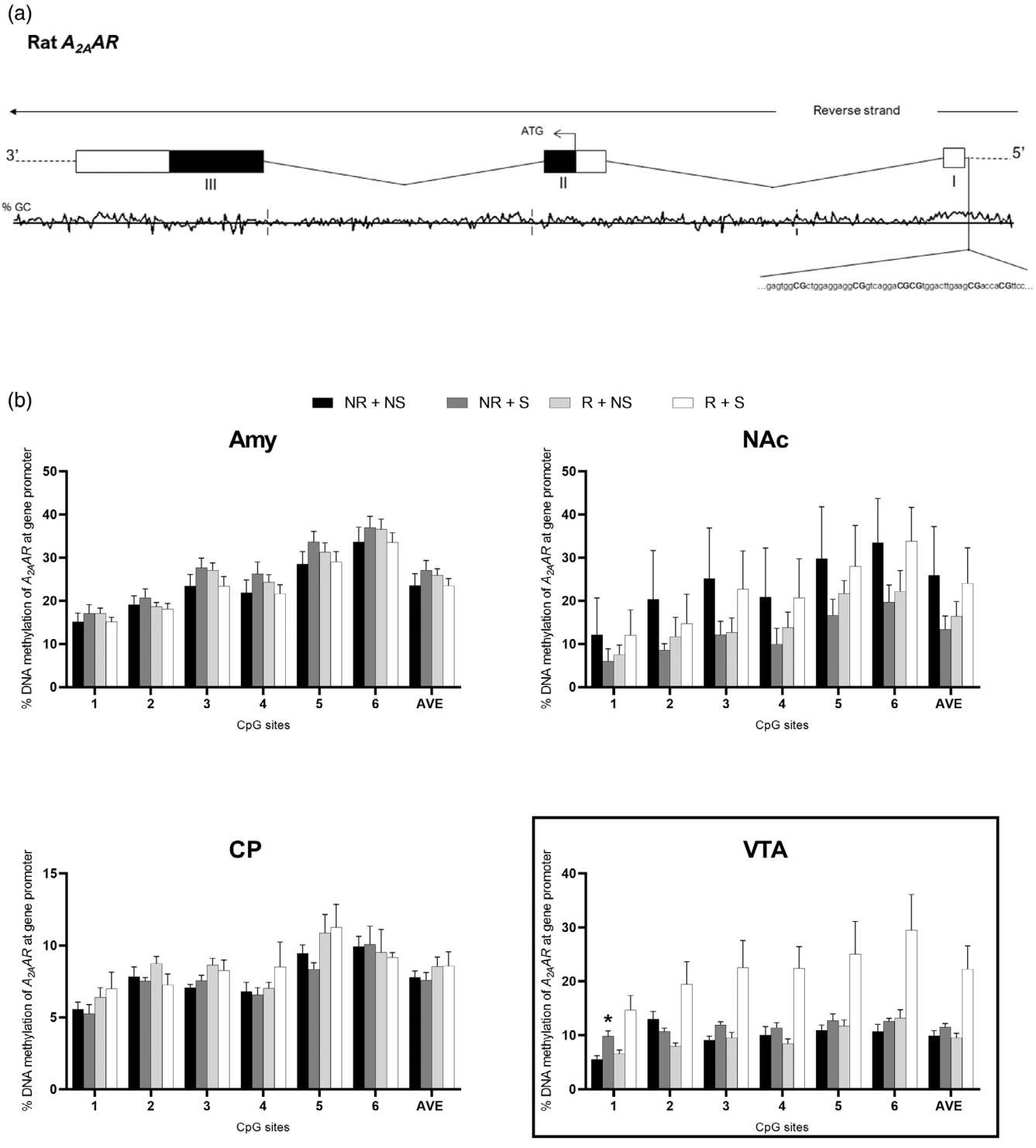

FIGURE 2 Schematic representation of rat $A_{2A}AR$ gene (a). Translation starts codon (ATG), exons and introns are depicted. Coding regions of exons are shown darker. Sequence of the CpG island under study is also reported. Bold text indicates the 6 CpG sites analyzed; (b) Percentage of DNA methylation at $A_{2A}AR$ promoter regions in the Amy, NAc, CP, and VTA. Values on the y-axis represent the % of methylation values of individual CpG sites under study, as well as of the average (AVE) of all CpG sites ± SEM with a sample size of $n = 5$ per group. Data were analyzed by multiple-*t*-test and corrected for multiple comparisons using the Sidak-Bonferroni method. Significant differences are indicated: $^*p < 0.05$ versus NR + NS.

$p < 0.05$). There was no significant interaction between these two factors ($F_{(1,16)} = 0.04$; $p = 0.847$). The multiple comparisons test, shown in Figure 1c, revealed a significant decrease in $A_{2A}AR$ mRNA levels between R + S group and NR + NS ($p = 0.0252$). Similarly, *D2R* mRNA levels exhibited a significant change in the R + S group. These changes were influenced by both stress ($F_{(1,16)} = 15.90$; $p < 0.05$) and food restriction ($F_{(1,16)} = 25.06$; $p < 0.05$), but not by the interaction between these two factors ($F_{(1,16)} = 2.27$; $p = 0.151$). The post hoc group differences showed a significant decrease in all the groups (NR + S, $p = 0.0079$; R + NS, $p = 0.0018$; R + S, $p < 0.0001$) compared to NR + NS (Figure 1d).

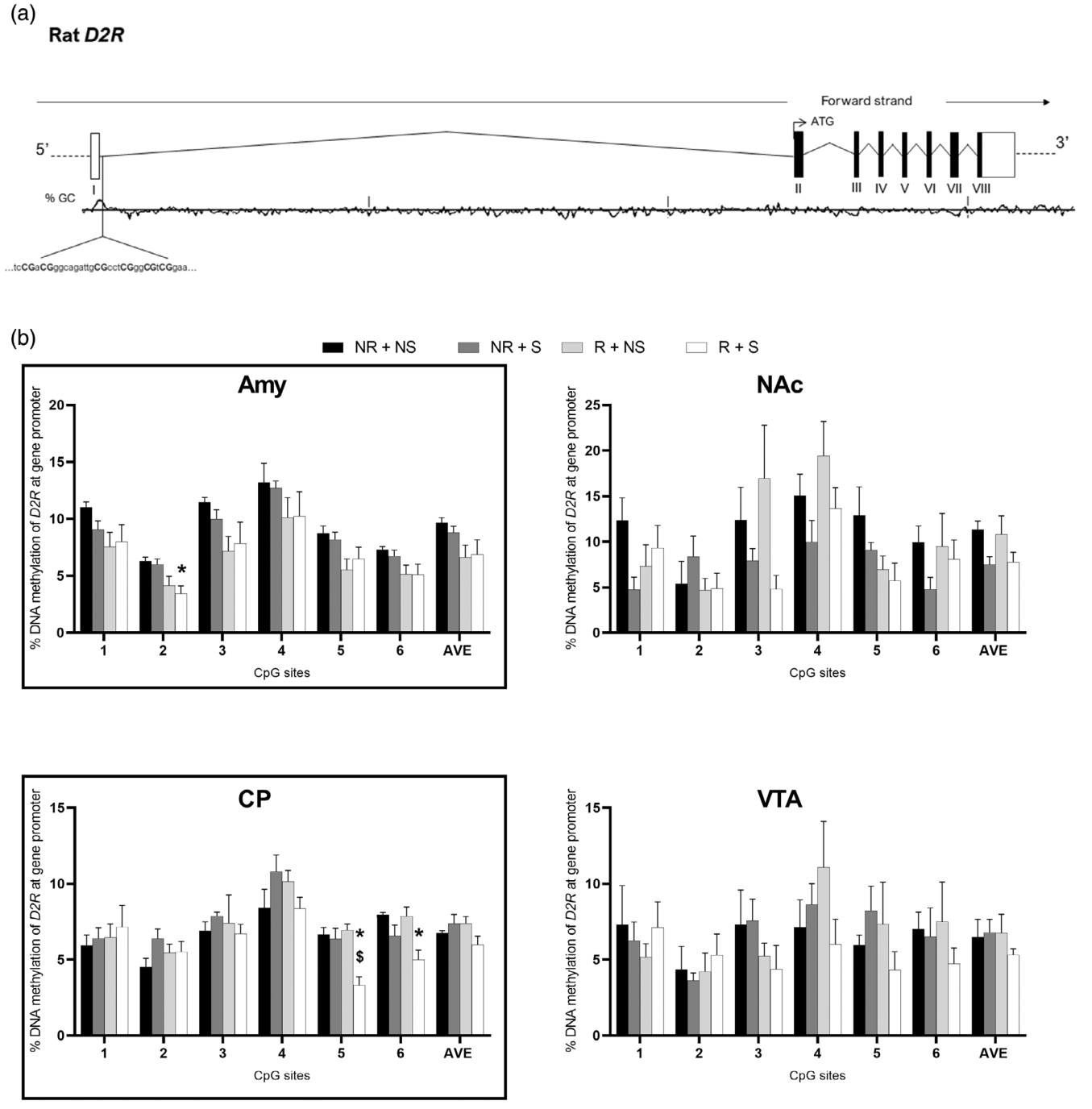

FIGURE 3 Schematic representation of rat *D2R* gene (a). Translation starts codon (ATG), exons and introns are depicted. Coding regions of exons are shown darker. Sequence of the CpG island under study is also reported. Bold text indicates the 6 CpG sites analyzed; (b) Percentage of DNA methylation level at *D2R* promoter regions in the Amy, NAc, CP, and VTA. Values on the y-axis represent the % of methylation values of individual CpG sites under study, as well as of the average (AVE) of all CpG sites ± SEM with a sample size of $n = 5$ per group. Data were analyzed by multiple-t-test and corrected for multiple comparisons using the Sidak-Bonferroni method. Significant differences are indicated: $^{*}p < 0.05$ versus NR + NS; $^{\$}p < 0.05$ versus R + NS.

In the CP, $A_{2A}AR$ mRNA levels were significantly affected by stress ($F_{(1,16)} = 17.45$; $p < 0.05$) but not by food restriction ($F_{(1,16)} = 0.16$; $p = 0.69$) or the interaction between these two factors ($F_{(1,16)} = 0.12$; $p = 0.728$). Multiple comparisons test revealed an increase of $A_{2A}AR$ mRNA levels in both groups NR + S ($p = 0.0332$) and R + S ($p = 0.0307$) with respect to NR + NS (Figure 1e). On the other hand, mRNA levels of *D2R* in the CP were influenced by both stress ($F_{(1,16)} = 52.40$; $p < 0.05$) and food restriction ($F_{(1,16)} = 9.39$; $p < 0.05$), but not by the interaction ($F_{(1,16)} = 2.70$; $p = 0.119$). The post hoc group differences, depicted in Figure 1f, presented a significant increase in R + S group compared to all the other groups (NR + NS, $p = 0.0001$; NR + S, $p = 0.0255$; R + NS, $p < 0.0001$) and also in NR + S versus NR + NS ($p = 0.0068$).

Finally, in the VTA, $A_{2A}AR$ gene exhibited significant changes in response to stress ($F_{(1,16)} = 8.01$; $p < 0.05$) and food restriction ($F_{(1,16)} = 13.51$; $p < 0.05$), but not by the interaction between these two factors ($F_{(1,16)} = 0.004$; $p = 0.94$). Meanwhile, the *D2R* gene in the VTA was also affected by stress ($F_{(1,16)} = 10.24$; $p < 0.05$) and food restriction ($F_{(1,16)} = 6.61$; $p < 0.05$) but also by the interaction between food restrictions and stress ($F_{(1,16)} = 17.39$; $p < 0.05$). It was observed a significant increase in $A_{2A}AR$ mRNA levels in R + S rats with respect to NR + NS ($p = 0.0018$). In addition, *D2R* mRNA levels were increased in the R + S group in relation to NR + NS ($p = 0.0052$), NR + S ($p = 0.0013$) and R + NS ($p = 0.0005$). All the specific post hoc differences were reported in Figure 1g, h.

To explore the potential role of epigenetic mechanisms in the regulation of gene expression in the BE model, the DNA methylation pattern in the promoter regions of genes in the different brain areas was analyzed. Results are reported in Figures 2 and 3.

No significant differences were found at the $A_{2A}AR$ gene in Amy, NAc, and CP. However, a noteworthy finding emerged when examining all CpG sites at the $A_{2A}AR$ promoter region in the VTA. A significant increase in DNA methylation was observed at CpG site 1 of the NR + S group ($p = 0.04$ versus NR + NS) (Figure 2b).

Furthermore, a correlation analysis was conducted between the expression of both genes and the HPF intake (BE behavior), calculated as the mean HPF intake during the 2 h time point of the four feeding tests in each experimental group (Supplementary Table 1). Significant correlations were observed in the Amy, NAc, and VTA. A positive correlation was identified between HPF intake and $A_{2A}AR$ gene expression (Spearman's $r = 0.4955$, $p = 0.0263$) in the Amy. Conversely, in the NAc, a negative correlation was found between HPF intake and *D2R* gene expression (Spearman's $r = -0.5459$, $p = 0.0128$). In the VTA, a positive correlation was found between HPF intake and $A_{2A}AR$ gene expression (Spearman's $r = 0.5167$, $p = 0.0197$), while a negative correlation was found with *D2R* gene expression (Spearman's $r = -0.6837$, $p = 0.0009$).

Conversely, when analyzing DNA methylation levels at CpG sites within the promoter region of the *D2R* gene, significant differences were observed in Amy and CP brain regions as shown in Figure 3b. In the Amy, a significant decrease in DNA methylation level was observed in the R + S group at CpG site 2 ($p = 0.036$ versus NR + NS). In CP, a significant change was detected in the R + S group, precisely

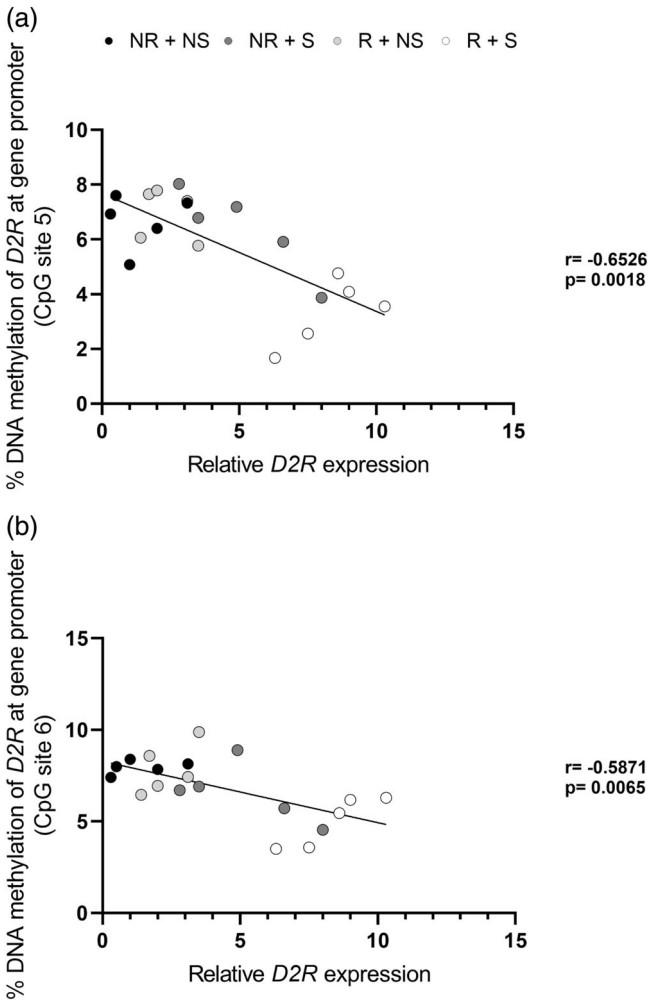

FIGURE 4 Correlation between *D2R* gene expression and DNA methylation level at CpG site 5 (a) and 6 (b) in CP. Data were compared by Spearman's rank correlation coefficient, *p*- and *r*-values are reported.

a decrease in the percentage of DNA methylation in the CpG sites 5 ($p = 0.01$ versus NR + NS; $p = 0.005$ versus R + NS) and 6 ($p = 0.01$ versus NR + NS).

An indirect correlation between *D2R* gene expression ($2^{-\Delta\Delta Ct}$ values) and DNA methylation was reported in the CP. Specifically, considering what observed in the fifth and sixth CpG sites analyzed, a negative correlation was found (CpG site 5: Spearman's $r = -0.6526$, $p = 0.0018$; CpG site 6: Spearman's $r = -0.5871$, $p = 0.0065$) (Figure 4). No significant results were observed correlating D2R CpG site 2 with gene expression (results not shown).

4 | DISCUSSION

The relationship between dieting and stress emerges as a crucial contribute to the onset of BE behavior and related eating disorders (American Psychiatric Association, 2022; Brewerton et al., 2000; Crowther et al., 2001; Grilo et al., 2001; Micioni Di Bonaventura et al., 2021; Micioni Di Bonaventura, Micioni Di Bonaventura, et al., 2020; Stice

et al., 2001). Accordingly, these two factors were recruited to induce BE episodes in preclinical models (Cifani et al., 2009; Hagan et al., 2002), including the BE protocol in female rats used in this work.

This study focuses on the transcriptional regulation of $A_{2A}AR$ and *D2R* genes, which were previously identified as involved in the development of a single BE episode in the Cifani et al. (2009) animal model.

Adenosine is an endogenous nucleoside and dopamine is a catecholamine neurotransmitter. They regulate several physiological functions and are involved in different diseases (Borea et al., 2018; Klein et al., 2019). Dopamine regulates feeding behaviors, and the dopamine reward system is acknowledged as the most important system that controls appetite, food craving, motivational and emotional drives to eat (Baik, 2021). Much attention has been given to the mesolimbic and mesocortical pathways in the dopamine reward system for controlling food intake in association with hedonic feeding (Baik, 2021). On the contrary, less is known about the involvement of central adenosine in mediating aspects of feeding, although studies found that the blockade of $A_{2A}AR$ significantly increased the consumption of a high-fat diet (Pritchett et al., 2010), while agonists of the same receptor blocked BE episodes (Micioni Di Bonaventura et al., 2019), and central injection of adenosine suppresses food intake in rats (Levine & Morley, 1983; Micioni Di Bonaventura, Cifani, et al., 2012).

The adenosine and dopamine systems are deeply interconnected because their receptors are co-localized, and adenosine has the ability to modulate dopamine release in the brain (Borgus et al., 2021).

In this research, we found alterations in these systems, specifically the $A_{2A}AR$ mRNA levels were selectively upregulated in the Amy of the R + S rats, (BE group), after recurrent episodes of BE, which is consistent with our previous results after a single episode (Micioni Di Bonaventura et al., 2019). This was supported by a selective enhancement of Fos immunoreactivity expression in central amygdala in R + S rats and by the mitigation of the BE episode after systemic or site-specific administration of $A_{2A}AR$ agonists into the same brain region.

Furthermore, other studies indicate the strong involvement of Amy in emotional reactivity, food-related behavior and excessive consumption of HPF (Blasio et al., 2013; Iemolo et al., 2013), and the $A_{2A}AR$ in this brain area plays an important role in fear memory processing (Simões et al., 2016, 2022, 2023), social interactions (López-Cruz et al., 2017) and anxiety (Chiu et al., 2014; López-Cruz et al., 2017). The results of $A_{2A}AR$ gene expression in the Amy are consistent with its upregulation in the VTA, which is relevant to better understand the interplay between VTA projections to post-synaptic targets in Amy. Of note, also *D2R* mRNA levels in the VTA were increased in the BE group. The VTA, located in the midbrain, is a primary source of dopamine neurons that project to cortical and limbic regions, playing an essential role in reward, stress processes (Farahimanesh et al., 2018), dietary cues (Polter & Kauer, 2014; Price & Drevets, 2010) and controls a variety of behaviors, including food reward processing and motivation for HPF (Meye & Adan, 2014). Palatability is seen as a representation of natural reward that could be sufficient to induce compulsive-like behavior towards food and impulsivity (de Macedo et al., 2016). The VTA was already found to be relevant in the modulation of *D2R*, potentially contributing to compulsive-like eating behaviors (Yu et al., 2022). In the striatum, a single BE episode did not induce alterations at the level of genes in the Nac and CP, (but only for both genes in Amy), while several changes were noted after repeated episodes of BE.

In the Nac, a brain region particularly susceptible to diet and stress (Campioni et al., 2009; Carr, 2020; Garcia-Keller et al., 2021), we found that both frustration stress and food restriction evoked a significant decrease in the expression of both $A_{2A}AR$ and *D2R* genes.

However, there was no significant interaction between these two conditions, suggesting that they may independently affect gene expression in the Nac. It has also been studied that pharmacological blockade of *D2R* in the Nac does not reduce the quantity of food ingested (Baldo et al., 2002); also, the inhibition of BE in animals, obtained with deep brain stimulation of the NAc shell, was significantly blunted by the use of raclopride (*D2R* antagonist), characterizing the involvement of *D2R* as fundamental in this feeding behavior (Halpern et al., 2013).

Besides, rats maintained on high-fat diets exhibit impulsivity phenotype and reduced accumbal expression of *D2R*, but not in the CP (Adams et al., 2015). In accordance with our model, in the CP, the mRNA levels of *D2R*, as well as $A_{2A}AR$, were significantly altered by repeated manipulation of frustration stress. Nevertheless, in our previous work, we did not observe any change in the expression of both genes after only one episode of BE (Micioni Di Bonaventura et al., 2019). Our data support the hypothesis of an adenosinergic control that may counteract dopaminergic activity in reward-related behavior through the existence of striatal $A_{2A}AR$-*D2R* complexes (Valle-León et al., 2021; Wydra et al., 2015). In addition, $A_{2A}AR$ agonists, which bind the adenosine receptor in the heteromer, decrease the affinity of *D2R* agonists through allosteric antagonistic interaction (Ferré et al., 2016).

To deeply analyze the transcriptional regulation of $A_{2A}AR$ and *D2R* genes, we assessed DNA methylation at gene promoters, observing selective changes for $A_{2A}AR$ only in the VTA and for *D2R* in the Amy and CP at specific CpG sites. Notably, in the CP, the reduction in DNA methylation at both CpG sites 5 and 6 was significantly inversely correlated with the increase in gene expression observed in the BE group.

Consumption of fat foods affects the methylation pattern of the dopamine system in the central reward circuitry, crucial to code the gratifying properties of HPF and hedonic feeding behavior (Vucetic et al., 2012). Studies show epigenetic dysregulation of *D2R* in mesolimbic areas in animals exposed to HPF (Rossetti et al., 2020), and this impacts the reward system (Ong & Muhlhausler, 2011). Endogenous and exogenous elements as nutrition influence the epigenome, and epigenetic changes may in turn modify animal phenotype (Zhang, 2015). The stability of DNA methylation changes with nutritional influences (Zhang, 2015), and DNA methyltransferases were suggested as possible therapeutic targets (Kozuka et al., 2017).

Interestingly, it was observed that prenatal stress in late gestation may increase the susceptibility of female offspring to develop BE like phenotype in adolescence, when there is a pre-existing epigenetic predisposition (Schroeder et al., 2017). Authors have observed hypomethylation of hypothalamic miR-1a and downstream dysregulation of the melanocortin system, which is implicated in stress and BE (Micioni Di

Bonaventura et al., 2022; Micioni Di Bonaventura, Botticelli, et al., 2020; Micioni Di Bonaventura, Micioni Di Bonaventura, et al., 2020).

In conclusion, this study provides insights into the complex interplay of stress, food restriction, gene expression, and epigenetic modifications in selected brain regions of the reward circuit associated with BE behavior. These findings underscore the importance of considering multiple factors, including epigenetic mechanisms, in understanding the neural basis of BE. This may encourage the search for new therapeutic targets for eating disorders and related conditions. Beside an approach focused on the maintenance of genomic DNA methylation, it would be relevant to target specific CpG sites within the promoters of loci whose methylation was found altered in specific conditions, as in our BE model for $A_{2A}AR$ and *D2R*. This approach was already developed using CRISPR-Cas9-based tools (Kang et al., 2019; Vojta et al., 2016) and further studies are needed to prove their efficacy in different conditions.

AUTHOR CONTRIBUTIONS

Francesca Mercante: Data curation; formal analysis; investigation; validation; visualization; writing – original draft; writing – review and editing. **Emanuela Micioni Di Bonaventura:** Data curation; formal analysis; investigation; validation; visualization; writing – original draft; writing – review and editing. **Mariangela Pucci:** Conceptualization; data curation; validation; visualization; writing – original draft; writing – review and editing. **Luca Botticelli:** Data curation; investigation; methodology; visualization; writing – review and editing. **Carlo Cifani:** Conceptualization; resources; supervision; validation; writing – review and editing. **Claudio D'Addario:** Conceptualization; resources; supervision; validation; writing – review and editing. **Maria Vittoria Micioni Di Bonaventura:** Data curation; formal analysis; investigation; methodology; supervision; validation; visualization; writing – original draft; writing – review and editing.

FUNDING INFORMATION

This work was supported by Italian Ministry of University and Research Project Code: PRIN2015KP7T2Y to C.C., by European Union – Next Generation EU: Project Code: PRIN2022LHMF43 to M.V.M.D.B. under Italian Ministry of University and Research and by European Union–Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy–VITALITY to C.D.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data were generated at the School of Pharmacy, Pharmacology Unit, University of Camerino and at the Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo. Derived data supporting the findings of this study are available from the corresponding authors on request.

ORCID

Francesca Mercante https://orcid.org/0000-0002-2260-5049
Emanuela Micioni Di Bonaventura https://orcid.org/0000-0001-8484-7513
Mariangela Pucci https://orcid.org/0000-0003-4657-6075
Luca Botticelli https://orcid.org/0000-0002-5414-2368
Carlo Cifani https://orcid.org/0000-0001-6180-828X
Claudio D'Addario https://orcid.org/0000-0002-1275-098X
Maria Vittoria Micioni Di Bonaventura https://orcid.org/0000-0002-8044-1206

REFERENCES

Adams, W. K., Sussman, J. L., Kaur, S., D'souza, A. M., Kieffer, T. J., & Winstanley, C. A. (2015). Long-term, calorie-restricted intake of a high-fat diet in rats reduces impulse control and ventral striatal D2 receptor signalling—Two markers of addiction vulnerability. *The European Journal of Neuroscience*, *42*(12), 3095–3104. https://doi.org/10.1111/ejn.13117

American Psychiatric Association. (2022). *Diagnostic and statistical manual of mental disorders*. American Psychiatric Association Publishing. https://doi.org/10.1176/appi.books.9780890425787

Avena, N. M., Bocarsly, M. E., Rada, P., Kim, A., & Hoebel, B. G. (2008). After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiology & Behavior*, *94*(3), 309–315. https://doi.org/10.1016/j.physbeh.2008.01.008

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience & Biobehavioral Reviews*, *32*(1), 20–39. https://doi.org/10.1016/j.neubiorev.2007.04.019

Baik, J.-H. (2021). Dopaminergic control of the feeding circuit. *Endocrinology and Metabolism (Seoul, Korea)*, *36*(2), 229–239. https://doi.org/10.3803/EnM.2021.979

Baldo, B. A., Sadeghian, K., Basso, A. M., & Kelley, A. E. (2002). Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behavioural Brain Research*, *137*(1–2), 165–177. https://doi.org/10.1016/s0166-4328(02)00293-0

Ballesteros-Yáñez, I., Castillo, C. A., Merighi, S., & Gessi, S. (2017). The role of adenosine receptors in psychostimulant addiction. *Frontiers in Pharmacology*, *8*, 985. https://doi.org/10.3389/fphar.2017.00985

Bello, N. T., & Hajnal, A. (2010). Dopamine and binge eating behaviors. *Pharmacology, Biochemistry, and Behavior*, *97*(1), 25–33. https://doi.org/10.1016/j.pbb.2010.04.016

Blasio, A., Iemolo, A., Sabino, V., Petrosino, S., Steardo, L., Rice, K. C., Orlando, P., Iannotti, F. A., Marzo, V. D., Zorrilla, E. P., & Cottone, P. (2013). Rimonabant precipitates anxiety in rats withdrawn from palatable food: Role of the central amygdala. *Neuropsychopharmacology*, *38*(12), 2498–2507. https://doi.org/10.1038/npp.2013.153

Bohon, C., & Stice, E. (2012). Negative affect and neural response to palatable food intake in bulimia nervosa. *Appetite*, *58*(3), 964–970. https://doi.org/10.1016/j.appet.2012.02.051

Borea, P. A., Gessi, S., Merighi, S., Vincenzi, F., & Varani, K. (2018). Pharmacology of adenosine receptors: The state of the art. *Physiological Reviews*, *98*(3), 1591–1625. https://doi.org/10.1152/physrev.00049.2017

Borgus, J. R., Wang, Y., DiScenza, D. J., & Venton, B. J. (2021). Spontaneous adenosine and dopamine Cotransmission in the caudate-putamen is regulated by adenosine receptors. *ACS Chemical Neuroscience*, *12*(23), 4371–4379. https://doi.org/10.1021/acschemneuro.1c00175

Brewerton, T. D., Dansky, B. S., Kilpatrick, D. G., & O'Neil, P. M. (2000). Which comes first in the pathogenesis of bulimia nervosa: Dieting or bingeing? *International Journal of Eating Disorders*, *28*(3), 259–264.

Campioni, M. R., Xu, M., & McGehee, D. S. (2009). Stress-induced changes in nucleus accumbens glutamate synaptic plasticity. *Journal of Neurophysiology*, *101*(6), 3192–3198. https://doi.org/10.1152/jn.91111.2008

Carr, K. D. (2020). Modulatory effects of food restriction on brain and behavioral effects of abused drugs. *Current Pharmaceutical Design*, *26*(20), 2363–2371. https://doi.org/10.2174/1381612826666200204141057

Chiu, G. S., Darmody, P. T., Walsh, J. P., Moon, M. L., Kwakwa, K. A., Bray, J. K., McCusker, R. H., & Freund, G. G. (2014). Adenosine through the A2A adenosine receptor increases IL-1β in the brain contributing to anxiety. *Brain, Behavior, and Immunity*, *41*, 218–231. https://doi.org/10.1016/j.bbi.2014.05.018

Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: Twenty-something years on. *Nature Protocols*, *1*, 581–585.

Cifani, C., Di Bonaventura, M. V. M., Ciccocioppo, R., & Massi, M. (2013). Binge eating in female rats induced by Yo-Yo dieting and stress. In N. M. Avena (Ed.), *Animal models of eating disorders* (Vol. 74, pp. 27–49). Humana Press. https://doi.org/10.1007/978-1-62703-104-2_3

Cifani, C., Micioni Di Bonaventura, E., Botticelli, L., Del Bello, F., Giorgioni, G., Pavletić, P., Piergentili, A., Quaglia, W., Bonifazi, A., Schepmann, D., Wünsch, B., Vistoli, G., & Micioni Di Bonaventura, M. V. (2020). Novel highly potent and selective Sigma1 receptor antagonists effectively block the binge eating episode in female rats. *ACS Chemical Neuroscience*, *11*(19), 3107–3116. https://doi.org/10.1021/acschemneuro.0c00456

Cifani, C., Micioni Di Bonaventura, M. V., Vitale, G., Ruggieri, V., Ciccocioppo, R., & Massi, M. (2010). Effect of salidroside, active principle of *Rhodiola rosea* extract, on binge eating. *Physiology & Behavior*, *101*(5), 555–562. https://doi.org/10.1016/j.physbeh.2010.09.006

Cifani, C., Polidori, C., Melotto, S., Ciccocioppo, R., & Massi, M. (2009). A preclinical model of binge eating elicited by yo-yo dieting and stressful exposure to food: Effect of sibutramine, fluoxetine, topiramate, and midazolam. *Psychopharmacology*, *204*(1), 113–125. https://doi.org/10.1007/s00213-008-1442-y

Crowther, J. H., Sanftner, J., Bonifazi, D. Z., & Shepherd, K. L. (2001). The role of daily hassles in binge eating. *International Journal of Eating Disorders*, *29*(4), 449–454. https://doi.org/10.1002/eat.1041

Cury, M. E. G., Berberian, A., Scarpato, B. S., Kerr-Gaffney, J., Santos, F. H., & Claudino, A. M. (2020). Scrutinizing domains of executive function in binge eating disorder: A systematic review and meta-analysis. *Frontiers in Psychiatry*, *11*, 288. https://doi.org/10.3389/fpsyt.2020.00288

D'Addario, C., Zaplatic, E., Giunti, E., Pucci, M., Bonaventura, M. V. M. D., Scherma, M., Dainese, E., Maccarrone, M., Nilsson, I. A., Cifani, C., & Fadda, P. (2020). Epigenetic regulation of the cannabinoid receptor CB1 in an activity-based rat model of anorexia nervosa. *International Journal of Eating Disorders*, *53*(5), 702–716. https://doi.org/10.1002/eat.23271

Davis, C., Levitan, R. D., Yilmaz, Z., Kaplan, A. S., Carter, J. C., & Kennedy, J. L. (2012). Binge eating disorder and the dopamine D2 receptor: Genotypes and sub-phenotypes. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *38*(2), 328–335. https://doi.org/10.1016/j.pnpbp.2012.05.002

Dawe, S., & Loxton, N. J. (2004). The role of impulsivity in the development of substance use and eating disorders. *Neuroscience & Biobehavioral Reviews*, *28*(3), 343–351. https://doi.org/10.1016/j.neubiorev.2004.03.007

de Macedo, I. C., de Freitas, J. S., & da Silva Torres, I. L. (2016). The influence of palatable diets in reward system activation: A mini review. *Advances in Pharmacological Sciences*, *2016*, 8677–8679. https://doi.org/10.1155/2016/7238679

Farahimanesh, S., Moradi, M., Nazari-Serenjeh, F., Zarrabian, S., & Haghparast, A. (2018). Role of D1-like and D2-like dopamine receptors within the ventral tegmental area in stress-induced and drug priming-induced reinstatement of morphine seeking in rats. *Behavioural Pharmacology*, *29*(5), 426–436. https://doi.org/10.1097/FBP.0000000000000381

Ferré, S., Bonaventura, J., Tomasi, D., Navarro, G., Moreno, E., Cortés, A., Lluís, C., Casadó, V., & Volkow, N. D. (2016). Allosteric mechanisms within the adenosine A2A-dopamine D2 receptor heterotetramer. *Neuropharmacology*, *104*, 154–160. https://doi.org/10.1016/j.neuropharm.2015.05.028

Ferré, S., Bonaventura, J., Zhu, W., Hatcher-Solis, C., Taura, J., Quiroz, C., Cai, N.-S., Moreno, E., Casadó-Anguera, V., Kravitz, A. V., Thompson, K. R., Tomasi, D. G., Navarro, G., Cordomí, A., Pardo, L., Lluís, C., Dessauer, C. W., Volkow, N. D., Casadó, V., … Zwilling, D. (2018). Essential control of the function of the striatopallidal neuron by pre-coupled complexes of adenosine A2A-dopamine D2 receptor heterotetramers and adenylyl cyclase. *Frontiers in Pharmacology*, *9*, 243. https://doi.org/10.3389/fphar.2018.00243

Ferré, S., Quiroz, C., Woods, A. S., Cunha, R., Popoli, P., Ciruela, F., Lluis, C., Franco, R., Azdad, K., & Schiffmann, S. N. (2008). An update on adenosine A2A-dopamine D2 receptor interactions: Implications for the function of G protein-coupled receptors. *Current Pharmaceutical Design*, *14*(15), 1468–1474. https://doi.org/10.2174/138161208784480108

Font, L., Mingote, S., Farrar, A. M., Pereira, M., Worden, L., Stopper, C., Port, R. G., & Salamone, J. D. (2008). Intra-accumbens injections of the adenosine A2A agonist CGS 21680 affect effort-related choice behavior in rats. *Psychopharmacology*, *199*(4), 515–526. https://doi.org/10.1007/s00213-008-1174-z

Fuxe, K., Marcellino, D., Borroto-Escuela, D. O., Guescini, M., Fernández-Dueñas, V., Tanganelli, S., Rivera, A., Ciruela, F., & Agnati, L. F. (2010). Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders. *CNS Neuroscience & Therapeutics*, *16*(3), e18–e42. https://doi.org/10.1111/j.1755-5949.2009.00126.x

Galarce, E. M., McDannald, M. A., & Holland, P. C. (2010). The basolateral amygdala mediates the effects of cues associated with meal interruption on feeding behavior. *Brain Research*, *1350*, 112–122. https://doi.org/10.1016/j.brainres.2010.02.042

Gallagher, M., & Chiba, A. A. (1996). The amygdala and emotion. *Current Opinion in Neurobiology*, *6*(2), 221–227. https://doi.org/10.1016/S0959-4388(96)80076-6

Garcia-Keller, C., Carter, J. S., Kruyer, A., Kearns, A. M., Hopkins, J. L., Hodebourg, R., Kalivas, P. W., & Reichel, C. M. (2021). Behavioral and accumbens synaptic plasticity induced by cues associated with restraint stress. *Neuropsychopharmacology*, *46*(10), 1848–1856. https://doi.org/10.1038/s41386-021-01074-7

Giel, K. E., Bulik, C. M., Fernandez-Aranda, F., Hay, P., Keski-Rahkonen, A., Schag, K., Schmidt, U., & Zipfel, S. (2022). Binge eating disorder. *Nature Reviews. Disease Primers*, *8*(1), 16. https://doi.org/10.1038/s41572-022-00344-y

Giel, K. E., Teufel, M., Junne, F., Zipfel, S., & Schag, K. (2017). Food-related impulsivity in obesity and binge eating disorder-a systematic update of the evidence. *Nutrients*, *9*(11), 1170. https://doi.org/10.3390/nu9111170

Goldschmidt, A. B., Smith, K. E., Crosby, R. D., Boyd, H. K., Dougherty, E., Engel, S. G., & Haedt-Matt, A. (2018). Ecological momentary assessment of maladaptive eating in children and adolescents with overweight or obesity. *International Journal of Eating Disorders*, *51*(6), 549–557. https://doi.org/10.1002/eat.22864

Grilo, C. M., Masheb, R. M., & Wilson, G. T. (2001). A comparison of different methods for assessing the features of eating disorders in patients with binge eating disorder. *Journal of Consulting and Clinical Psychology*, *69*(2), 317–322. https://doi.org/10.1037/0022-006X.69.2.317

Hagan, M. M., Wauford, P. K., Chandler, P. C., Jarrett, L. A., Rybak, R. J., & Blackburn, K. (2002). A new animal model of binge eating: Key synergistic role of past caloric restriction and stress. *Physiology & Behavior*, *77*(1), 45–54. https://doi.org/10.1016/s0031-9384(02)00809-0

Halpern, C. H., Tekriwal, A., Santollo, J., Keating, J. G., Wolf, J. A., Daniels, D., & Bale, T. L. (2013). Amelioration of binge eating by nucleus accumbens shell deep brain stimulation in mice involves D2 receptor modulation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(17), 7122–7129. https://doi.org/10.1523/JNEUROSCI.3237-12.2013

Heo, Y.-A., & Duggan, S. T. (2017). Lisdexamfetamine: A review in binge eating disorder. *CNS Drugs*, *31*(11), 1015–1022. https://doi.org/10.1007/s40263-017-0477-1

Holland, P. C., & Gallagher, M. (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and pavlovian-instrumental transfer. *European Journal of Neuroscience*, *17*(8), 1680–1694. https://doi.org/10.1046/j.1460-9568.2003.02585.x

Iceta, S., Rodrigue, C., Legendre, M., Daoust, J., Flaudias, V., Michaud, A., & Bégin, C. (2021). Cognitive function in binge eating disorder and food addiction: A systematic review and three-level meta-analysis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *111*, 110400. https://doi.org/10.1016/j.pnpbp.2021.110400

Iemolo, A., Blasio, A., Cyr, S. A. S., Jiang, F., Rice, K. C., Sabino, V., & Cottone, P. (2013). CRF-CRF1 receptor system in the central and basolateral nuclei of the amygdala differentially mediates excessive eating of palatable food. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *38*(12), 2456–2466. https://doi.org/10.1038/npp.2013.147

Johnson, P. M., & Kenny, P. J. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nature Neuroscience*, *13*(5), 635–641. https://doi.org/10.1038/nn.2519

Kang, J. G., Park, J. S., Ko, J.-H., & Kim, Y.-S. (2019). Regulation of gene expression by altered promoter methylation using a CRISPR/Cas9-mediated epigenetic editing system. *Scientific Reports*, *9*(1), 11960. https://doi.org/10.1038/s41598-019-48130-3

Kavanagh, K. A., Schreiner, D. C., Levis, S. C., O'Neill, C. E., & Bachtell, R. K. (2015). Role of adenosine receptor subtypes in methamphetamine reward and reinforcement. *Neuropharmacology*, *89*, 265–273. https://doi.org/10.1016/j.neuropharm.2014.09.030

Kenny, P. J., Voren, G., & Johnson, P. M. (2013). Dopamine D2 receptors and striatopallidal transmission in addiction and obesity. *Current Opinion in Neurobiology*, *23*(4), 535–538. https://doi.org/10.1016/j.conb.2013.04.012

Keski-Rahkonen, A. (2021). Epidemiology of binge eating disorder: Prevalence, course, comorbidity, and risk factors. *Current Opinion in Psychiatry*, *34*(6), 525–531. https://doi.org/10.1097/YCO.0000000000000750

Kessler, R. M., Hutson, P. H., Herman, B. K., & Potenza, M. N. (2016). The neurobiological basis of binge-eating disorder. *Neuroscience & Biobehavioral Reviews*, *63*, 223–238. https://doi.org/10.1016/j.neubiorev.2016.01.013

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., & Correa, R. G. (2019). Dopamine: Functions, signaling, and association with neurological diseases. *Cellular and Molecular Neurobiology*, *39*(1), 31–59. https://doi.org/10.1007/s10571-018-0632-3

Kozuka, C., Kaname, T., Shimizu-Okabe, C., Takayama, C., Tsutsui, M., Matsushita, M., Abe, K., & Masuzaki, H. (2017). Impact of brown rice-specific γ-oryzanol on epigenetic modulation of dopamine D2 receptors in brain striatum in high-fat-diet-induced obesity in mice. *Diabetologia*, *60*(8), 1502–1511. https://doi.org/10.1007/s00125-017-4305-4

Leenaerts, N., Jongen, D., Ceccarini, J., van Oudenhove, L., & Vrieze, E. (2022). The neurobiological reward system and binge eating: A critical systematic review of neuroimaging studies. *The International Journal of Eating Disorders*, *55*(11), 1421–1458. https://doi.org/10.1002/eat.23776

Levine, A. S., & Morley, J. E. (1983). Effect of intraventricular adenosine on food intake in rats. *Pharmacology, Biochemistry, and Behavior*, *19*(1), 23–26. https://doi.org/10.1016/0091-3057(83)90305-2

Lewis, R. G., Florio, E., Punzo, D., & Borrelli, E. (2021). The Brain's reward system in health and disease. *Advances in Experimental Medicine and Biology*, *1344*, 57–69. https://doi.org/10.1007/978-3-030-81147-1_4

Li, L.-C., & Dahiya, R. (2002). MethPrimer: Designing primers for methylation PCRs. *Bioinformatics*, *18*(11), 1427–1431. https://doi.org/10.1093/bioinformatics/18.11.1427

Li, N., & Jasanoff, A. (2020). Local and global consequences of reward-evoked striatal dopamine release. *Nature*, *580*(7802), 239–244. https://doi.org/10.1038/s41586-020-2158-3

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

López-Cruz, L., Carbó-Gas, M., Pardo, M., Bayarri, P., Valverde, O., Ledent, C., Salamone, J. D., & Correa, M. (2017). Adenosine A2A receptor deletion affects social behaviors and anxiety in mice: Involvement of anterior cingulate cortex and amygdala. *Behavioural Brain Research*, *321*, 8–17. https://doi.org/10.1016/j.bbr.2016.12.020

Manfredi, L., Accoto, A., Couyoumdjian, A., & Conversi, D. (2021). A systematic review of genetic polymorphisms associated with binge eating disorder. *Nutrients*, *13*(3), 848. https://doi.org/10.3390/nu13030848

Meye, F. J., & Adan, R. A. H. (2014). Feelings about food: The ventral tegmental area in food reward and emotional eating. *Trends in Pharmacological Sciences*, *35*(1), 31–40. https://doi.org/10.1016/j.tips.2013.11.003

Micioni Di Bonaventura, E., Botticelli, L., Del Bello, F., Giorgioni, G., Piergentili, A., Quaglia, W., Romano, A., Gaetani, S., Micioni Di Bonaventura, M. V., & Cifani, C. (2022). Investigating the role of the central melanocortin system in stress and stress-related disorders. *Pharmacological Research*, *185*, 106521. https://doi.org/10.1016/j.phrs.2022.106521

Micioni Di Bonaventura, E., Botticelli, L., Tomassoni, D., Tayebati, S. K., Micioni Di Bonaventura, M. V., & Cifani, C. (2020). The Melanocortin system behind the dysfunctional eating behaviors. *Nutrients*, *12*(11), 3502. https://doi.org/10.3390/nu12113502

Micioni Di Bonaventura, M. V., Micioni Di Bonaventura, E., Botticelli, L., & Cifani, C. (2021). Impact of a history of caloric restriction and a frustration stress manipulation on binge-like eating behavior in female rats: Preclinical results *Animal Models of Eating Disorders*, Springer (pp. 239–260). https://doi.org/10.1007/978-1-0716-0924-8_13

Micioni Di Bonaventura, M. V., Cifani, C., Lambertucci, C., Volpini, R., Cristalli, G., & Massi, M. (2012). A2A adenosine receptor agonists reduce both high-palatability and low-palatability food intake in female rats. *Behavioural Pharmacology*, *23*(5–6), 567–574. https://doi.org/10.1097/FBP.0b013e3283566a60

Micioni Di Bonaventura, M. V., Lutz, T. A., Romano, A., Pucci, M., Geary, N., Asarian, L., & Cifani, C. (2017). Estrogenic suppression of binge-like eating elicited by cyclic food restriction and frustrative-nonreward stress in female rats. *The International Journal of Eating Disorders*, *50*(6), 624–635. https://doi.org/10.1002/eat.22687

Micioni Di Bonaventura, M. V., Micioni Di Bonaventura, E., Polidori, C., & Cifani, C. (2020). Preclinical models of stress and environmental influences on binge eating. In *Binge eating* (pp. 85–101). Springer International Publishing. https://doi.org/10.1007/978-3-030-43562-2_7

Micioni Di Bonaventura, M. V., Pucci, M., Giusepponi, M. E., Romano, A., Lambertucci, C., Volpini, R., Micioni Di Bonaventura, E., Gaetani, S., Maccarrone, M., D'Addario, C., & Cifani, C. (2019). Regulation of adenosine A2A receptor gene expression in a model of binge eating in the amygdaloid complex of female rats. *Journal of Psychopharmacology (Oxford, England)*, *33*(12), 1550–1561. https://doi.org/10.1177/0269881119845798

Micioni Di Bonaventura, M. V., Ubaldi, M., Giusepponi, M. E., Rice, K. C., Massi, M., Ciccocioppo, R., & Cifani, C. (2017). Hypothalamic CRF1 receptor mechanisms are not sufficient to account for binge-like palatable food consumption in female rats. *The International Journal of Eating Disorders*, *50*(10), 1194–1204. https://doi.org/10.1002/eat.22767

Micioni Di Bonaventura, M. V., Vitale, G., Massi, M., & Cifani, C. (2012). Effect of Hypericum perforatum extract in an experimental model of binge eating in female rats. *Journal of Obesity*, *2012*, 956137. https://doi.org/10.1155/2012/956137

Mingote, S., Pereira, M., Farrar, A. M., McLaughlin, P. J., & Salamone, J. D. (2008). Systemic administration of the adenosine a(2A) agonist CGS

21680 induces sedation at doses that suppress lever pressing and food intake. *Pharmacology, Biochemistry, and Behavior*, *89*(3), 345–351. https://doi.org/10.1016/j.pbb.2008.01.006

Nunes, E. J., Randall, P. A., Podurgiel, S., Correa, M., & Salamone, J. D. (2013). Nucleus accumbens neurotransmission and effort-related choice behavior in food motivation: Effects of drugs acting on dopamine, adenosine, and muscarinic acetylcholine receptors. *Neuroscience & Biobehavioral Reviews*, *37*(9), 2015–2025. https://doi.org/10.1016/j.neubiorev.2013.04.002

Ong, Z. Y., & Muhlhausler, B. S. (2011). Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. *The FASEB Journal*, *25*(7), 2167–2179. https://doi.org/10.1096/fj.10-178392

Pankevich, D. E., Teegarden, S. L., Hedin, A. D., Jensen, C. L., & Bale, T. L. (2010). Caloric restriction experience reprograms stress and orexigenic pathways and promotes binge eating. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*(48), 16399–16407. https://doi.org/10.1523/JNEUROSCI.1955-10.2010

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., & Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: Feedforward and feedback effects of chronic stress. *Endocrinology*, *145*(8), 3754–3762. https://doi.org/10.1210/en.2004-0305

Piccoli, L., Bonaventura, M. V. M. D., Cifani, C., Costantini, V. J. A., Massagrande, M., Montanari, D., Martinelli, P., Antolini, M., Ciccocioppo, R., Massi, M., Merlo-Pich, E., Fabio, R. D., & Corsi, M. (2012). Role of orexin-1 receptor mechanisms on compulsive food consumption in a model of binge eating in female rats. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *37*(9), 1999–2011. https://doi.org/10.1038/npp.2012.48

Polivy, J., Zeitlin, S. B., Herman, C. P., & Beal, A. L. (1994). Food restriction and binge eating: A study of former prisoners of war. *Journal of Abnormal Psychology*, *103*(2), 409–411. https://doi.org/10.1037//0021-843x.103.2.409

Polter, A. M., & Kauer, J. A. (2014). Stress and VTA synapses: Implications for addiction and depression. *European Journal of Neuroscience*, *39*(7), 1179–1188. https://doi.org/10.1111/ejn.12490

Price, J. L., & Drevets, W. C. (2010). Neurocircuitry of mood disorders. *Neuropsychopharmacology*, *35*(1), 192–216. https://doi.org/10.1038/npp.2009.104

Pringle, A., Ashworth, F., Harmer, C. J., Norbury, R., & Cooper, M. J. (2011). Neural correlates of the processing of self-referent emotional information in bulimia nervosa. *Neuropsychologia*, *49*(12), 3272–3278. https://doi.org/10.1016/j.neuropsychologia.2011.07.032

Pritchett, C. E., Pardee, A. L., McGuirk, S. R., & Will, M. J. (2010). The role of nucleus accumbens adenosine-opioid interaction in mediating palatable food intake. *Brain Research*, *1306*, 85–92. https://doi.org/10.1016/j.brainres.2009.09.115

Pucci, M., Bonaventura, M. V. M. D., Zaplatic, E., Bellia, F., Maccarrone, M., Cifani, C., & D'Addario, C. (2019). Transcriptional regulation of the endocannabinoid system in a rat model of binge-eating behavior reveals a selective modulation of the hypothalamic fatty acid amide hydrolase gene. *International Journal of Eating Disorders*, *52*(1), 51–60. https://doi.org/10.1002/eat.22989

Pucci, M., D'Addario, C., Micioni Di Bonaventura, E., Mercante, F., Annunzi, E., Fanti, F., Sergi, M., Botticelli, L., Einaudi, G., Cifani, C., & Bonaventura, M. V. M. D. (2022). Endocannabinoid system regulation in female rats with recurrent episodes of binge eating. *International Journal of Molecular Sciences*, *23*(23), 15228. https://doi.org/10.3390/ijms232315228

Pucci, M., Micioni Di Bonaventura, M. V., Giusepponi, M. E., Romano, A., Filaferro, M., Maccarrone, M., Ciccocioppo, R., Cifani, C., & D'Addario, C. (2016). Epigenetic regulation of nociceptin/orphanin FQ and corticotropin-releasing factor system genes in frustration stress-induced binge-like palatable food consumption. *Addiction Biology*, *21*(6), 1168–1185. https://doi.org/10.1111/adb.12303

Randall, P. A., Pardo, M., Nunes, E. J., López Cruz, L., Vemuri, V. K., Makriyannis, A., Baqi, Y., Müller, C. E., Correa, M., & Salamone, J. D. (2012). Dopaminergic modulation of effort-related choice behavior as assessed by a progressive ratio chow feeding choice task: Pharmacological studies and the role of individual differences. *PLoS One*, *7*(10), e47934. https://doi.org/10.1371/journal.pone.0047934

Romano, A., Micioni Di Bonaventura, M. V., Gallelli, C. A., Koczwara, J. B., Smeets, D., Giusepponi, M. E., de Ceglia, M., Friuli, M., Micioni Di Bonaventura, E., Scuderi, C., Vitalone, A., Tramutola, A., Altieri, F., Lutz, T. A., Giudetti, A. M., Cassano, T., Cifani, C., & Gaetani, S. (2020). Oleoylethanolamide decreases frustration stress-induced binge-like eating in female rats: A novel potential treatment for binge eating disorder. *Neuropsychopharmacology*, *45*(11), 1931–1941. https://doi.org/10.1038/s41386-020-0686-z

Rossetti, M. F., Schumacher, R., Gastiazoro, M. P., Lazzarino, G. P., Andreoli, M. F., Stoker, C., Varayoud, J., & Ramos, J. G. (2020). Epigenetic dysregulation of dopaminergic system by maternal cafeteria diet during early postnatal development. *Neuroscience*, *424*, 12–23. https://doi.org/10.1016/j.neuroscience.2019.09.016

Salamone, J. D., & Correa, M. (2009). Dopamine/adenosine interactions involved in effort-related aspects of food motivation. *Appetite*, *53*(3), 422–425. https://doi.org/10.1016/j.appet.2009.07.018

Salamone, J. D., Correa, M., Ferrigno, S., Yang, J.-H., Rotolo, R. A., & Presby, R. E. (2018). The psychopharmacology of effort-related decision making: Dopamine, adenosine, and insights into the neurochemistry of motivation. *Pharmacological Reviews*, *70*(4), 747–762. https://doi.org/10.1124/pr.117.015107

Schneider, E., Higgs, S., & Dourish, C. T. (2021). Lisdexamfetamine and binge-eating disorder: A systematic review and meta-analysis of the preclinical and clinical data with a focus on mechanism of drug action in treating the disorder. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *53*, 49–78. https://doi.org/10.1016/j.euroneuro.2021.08.001

Schroeder, M., Jakovcevski, M., Polacheck, T., Lebow, M., Drori, Y., Engel, M., Ben-Dor, S., & Chen, A. (2017). A methyl-balanced diet prevents CRF-induced prenatal stress-triggered predisposition to binge eating-like phenotype. *Cell Metabolism*, *25*(6), 1269–1281.e6. https://doi.org/10.1016/j.cmet.2017.05.001

Schultz, W. (2015). Neuronal reward and decision signals: From theories to data. *Physiological Reviews*, *95*(3), 853–951. https://doi.org/10.1152/physrev.00023.2014

Simões, A. P., Gonçalves, F. Q., Rial, D., Ferreira, S. G., Lopes, J. P., Canas, P. M., & Cunha, R. A. (2022). CD73-mediated formation of extracellular adenosine is responsible for adenosine A2A receptor-mediated control of fear memory and amygdala plasticity. *International Journal of Molecular Sciences*, *23*(21), 12826. https://doi.org/10.3390/ijms232112826

Simões, A. P., Machado, N. J., Gonçalves, N., Kaster, M. P., Simões, A. T., Nunes, A., Pereira de Almeida, L., Goosens, K. A., Rial, D., & Cunha, R. A. (2016). Adenosine A2A receptors in the amygdala control synaptic plasticity and contextual fear memory. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *41*(12), 2862–2871. https://doi.org/10.1038/npp.2016.98

Simões, A. P., Portes, M. A. M., Lopes, C. R., Vanz, F., Lourenço, V. S., Pliássova, A., Gaspar, I. L., Silva, H. B., Tomé, Â. R., Canas, P. M., Prediger, R. D., & Cunha, R. A. (2023). Adenosine A2A receptors control generalization of contextual fear in rats. *Translational Psychiatry*, *13*(1), 316. https://doi.org/10.1038/s41398-023-02613-0

Stice, E., Agras, W. S., Telch, C. F., Halmi, K. A., Mitchell, J. E., & Wilson, T. (2001). Subtyping binge eating-disordered women along dieting and negative affect dimensions. *International Journal of Eating Disorders*, *30*(1), 11–27. https://doi.org/10.1002/eat.1050

Teegarden, S. L., & Bale, T. L. (2008). Effects of stress on dietary preference and intake are dependent on access and stress sensitivity. *Physiology & Behavior*, *93*(4–5), 713–723. https://doi.org/10.1016/j.physbeh.2007.11.030

Valle-León, M., Callado, L. F., Aso, E., Cajiao-Manrique, M. M., Sahlholm, K., López-Cano, M., Soler, C., Altafaj, X., Watanabe, M., Ferré, S., Fernández-Dueñas, V., Menchón, J. M., & Ciruela, F. (2021). Decreased striatal adenosine A2A-dopamine D2 receptor heteromerization in schizophrenia. *Neuropsychopharmacology*, *46*(3), 665–672. https://doi.org/10.1038/s41386-020-00872-9

Vojta, A., Dobrinić, P., Tadić, V., Bočkor, L., Korać, P., Julg, B., Klasić, M., & Zoldoš, V. (2016). Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Research*, *44*(12), 5615–5628. https://doi.org/10.1093/nar/gkw159

Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., & Telang, F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology*, *56*, 3–8. https://doi.org/10.1016/j.neuropharm.2008.05.022

Vucetic, Z., Carlin, J. L., Totoki, K., & Reyes, T. M. (2012). Epigenetic dysregulation of the dopamine system in diet-induced obesity. *Journal of Neurochemistry*, *120*(6), 891–898. https://doi.org/10.1111/j.1471-4159.2012.07649.x

Wang, G.-J., Geliebter, A., Volkow, N. D., Telang, F. W., Logan, J., Jayne, M. C., Galanti, K., Selig, P. A., Han, H., Zhu, W., Wong, C. T., & Fowler, J. S. (2011). Enhanced striatal dopamine release during food stimulation in binge eating disorder. *Obesity*, *19*(8), 1601–1608. https://doi.org/10.1038/oby.2011.27

Watabe-Uchida, M., Zhu, L., Ogawa, S. K., Vamanrao, A., & Uchida, N. (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron*, *74*(5), 858–873. https://doi.org/10.1016/j.neuron.2012.03.017

Witt, A. A., & Lowe, M. R. (2014). Hedonic hunger and binge eating among women with eating disorders. *International Journal of Eating Disorders*, *47*(3), 273–280. https://doi.org/10.1002/eat.22171

Wydra, K., Suder, A., Borroto-Escuela, D. O., Filip, M., & Fuxe, K. (2015). On the role of A2A and D2 receptors in control of cocaine and food-seeking behaviors in rats. *Psychopharmacology*, *232*(10), 1767–1778. https://doi.org/10.1007/s00213-014-3818-5

Yu, Y., Miller, R., & Groth, S. W. (2022). A literature review of dopamine in binge eating. *Journal of Eating Disorders*, *10*(1), 11. https://doi.org/10.1186/s40337-022-00531-y

Zhang, N. (2015). Epigenetic modulation of DNA methylation by nutrition and its mechanisms in animals. *Animal Nutrition*, *1*(3), 144–151. https://doi.org/10.1016/j.aninu.2015.09.002

Zhang, P., Liu, Y., Lv, H., Li, M.-Y., Yu, F.-X., Wang, Z., Ding, H.-Y., Wang, L.-X., Zhao, K.-X., Zhang, Z.-Y., Zhao, P.-F., Li, J., Yang, Z.-H., Zhang, Z.-T., & Wang, Z.-C. (2019). Integration of neural reward processing and appetite-related signaling in obese females: Evidence from resting-state fMRI. *Journal of Magnetic Resonance Imaging*, *50*(2), 541–551. https://doi.org/10.1002/jmri.26576

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Mercante, F., Micioni Di Bonaventura, E., Pucci, M., Botticelli, L., Cifani, C., D'Addario, C., & Micioni Di Bonaventura, M. V. (2024). Repeated binge-like eating episodes in female rats alter adenosine A_{2A} and dopamine *D2* receptor genes regulation in the brain reward system. *International Journal of Eating Disorders*, *57*(7), 1433–1446. https://doi.org/10.1002/eat.24216