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Central and peripheral monoamine changes in a rat model of Gaming Disorder

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ABSTRACT

Gaming Disorder (GD) is a mental disorder primarily affecting adolescents. Prolonged video game use can lead to behavioral alterations, such as loss of control, increased compulsivity, and symptoms of depression and anxiety. Studies in humans have shown changes in brain areas involved in emotional regulation and reward circuits, including the lateral hypothalamus (LH), ventral tegmental area (VTA), and dorsal (DRN) and median (MnR) raphe nuclei. These alterations appear to be linked to disrupted activity of orexin- (ORX), dopamine- (DA), and serotonin- (5-HT) positive (+) neurons.

Our laboratory developed a rat model that mimics some symptoms of GD. Using immunohistochemistry and high-performance liquid chromatography, we analyzed ORX, DA, and 5-HT levels in rats exposed to the GD protocol. Compared to control groups, GD rats of both sexes exhibited a decreased density and number of ORX+, DA+, and 5-HT+ cells in the analyzed nuclei. Additionally, the reduction in DA+ and 5-HT+ neurons was associated with peripheral changes in their plasma levels and alterations in the tryptophan signaling pathway, accompanied by an increase in its inflammatory metabolites.

These three systems are crucial for proper brain function, decision-making regulation, and the processing of emotional and reward-related stimuli. Our findings support the hypothesis that neurotransmitter dysfunction plays a key role in GD and provide a foundation for further research on the cerebral and behavioral changes observed in GD patients.

1. Introduction

Adolescents are increasingly immersed in surfing the Internet or using new equipment, such as smartphones and computers. Faster communication, faster access to information, or the ability to find new sources of entertainment and amusement are among the motivations that drive individuals to use these new technologies (Bediou et al., 2018). In particular, video games are certainly a very popular form of entertainment among young people (Cudo et al., 2022).

However, the excessive use of video games becomes a risk factor for young people, as it interferes with the individual's own normal psychological and social functioning. The possibility of developing a

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dysfunctional emotional attachment to this activity is high, and it is related both to the psychological condition of the players and to the excessive and prolonged use of the device (Ostinelli et al., 2021). Nonetheless, only in 2013 (Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed, 2013), the World Health Organization (WHO) recognized "Gaming Disorder" (GD) and then included it in the 11th final revision of the International Classification of Diseases (ICD-11), finally defining it as a mental disorder in 2018 ("International classification of diseases for mortality and morbidity statistics (11th Revision)", 2018).

In the ICD-11, individuals with GD are defined as gamers who, in addition to persistent and recurrent use of video games for at least 12 months, present other symptoms, such as loss of control over the game, little interest in other activities, increased anxiety, accompanied by the development of depressive symptoms (Blashfield and Fuller, 2016; Sarmiento and Lau, 2020).

Previous studies show that GD occurs at a higher probability during adolescence (Nobre et al., 2020; Paulus et al., 2018). An imbalance in impulse control ability and an alteration in reward circuits are among the various neurobiological hypotheses implicated in this disorder (Li et al., 2020). Multiple studies conducted in adolescents diagnosed with GD have demonstrated a decrease in cognitive control functions and an increase in behaviors analogous to addiction-seeking (Li et al., 2020). These two symptoms are associated with changes in the activity of the serotonergic and dopaminergic systems, two of the most important systems regulating emotion and cognitive functions (Cools et al., 2019).

At the cortical level, serotonin (5-HT) modulates the function of various brain regions through its receptors, exerting inhibitory effects via 5-HT1A receptors and excitatory effects via 5-HT2A receptors. The regions affected include: the anterior cingulate cortex (ACC), which is involved in the regulation of reward-seeking behavior; the orbitofrontal cortex (OFC), which integrates sensory and emotional information; and the prelimbic cortex (PRL), which is responsible for the regulation of decision-making processes (Kranz et al., 2010).

Dopamine (DA) regulates the activity of the nucleus accumbens (NAc) and striatum, which are involved in the control of decision-making and motivational behaviors, via its D1, D2, and D3 receptors. These nuclei project to the ACC and PRL, regulating their functions. Additionally, DA is linked to the functioning of other limbic regions that regulate the processing of emotional information, such as the amygdala and ventral hippocampus (Arias-Carrión et al., 2010; Assadi et al., 2009).

GD patients show alterations in these brain areas and, particularly in the expression of DA and 5-HT receptors and transporters (Hong et al., 2018; Tian et al., 2014). Disruption of these two systems in GD patients is also associated with the onset of other mental disorders that have been described in co-morbidity with GD (Jorgenson et al., 2016; Schou Andreassen et al., 2016), including anxiety, depression, and bipolar disorder (Furuyashiki and Kitaoka, 2019; Hong et al., 2018; Tian et al., 2014). Indirect evidence of the involvement of the DA and 5-HT systems in GD relies on the fact that drugs that increase cerebral levels of 5-HT and DA, such as fluoxetine or bupropion, respectively, reduce the symptoms of GD, including impulsivity, addiction toward gambling, and depression (de Sá et al., 2023).

Another important neurotransmitter that seems to be dysfunctional in GD patients is orexin (ORX). Individuals with GD show an increase in the blood levels of ORX correlated with enhanced symptoms typical of this mental disorder (Choi et al., 2020; Zhou et al., 2021). ORX modulates several physiological functions, such as motivated behavior, feeding, mood, and stress (James et al., 2017; Sargin, 2019). Dysfunction in the ORX system has been linked to increased addictive behavior (James and Aston-Jones, 2022; James et al., 2017) and mood dysregulation (Khairuddin et al., 2020), and predisposes more in general to the development of mental disorders (Akça Ö et al., 2020; Katzman and Katzman, 2022). ORX is a potent regulator of DA function (Calipari and España, 2012). Increased ORX activity leads to increased DA activity by influencing the perception of pleasure and reinforcement of addiction-related experiences (Cason et al., 2010). Like DA (Esposito et al., 2008), ORX is also regulated by and, in turn, regulates 5-HT activity (Muraki et al., 2004; Wang et al., 2005). These two neurotransmitters are interconnected and form a dynamic balance in regulating the functions of sleep-wake (Saito et al., 2018; Sakurai et al., 2010), stress reduction (James et al., 2017), and regulation of emotional states (Mavanji et al., 2022; Tsujino and Sakurai, 2013).

Finally, GD and mental disorders that often occur in comorbidity (Dullur et al., 2021; Furuyashiki and Kitaoka, 2019; Hong et al., 2018; Koncz et al., 2023; Tian et al., 2014) are also related to the activation of the immune system and cytokine production under stress conditions (Benedetti et al., 2017; Comai et al., 2020). Among the pathways activated by proinflammatory cytokines and stress there is the kynurenine (KYN) pathway of tryptophan (Trp) (Comai et al., 2022). Increased conversion of Trp into KYN not only reduces 5-HT availability in the brain, but also increases the formation of quinolinic acid (QA), a neurotoxic molecule N-methyl-D-aspartate (NMDA) agonist, affecting the glutamatergic system (Comai et al., 2022; Schwarcz et al., 2012). The excitotoxicity of QA also has a direct effect on 5-HT, DA, and ORX neurons by reducing their numbers and activity (Cummings and Walker, 1996; Katsuki and Akaike, 2004; Stepanova et al., 2020), increasing depressive symptoms, and predisposing individuals with increased susceptibility to stress to more likely develop psychiatric disorders (Firk and Markus, 2007; Grafe and Bhatnagar, 2018; Moghaddam, 2002).

The possibility of investigating changes in the DA, 5-HT, and ORX systems in humans is limited to the analysis of peripheral biomarkers, whose relevance for what is happening in the brain is still debated (Marraudino et al., 2022), or to analysis of the brain activity using functional magnetic resonance imaging. However, these techniques have some limitations in that they do not provide data on the functioning of specific neurons.

Recently, in our laboratory, we have developed an animal model recapitulating the main features present in GD patients, including changes in brain activity in areas related to cognitive control functions, an increase in compulsive and seeking behaviors, and alterations of social skills. In addition, the model exhibits persistent loss of control and hyperactivity during play, which becomes evident after 5 weeks of training and are intensified when the expected reward is withheld. These patterns are consistently observed in the play context and are absent in control rats (Casile et al., 2024). Taking advantage of this new animal model, here, we investigated the potential correlation between the development of GD symptoms and the alteration of the DA, 5-HT, and ORX systems at central and peripheral levels. In particular, by immunohistochemical techniques, we quantified DA, 5-HT, and ORX immunoreactivity within specific brain nuclei. The analysis of these neurotransmitters in the brain was complemented by the quantification of DA and Trp metabolites at the peripheral level, in plasma samples, through both 5-HT and KYN pathways.

2. Materials and methods

2.1. Specific aim and experimental design

In this study, we aimed to investigate the impact of GD on the monoamine system at both peripheral and central levels. Specifically, we focused on DA, 5-HT, and their key regulator, ORX.

First, we established the GD model in male and female rats. Then, we collected tissues (*i.e.*, plasma and brains) to proceed with the following analyses:

1) Immunohistochemical evaluation of DA-, 5-HT-, and ORX-immunoreactivity within specific brain nuclei.
2) Evaluation of plasma levels of Trp, Trp-metabolites along the 5-HT and KYN pathways, and DA through liquid chromatography-mass spectrometry (LC-MS/MS).

Detailed procedures are described in the following paragraphs.

2.2. Animals

Animal samples analyzed in this study were originally collected for a previously published study (Casile et al., 2024).

Wistar Kyoto rats from our colony at the Neuroscience Institute Cavalieri Ottolenghi (originally purchased from Charles River - Charles River Laboratories Italia S.r.l., Milan, Italy) were housed in standard conditions at 22 ± 2 °C, under a 12:12 h light-dark cycle (lights on at 08:00 a.m.). Food (standard chow diet, VRF1, SDS Charles River Laboratories) and water were provided *ad libitum* throughout the study.

Specifically, at weaning (*i.e.,* postnatal day 28, PND28), 64 rats (30 males and 34 females) were housed in separate cages containing four same-sex rats.

The rats were randomly subdivided into four experimental groups:

- Control males (CON M, n = 12);
- Control females (CON F, n = 13);
- Males subjected to the GD protocol (GD M, n = 18);
- Females subjected to the GD protocol (GD F, n = 21).

Animal care and handling were according to the European Union Council Directive of 22nd September 2010 (2010/63/UE); all the procedures reported in the present study were approved by the Italian Ministry of Health (authorization n. 1035/2020-PR). The experimental design conforms to the "ARRIVE guidelines originally published by Kilkenny et al. (2010)".

For behavioral experiments (Casile et al., 2024), sample size was estimated using G*Power to detect medium-to-large effect sizes (Cohen's d = 0.8) with a statistical power of 0.80 and α level of 0.05. This analysis indicated a minimum of 12 animals per group. Slightly larger group sizes were used in the GD groups to account for individual variability and to ensure reliable detection of behavioral changes.

For immunohistochemical (Aspesi et al., 2021; Bonaldo et al., 2024) and HPLC (Bilel et al., 2025; Inserra et al., 2023; Tassan Mazzocco et al., 2021) analyses, sample sizes were determined based on our previous works, in which medium-to-large effect sizes in brain monoamine levels were consistently detected following pharmacological treatments or in genetic knockout models.

2.2.1. GD protocol

Rats in the GD group were subjected to the GD protocol as described in Casile et al. (2024).

Briefly, after weaning, all rats were habituated to the operator handling and to the experimental apparatus (from PND44 to PND55). From PND55 to PND87 only the GD group of both sexes were subjected to the GD protocol for 5 weeks.

This protocol involved training the rats to touch a visual stimulus (white circle) on a touchscreen to receive a reward. At the end of the 5 weeks of training, during which rats interacted with the screen for 5 min per day, for 5 days a week, they were subjected to a series of behavioral tests.

These tests aimed to assess whether the rats had developed compulsive and hyperactive behavior toward the game, and whether such behavior persisted in the presence of other stimuli (a new object, an unfamiliar conspecific of the opposite sex, or of the same sex), or in the absence of reward following a period of play interruption. All tests were used to positively assess the development of GD-like behavior (data not shown, for details, see Casile et al. (2024).

At the end of the behavioral tests, rats were sacrificed after a final 10-min session, and brain and blood samples were collected as described below.

2.3. Fixation and tissue sampling for immunohistochemical analysis

Part of the animals (CON M = 6; CON F = 6; GD M = 11; GD F = 10) were sacrificed by deep irreversible anesthesia (intraperitoneal injection of Zoletil 80 mg/kg and Rompum 10 mg/kg) and transcardially perfused with a 4% paraformaldehyde (PFA) solution. Females were sacrificed in the estrus phase previous assessment by vaginal smear (Bonaldo et al., 2023; Bonaldo et al., 2023).

Brains were removed and stored in 4% PFA solution for 24 h at +4°C, followed by several washes in 0.01 M phosphate-buffered saline (PBS). Finally, they were stored in 30% sucrose solution in PBS at +4 °C, frozen in pre-cooled isopentane on dry ice at −35 °C, and stored in a deep freezer at −80 °C until sectioning.

Brains (n = 6–11/group) were serially cut in the coronal plane at 30 μm thickness using a cryostat in three series. The sectioning plane was oriented to match the corresponding patterns to the coronal sections of the rat brain atlas (Paxinos and Watson, 2004). Sections were collected in a cryoprotective solution and stored at −20 °C. Different areas from one series were selected and processed, using the free-floating technique, for ORX, DA, and 5-HT immunoreactivity, respectively.

2.4. Immunohistochemistry

The presence (*i.e.*, immunoreactivity, ir) of ORX, DA (evaluated as Aromatic L-Amino Acid Decarboxylase, AADC), and 5-HT was detected by immunohistochemistry performed on free-floating sections.

First, all sections were washed overnight in 0.01 M PBS pH 7.3–7.4. Before starting, the sections selected for the 5-HT analysis were first incubated in citrate buffer (10 mM citric acid, 0.05% Tween, pH 6.0) that was previously heated to 95 °C for antigen retrieval. All sections were washed in PBS pH 7.3–7.4 containing 0.5% Triton X-100 for 30 min and then treated with a solution of PBS containing methanol/hydrogen peroxide for 20 min to inhibit endogenous peroxidase activity. Nonspecific binding was blocked by incubating the sections for 30 min at room temperature in a blocking solution prepared diluting 1.5% normal goat serum (NGS, Vector Laboratories, Burlingame, CA, USA) in PBS pH 7.3–7.4, for the ORX-ir and the DA-ir sections, and diluting 1.5 % normal horse serum (NHS, Vector Laboratories, Burlingame, CA, USA) and 0.2% bovine serum albumin (BSA, Sigma-Aldrich, Milan, Italy) in PBS pH 7.3–7.4 and 0.5% Triton X-100, for the 5-HT ones. The sections were then incubated two overnights at +4 °C with primary antibodies diluted in PBS pH 7.3–7.4 containing 0.2% Triton X-100 (*i.e.*, anti-AADC antibody, Rabbit, 1:30000, Cat. No. TE102, Eugene Tech International, New Jersey, USA; anti-ORX-A, Rabbit, 1:1000, Cat. No. D55078, Merck-Millipore, Milan, Italy) or in blocking solution (*i.e.*, anti-5-HT antibody, Goat, 1:5000, Immunostar, #20079). Next, sections were incubated in biotinylated secondary antibody (*i.e.*, goat anti-rabbit or horse anti-goat, Vector Laboratories, Burlingame, CA, USA) diluted 1:200 in PBS pH 7.3–7.4 containing 0.2% Triton X-100, for 60 min at room temperature. The antigen-antibody reaction was revealed after a 60 min incubation with avidin–peroxidase complex (Vectastain ABC Kit Elite, Vector Laboratories, Burlingame, CA, USA). The peroxidase activity was visualized with a solution containing 0.400 mg/ml 3,3-diamino-benzidine (Sigma-Aldrich, Milan, Italy) and 0.004% hydrogen peroxide in 0.05 M Tris–HCl buffer at pH 7.6. Sections were mounted on chromallum-coated slides, air-dried, cleared in xylene, and cover-slipped with New-Entellan (Merck, Milano, Italy).

These antibodies were successfully used in previous studies (Aspesi et al., 2021; Bonaldo et al., 2024). As a further control, we omitted the primary antisera or the biotinylated secondary ones and replaced them with PBS. In both cases, positive cell bodies and fibers were totally absent.

2.5. Plasma levels of tryptophan, tryptophan-metabolites, and dopamine

Blood was taken directly from the heart before the sacrifice

procedures began and collected in EDTA-treated vials. After collecting, the blood was centrifuged for 10 min at 3000g to separate the plasma.

A total of 42 rats (CON M = 8; CON F = 9; GD M = 13; GD F = 12) were sacrificed to quantify plasma levels of DA and the following Trp metabolites along the 5-HT and Kyn pathways: Trp, 5-HT, melatonin (MLT), KYN, 3-hydroxykynurenine (3-HK), kynurenic acid (KYNA), and quinolinic acid (QA) In brief, the biomarkers were quantified by LC-MS/MS using the method described in Sapienza et al. (2024) and Tassan Mazzocco et al. (2023) on a Varian system consisting of a binary Prostar pump, a 410 autosampler, and an MS320 triple quadrupole mass spectrometer equipped with an Electro Spray ion source, and using alpha-methyl tryptophan as an internal standard. The instrument operated in multiple reaction monitoring mode, working in positive ion mode, except for quinolinic acid, which was analyzed in negative mode. LC analysis was performed using an Agilent Eclipse XDB-C8 column (3 × 150 mm, 3.5 μm) and gradient elution with (A) water 1 % formic acid and (B) acetonitrile (0 min: 95% A; 5 min: 30% A; 8.3 min: 10% A; 10 min: 10% A; 11 min: 95% A; 15 min: 95% A) at a flow rate of 400 μL/min.

The following ratios were also calculated as a proxy of the activity of some of the enzymes involved in the metabolic steps of the KYN pathway: KYN/Trp ratio as an index of the activity of the indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) activities; 3-HK/KYN as an index of kynurenine 3-monooxygenase (KMO) activity; KYNA/KYN as an index of kynurenine aminotransferases (KAT) activity. Finally, the KYNA/QA ratio was calculated as an index of neuroprotection, and the KYNA/3-HK ratio was used as an index of the balance between neuroprotective and neurotoxic branches of the KYN pathway (Comai et al., 2020).

2.6. Quantitative analysis of DA-, 5-HT-, and ORX-ir

For quantitative analysis, standardized sections of the brain areas, summarized in Supplementary Tables 1, 2, and 3 and selected according to the rat brain atlas (Paxinos and Watson, 2004), were acquired with the Axioscan Z1 scanner (ZEISS, Oberkochen, DE) at both low and high magnification (5x and 20x, respectively). The digital images were processed and analyzed with ImageJ (version 2.10/1.53c; Wayne Rasband, NIH, Bethesda, MD, USA). Measurements were performed within predetermined fields (region of interest, ROI), boxes of fixed shape and size inserted within each considered labeled brain area (see Supplementary Tables 1, 2, and 3 for details). Specifically, we counted the number of positive cells, and we evaluated the immunoreactivity of cell bodies, dendrites, and fibers in all the selected nuclei as area fraction (AF) covered by immunopositive material (Aspesi et al., 2021; Bonaldo et al., 2021, 2024, 2025). When two comparable levels were analyzed, their mean was used.

2.7. Statistical analysis

Quantitative data were analyzed with SPSS 27 statistical software (SPSS Inc, Chicago, IL, USA) by two-way analysis of variance (ANOVA), considering sex and treatment as independent variables. If ANOVA indicated statistical significance, the *post hoc* analysis was performed using Tukey's HSD (honestly significant difference) test. Data is presented as mean ± SEM, and differences between groups are considered significant for p values < 0.05.

3. Results

The complete analysis of the ir for ORX, AADC, and 5-HT is summarized in Supplementary Tables 4, 5, and 6. Here we focus on some particularly relevant results.

3.1. Reduction of the orexin immunoreactivity in GD rats

ORX neurons are mainly located in the lateral hypothalamus (LH), but their fibers extend into different brain regions (Marcus and Elmquist, 2005). The GD protocol caused an overall reduction in ORX-ir in all nuclei analyzed (Supplementary Table 1) compared to CON rats, as shown by the coronal brain sections depicted in Fig. 1A.

First of all, quantitative analysis performed in LH (Bregma −2.56 mm) showed a sex difference in ORX-ir. CON M showed more ORX+ cells than CON F ($p = 0.004$). In contrast, the GD protocol abolished this sex difference in these rats ($p = 0.999$). Furthermore, the GD M showed a reduction compared with the same-sex controls (males, $p < 0.0001$), but no differences were found between CON and GD females ($p = 0.628$) (Fig. 1B).

The analysis of the AF in the LH revealed further differences. In fact, both CON and GD groups showed a sex difference, with a higher AF in males than females (CON, $p < 0.0001$; GD, $p = 0.003$). Furthermore, in line with the number of ORX+ cells, it was possible to observe a reduction in AF in both GD groups compared with the same-sex control group (males, $p < 0.0001$; females, $p = 0.040$) (Fig. 1C).

3.2. Reduction of dopamine immunoreactivity in GD rats

Most dopaminergic neurons reside in the VTA and SN, sending their projections to different brain regions (Bayer et al., 1995). After the development of the GD model, rats of both sexes showed an alteration of the dopaminergic system with a consequent reduction in DA-ir in different nuclei (Supplementary Table 5).

First, as shown by images of coronal sections of rat brain (Fig. 2A), the AADC-ir within the VTA was sexually dimorphic (Bregma −5.80 mm). Indeed, CON F showed fewer AADC+ cells than males ($p = 0.022$), and this difference was also maintained in the GD group ($p = 0.0002$). The GD protocol resulted in a reduction in the AADC+ cells in both sexes, compared with the controls (males, $p = 0.002$; females, $p < 0.0001$) (Fig. 2B). The analysis of the AF, on the other hand, revealed no sex differences in the control groups ($p = 0.859$). In contrast, GD females showed a lower AF compared with GD males ($p = 0.020$) due to the overall effects of the GD protocol that led to a significant reduction of AF in both sexes compared to controls (males, $p = 0.016$; females, $p < 0.0001$) (Fig. 2C) (Supplementary Table 5).

The analysis also highlighted a reduction in AADC+ cells also in the substantia nigra pars compacta (SNc). This subnucleus showed a sexual dimorphism, maintained also in the GD group: males showed more AADC+ cells than females (CON, $p < 0.0001$; GD, $p = 0.005$). The GD protocol reduced the number of AADC+ cells in GD groups compared with same-sex controls (males, $p < 0.0001$; females, $p < 0.0001$) (Fig. 3B). The reduced AADC-ir in the SNc is also reflected by a decrease in AF in the GD groups compared with the same-sex controls (males, $p = 0.005$; females, $p < 0.0001$) (Fig. 3C). In addition, the reduction in AF abolished the sex difference between the GD groups ($p = 0.994$) (Supplementary Table 5).

In the substantia nigra pars reticulata (SNr), the ANOVA revealed a difference in the AF ($p < 0.0001$) (Fig. 3E), but not in the number of AADC+ cells ($p = 0.834$) (Fig. 3D) between groups. Specifically, the GD protocol reduced AF in females, thereby resulting in a sex difference ($p < 0.0001$) that was not present in the controls ($p = 0.154$). In addition, both groups exposed to the GD protocol showed lower AF compared with CON groups of the same sex (males, $p = 0.023$; females, $p < 0.0001$) (Fig. 3E).

3.3. Reduction of the serotonin immunoreactivity in GD rats

The serotonergic neurons are mainly located in the most caudal area of the brain, more specifically in the dorsal (DRN) and median (MnR) raphe nuclei (Molliver, 1987). At the end of the GD protocol, rats of both sexes showed a strong reduction of 5-HT-ir (Supplementary Table 6)

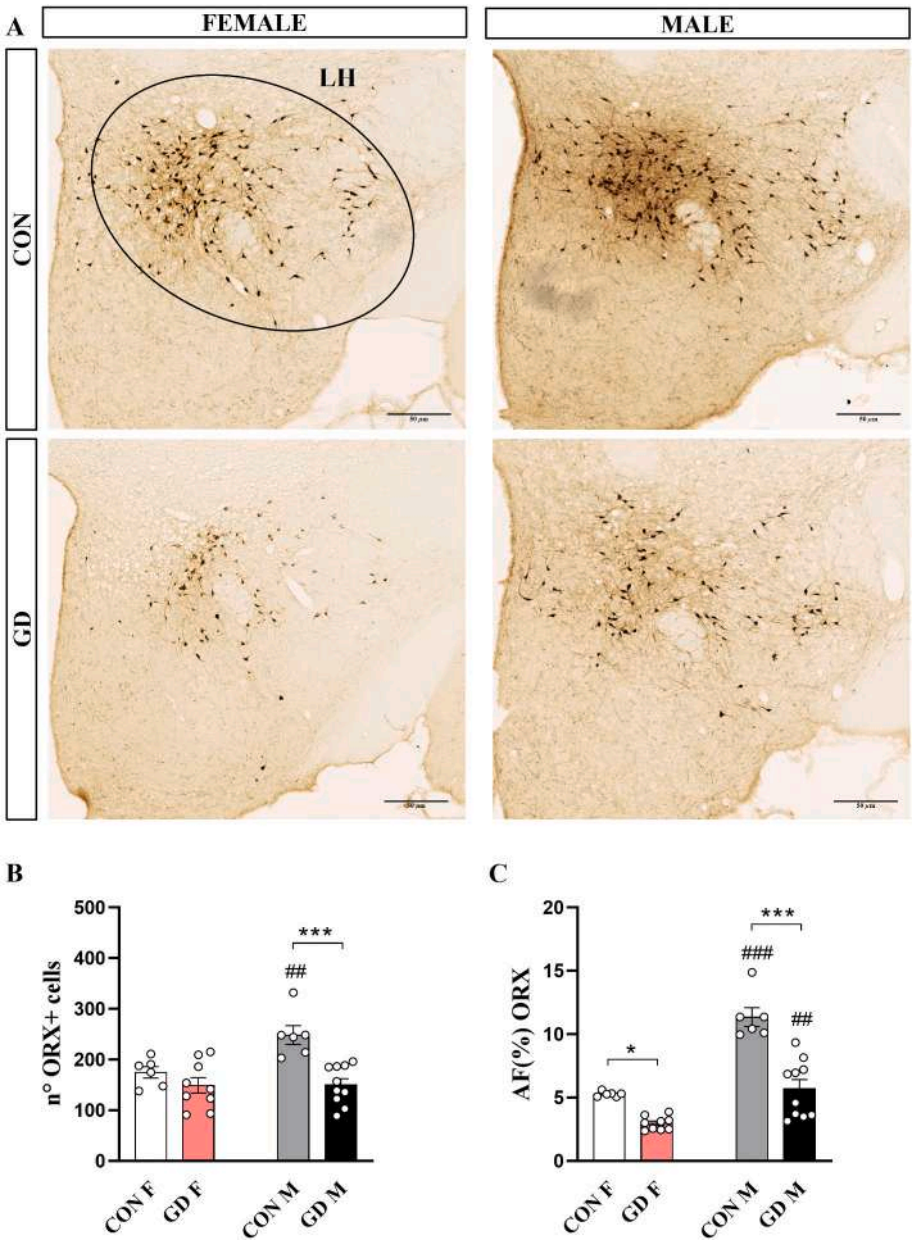

Fig. 1. Analysis of ORX immunoreactivity in the LH. **(A)** Representative images of ORX-ir in coronal sections of rat LH. Quantification of **(B)** number of ORX+ cells and **(C)** AF in the LH. Data is expressed as mean ± SEM. Statistical analysis revealed a significant effect for $p < 0.05$ (*comparison between different groups; #comparison between sexes). + = positive; ORX = orexin; AF = area fraction; LH = lateral hypothalamus; CON = control; GD = gaming disorder.

throughout the rostro-caudal development of these nuclei. Alterations were particularly significant in the central portion of the nuclei (Bregma −7.80 mm) (Fig. 4A) and are described in detail below.

Firstly, in the DRN, the statistical analysis of 5-HT-ir highlighted a sex difference in the dorsal part of the nucleus (DRD). In fact, CON males showed more 5-HT+ cells than CON females ($p = 0.033$). This sex difference is maintained among the GD groups ($p = 0.045$). However, the GD rats showed an overall reduction in the number of 5-HT+ cells in both sexes, compared with the same-sex control group (males, $p < 0.0001$; females, $p = 0.001$) (Fig. 4B). In the ventral part of the dorsal raphe nucleus (DRV), there are no sex differences between CON ($p = 0.999$) or GD ($p = 0.832$) groups.

Interestingly, the GD protocol induced a reduction of 5-HT+ cells compared to same-sex controls in both the DRV subnucleus (males, $p = 0.005$; females, $p = 0.049$) (Fig. 4C) and the DRN totality (males, $p < 0.0001$; and females, $p = 0.0007$) (Fig. 4D). The analysis of the AR showed the same results in the DRD. However, in contrast to the quantification of the number of 5-HT+ cells in the DRV, only the female GD group showed a reduction in the AF compared with the same-sex control group (males, $p = 0.136$; and females, $p < 0.0001$) (Supplementary Table 6).

Similar outcomes are shown in the MnR. However, unlike the control groups, the GD groups showed a sex difference in the MnR, with more 5-HT+ cells in males than females (GD, $p = 0.003$; CON, $p = 0.997$). Moreover, GD protocol produced a reduction in the number of 5-HT+ cells in GD groups compared to same-sex controls (males, $p = 0.039$; females, $p < 0.0001$) (Fig. 4E). Comparing the AF, the control group showed a sex difference, with higher values in males than females ($p = 0.001$). The GD protocol abolished this sex difference in GD groups ($p = 0.270$), and caused a significant AF reduction in both sexes compared to controls (males, $p = 0.030$; females, $p < 0.0001$) (Supplementary Table 6).

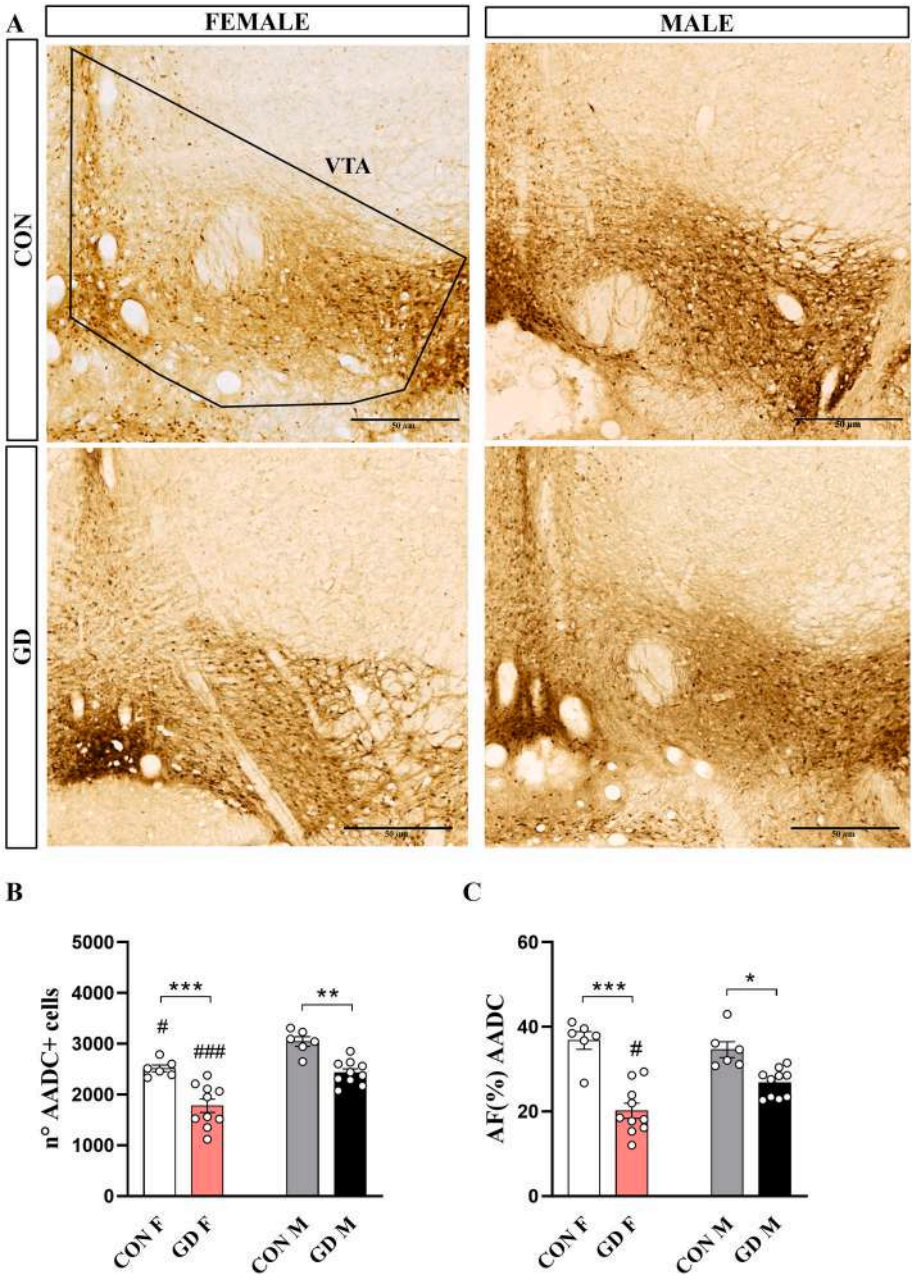

Fig. 2. Analysis of AADC immunoreactivity in the ventral tegmental area. **(A)** Representative images of AADC-ir in coronal sections of rat VTA. Quantification of **(B)** the number of AADC+ and **(C)** AF in the VTA. Data is expressed as mean ± SEM. Statistical analysis revealed a significant effect for $p < 0.05$ (* comparison between different groups; # comparison between sexes). Scale bar = 50 μm. + = positive; AADC = Aromatic L-Amino Acid Decarboxylase; VTA = ventral tegmental area; AF = area fraction; CON = control; GD = gaming disorder.

3.4. GD induces changes in plasma levels of Trp and its metabolites via 5-HT and KYN

Rats subjected to the GD model showed significant changes in plasma levels of Trp and its metabolites along the 5-HT and KYN pathways.

GD groups of both sexes showed an increase in plasma Trp levels after the game session compared with sex-matched control groups (males, $p = 0.028$; females, $p = 0.045$) (Fig. 5B). The GD groups displayed a strong reduction in plasma 5-HT levels compared to controls (males, $p = 0.0002$; females, $p = 0.003$). Interestingly, while 5-HT plasma levels were higher in males than in females control animals ($p = 0.002$), this sex difference was not present in GD rats ($p = 1.000$) (Fig. 5C). In both sexes, the control groups, despite having a lower Trp level than the GD groups, showed higher Trp to 5-HT conversion than the GD groups (males, $p = 0.001$; females, $p = 0.049$) (Fig. 5D).

Similar to 5-HT, GD rats had a strong reduction in KYN plasma levels compared to sex-matched controls (males, $p = 0.0007$; females, $p = 0.017$). However, while CON males had higher KYN levels than CON females ($p = 0.002$), this sex difference was not observed in GD rats ($p = 0.939$) (Fig. 5E).

Regarding downstream metabolites of KYN, both 3-HK and QA plasma levels were higher in CON males than in CON females (3-HK, $p = 0.029$; QA, $p = 0.062$) (Fig. 5F and G). This sex difference was absent in GD rats (3-HK, $p = 0.879$; QA, $p = 0.768$). Interestingly, while GD males showed a reduction in plasma 3-HK (males, $p = 0.045$) and QA (males, $p = 0.003$) compared to CON male rats, no difference for these two metabolites was present comparing GD versus CON females (3-HK, $p = 0.691$; QA, $p = 0.258$) (Fig. 5F and G).

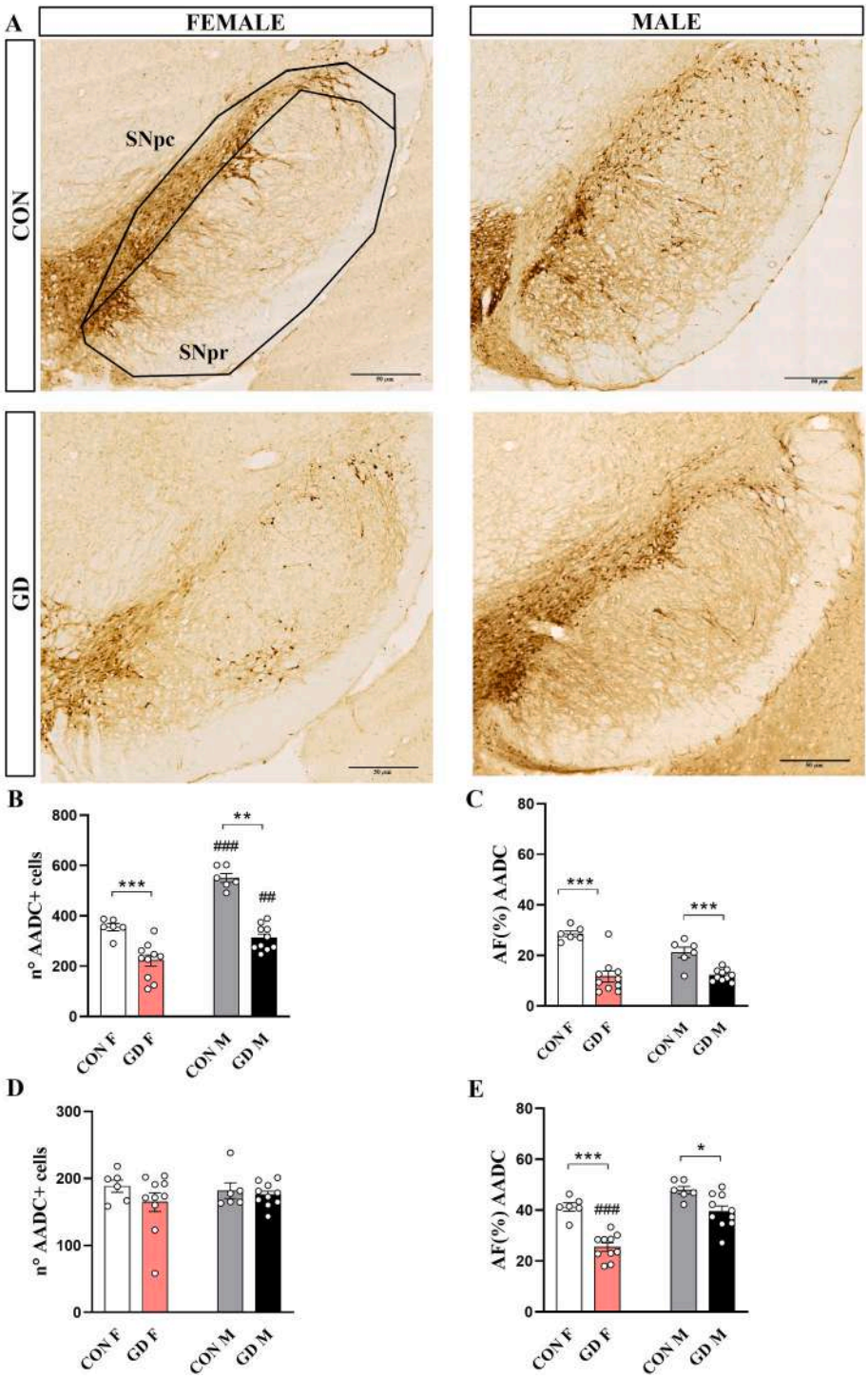

Fig. 3. Analysis of AADC immunoreactivity in the substantia nigra. **(A)** Representative images of AADC-ir in coronal sections of rat SN. Quantification includes AADC+ cells and AF in SNc (**B, C**) and in the SNr (**D, E**). Data is expressed as mean ± SEM. Statistical analysis revealed a significant effect for $p < 0.05$ (*comparison between different groups; #comparison between sexes). Scale bar = 50 μm.+ = positive; SN = substantia nigra; AADC=Aromatic L-Amino Acid Decarboxylase; AF = Area Fraction; SNc = Substantia nigra compact part; SNr = Substantia nigra pars reticulata; CON = control; GD = gaming disorder.

In CON females, there was a trend toward higher plasma KYNA levels compared to CON males ($p = 0.084$), while in GD females, a significant increase was observed compared to GD males ($p = 0.010$). In addition, in both sexes, KYNA levels increased significantly in GD compared to sex-matched CON rats (males, $p = 0.044$; females, $p = 0.013$) (Fig. 5H).

We also indirectly assessed the activity of key enzymes involved in the KYN pathway. A significantly lower KYN/Trp ratio was observed in GD rats compare to sex-matched CON (males, $p = 0.001$; females, $p = 0.007$) rats, suggesting reduced IDO/TDO activity (Fig. 5I).

In contrast, the KYNA/KYN ratio was significantly higher in GD than in sex-matched CON rats (males, $p = 0.001$; females, $p = 0.020$), indicating a likely increased KAT activity in GD rats (Fig. 5L). Interestingly, in GD female but not male rats, we found a greater conversion of KYN into 3-HK (a higher 3-HK/KYN ratio used as a proxy of KMO enzyme activity) compared to sex-matched CON rats (males, $p = 1.000$; females, $p = 0.036$) (Fig. 5M). To further evaluate the balance of the amount of

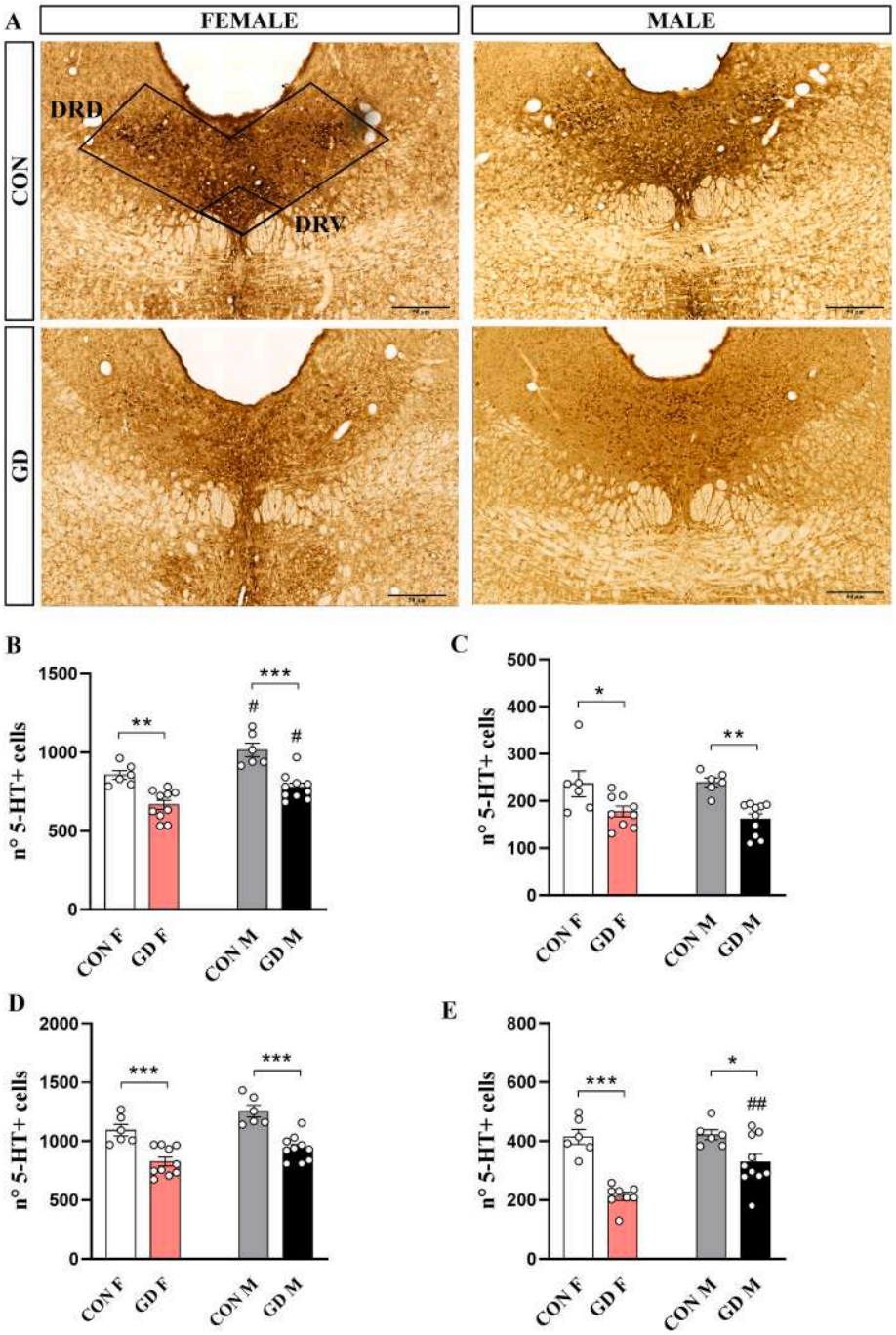

Fig. 4. Analysis of 5-HT immunoreactivity in the dorsal raphe nucleus. **(A)** Representative images of 5-HT-ir in coronal sections of rat DRN. Quantification of the number of 5-HT+ cells in **(B)** DRD, **(C)** DRV, (**D**) DRN, and **(E)** MnR. Data is expressed as mean ± SEM. Statistical analysis revealed a significant effect for $p < 0.05$ (*comparison between different groups; #comparison between sexes). Scale bar = 50 μm. + = positive; 5-HT = serotonin; DRN = dorsal raphe nucleus; DRD = dorsal raphe nucleus, dorsal part; DRV = dorsal raphe nucleus, ventral part; MnR = median raphe nucleus; CON = control; GD = gaming disorder.

KYN converted in the neuroprotective and neurotoxic branch of the pathway, we calculated the KYNA/3-HK*1000 ratio (Fig. 5N). GD females showed no difference compared to CON females ($p = 1.000$), whereas GD males exhibited a significant higher KYNA/3-HK ratio compared to CON males ($p = 0.007$), indicating a relative shift toward the neuroprotective branch of the pathway in male but not female GD rats. Finally, the KYNA/QA ratio, which reflects the balance between neuroprotection and neurotoxicity, was lower in both female and male GD rats compared to sex-matched CON rats (males, $p = 0.007$; females, $p = 0.045$) (Fig. 5O). Finally, we examined plasma levels of DA. Similarly to 5-HT, we found higher DA levels in CON males than in CON females ($p = 0.0003$), and no sex-difference between male and females GD rats. Moreover, both male and female GD rats had lower plasma DA levels than their respective CON male ($p = 0.0007$) and female ($p = 0.049$) rats (Fig. 5Q).

4. Discussion

GD is a mental disorder that primarily affects adolescents. Patients with GD show several symptoms, among the best known being loss of control over play, increased compulsive and hyperactive behaviors, anxiety, and social isolation (Harrison et al., 2021; "The ICD-11

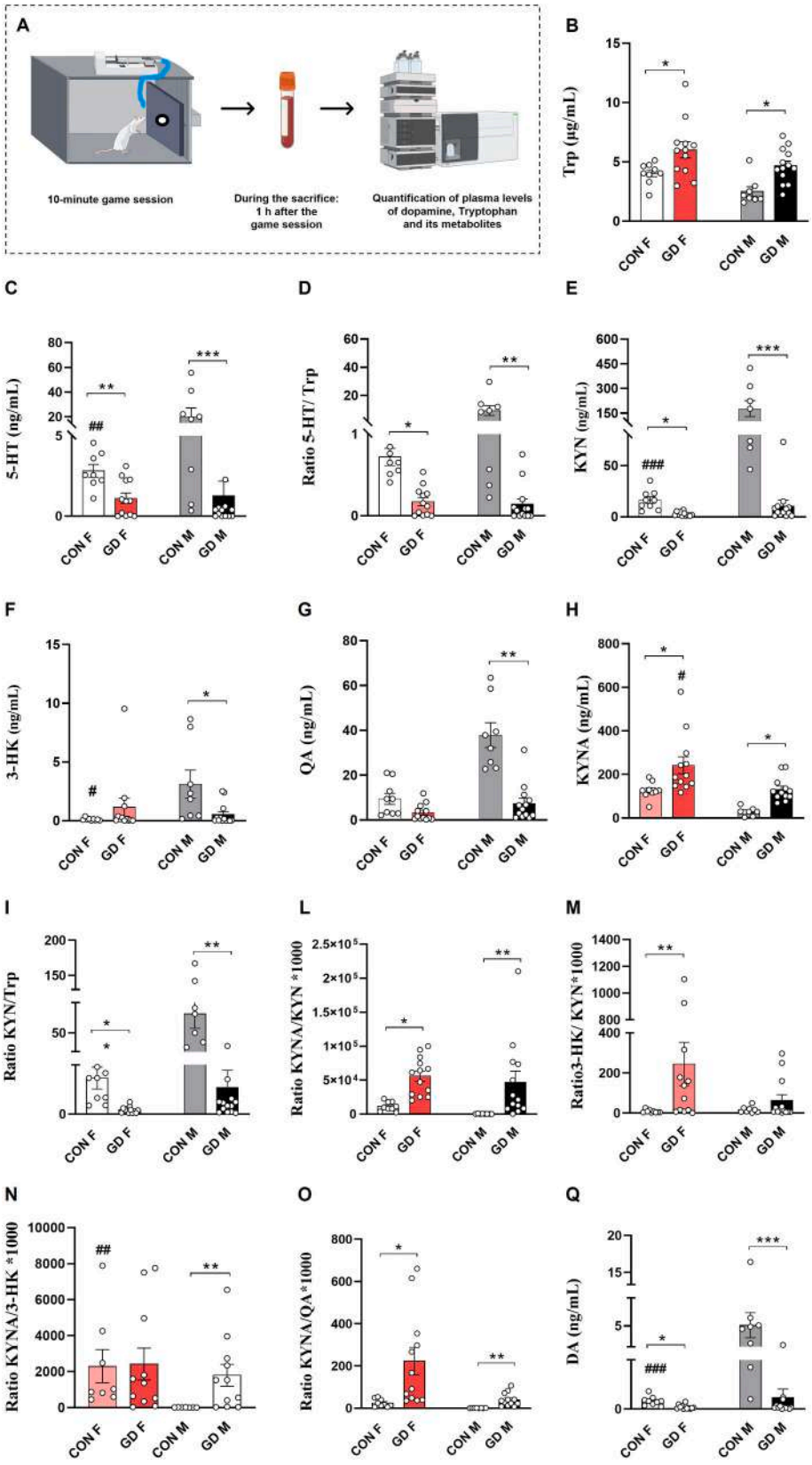

Fig. 5. HPLC-mediated determination of plasma levels of dopamine, tryptophan, and its metabolites. (**A**) Schematic representation of the timing of sample collection and processing. Quantification of (**B**) tryptophan, (**C**) serotonin, (**D**) serotonin/tryptophan ratio, (**E**) kynurenine, (**F**) 3-hydroxykynurenine, (**G**) quinolinic acid, (**H**) kynurenic acid, (**I**) kynurenine/tryptophan ratio, (**L**) kynurenic acid/kynurenine ratio, (**M**) 3-hydroxykynurenine/kynurenine ratio **(N)** kynurenic acid/3-hydroxykynurenine ratio, and (**O**) kynurenic acid/quinolinic acid ratio, and (**Q**) dopamine in rat plasma samples. Ratios KYN/Trp, KYNA/KYN, 3-HK/KYN, KYNA/3-HK, and KYNA/QA were multiplied by 1000 to facilitate visualization. Data is expressed as mean ± SEM. Statistical analysis revealed a significant effect for $p < 0.05$

(*comparison between different groups; #comparison between sexes). Ratios in panels I, L, M, and N are multiplied by 1000 for clarity. Trp = tryptophan; 5-HT = serotonin; KYN = kynurenine; 3-HK = 3-hydroxykynurenine; QA = quinolinic acid; KYNA = kynurenic acid; DA = dopamine.

Classification of Mental and Behavioral Disorders: Diagnostic Criteria for Research," 2018). These symptoms are associated with a reduction in neural activity in areas suitable for inhibitory control or regulation of emotional state (Li et al., 2020).

GD is a currently under-explored mental disorder whose causes are still poorly understood. Among the possible causes of the development and maintenance of this mental disorder is the potential alteration of the dopaminergic, serotonergic and orexinergic systems (Ariatama et al., 2019; Choi et al., 2020; Hong et al., 2018; Li et al., 2020; Tian et al., 2014). These three systems are fundamental for the proper functioning of different brain areas, regulating decision-making functions, as well as the perception and processing of emotional stimuli, including reward processing (Arias-Carrión et al., 2010; Assadi et al., 2009; Homberg, 2012).

Patients with psychiatric disorders or mental illnesses have decreased expression of DA, 5-HT, or ORX transporters and receptors (Cummings and Walker, 1996; Katsuki and Akaike, 2004; Stepanova et al., 2020). Impairment of these systems predisposes individuals to the development of various mental disorders that show high comorbidity with GD (*i.e.*, ADHD, anxiety, depression, and bipolar disorders (Dullur et al., 2021; Furuyashiki and Kitaoka, 2019; Hong et al., 2018; Koncz et al., 2023; Tian et al., 2014). Pharmacological treatments that enhance cerebral levels of DA and 5-HT alleviate typical symptoms of GD, such as loss of control over play, hyperactivity, and depressive states (Sá et al., 2023).

In a previous work, we developed a rat model that mimics certain behavioral traits found in GD patients. In this model, rats subjected to the GD protocol are trained to play with a touch screen platform, toward which they develop imperative and compulsive behaviors (Casile et al., 2024).

We also demonstrated that GD behavior led to altered activation of specific brain circuits in rats, mainly related to the control of decision-making processing, emotional, motivated, and reward-seeking behaviors (Casile et al., 2024). These alterations in brain activity could reflect the behavioral changes observed in the GD rats, which include a hyperactive and compulsive behavioral phenotype during task performance, accompanied by a loss of interest in social novelty and impaired social interaction. Notably, these findings mirror neural dysfunctions already described in GD patients (Li et al., 2020).

The main purpose of this work was to investigate the possible involvement of the dopaminergic, serotonergic, and orexinergic systems in the development of GD, using this rat model. We have shown that both male and female GD rats exhibit central and peripheral impairment in these neurotransmitters.

GD rats showed a reduction of 5-HT-ir in the DRN and MnR like that found in rats developing alcohol dependence (Ohta et al., 2010). Alterations in this system may impair signal transduction, leading to reduced 5-HT release. A decrease in the cell number or amount of circulating serotonin causes persistent disruptions in neurocognitive pathway involved in emotion processing, reward mechanism, and executive control (Adjimann et al., 2021; Choi et al., 2020; Malave et al., 2022). GD rats showed a dysregulated reward response during play, which is not restored even in the presence of a social stimulus with a positive valence (Casile et al., 2024). These behavioral modifications are common in various psychiatric disorders and make individuals more susceptible to the development of depression, anxiety, and bipolar disorder (Lis et al., 2025).

Dopaminergic neurons in the VTA are involved in several cognitive functions, including motivated learning, reward prediction, and the regulation to initiate or stop an action (Bromberg-Martin et al., 2010; Kaufling, 2019). A reduction in both the number and fibers of dopaminergic neurons in the VTA in GD rats could be underlie the loss of control during play and the reduced ability to predictive reward outcomes (Casile et al., 2024).

Similar cognitive and behavioral alterations have been reported in animal models of neuropsychiatric disorders, such as depression or schizophrenia (Douma and de Kloet, 2020; Kaufling, 2019; Morikawa and Paladini, 2011). In addition, several studies have demonstrated dopaminergic system deficits in rodent models exposed to substances of abuse, such as alcohol or drugs (Bass et al., 2013; Bocklisch et al., 2013; Matthews and German, 1984). A comparable reduction in the number of DA+ neurons was also found in the SN of GD rats. Specifically, the GD group of both sexes exhibited a decrease in AF and the number of DA+ cells in the SNr and SNc compared with the same-sex controls. In psychiatric diseases, such as schizophrenia, it has been described that a reduction in the number of DA+ cells in the SN (Birtwistle and Baldwin, 1998; Rice et al., 2016) is often correlated with a reduction in brain-derived neurotrophic factor (BDNF), which regulates its survival and differentiation and synaptic plasticity (Baquet et al., 2005).

ORX plays an important role in learning, memory acquisition, and cognitive functions through the activation of monoaminergic systems (Al-Kuraishy et al., 2020; James et al., 2017). Compared to control rats, GD groups showed a reduction in AF and the number of ORX+ cells in the LH. Repeated or chronic stress can lead to a decrease in the functionality of the ORX system generating a behavioral phenotype of low motivated arousal or depressive-like behavior (James et al., 2017; Murgatroyd et al., 2015).

In fact, the diminish of the ORX pathway is typical in depression or anxiety and it is associated with deficits in DA and 5-HT production (Abbas et al., 2015; James et al., 2014; Johnson et al., 2012). Trp is an essential amino acid required for protein biosynthesis, but also the biochemical precursor of several metabolites that regulate immune, metabolic, and nervous system functions (Comai et al., 2020). The two major routes of Trp metabolism are the 5-HT/MLT and the KYN pathways which are actively studied as potential pharmacological targets for different peripheral and central disorders (Modoux et al., 2021). Variations in the levels of Trp metabolites, along with high levels of pro-inflammatory factors, are involved in the development of depression and bipolar disorder (Pisanu et al., 2022) but also of other psychiatric disorders (Comai et al., 2020). We have observed that the GD protocol induces an alteration in the metabolism of Trp along both the 5-HT and the KYN pathways. Specifically, GD rats exhibited high levels of Trp likely as the result of reduced conversion of the amino acid into 5-HT and KYN. Within the context of the KYN pathway, in which its activation could lead to an imbalance between the neurotoxic (formation of QA and 3-HK) and the neuroprotective (formation of KYNA) branches, we observed that GD rats compared to CON rats exhibit on one side low conversion of Trp into KYN (lower KYN/Trp ratio), on the other side they showed increased conversion of KYN into 3-HK (higher 3-HK/KYN ratio), the precursor of QA, indicating a shift toward the neurotoxic branch. However, despite this apparent shift, GD rats also showed an elevated KYNA/QA ratio, suggesting that, overall, the pathway was preferentially directed towards the production of the neuroprotective metabolite KYNA. It should be noted, though, that elevated KYNA levels, although generally considered neuroprotective, may lead to excessive antagonism of NMDA, resulting in NMDA hypofunction. This has been associated to psychotic-like behavior and deficits in prefrontal cortex-dependent cognitive and behavioral functions.

The increased KYNA/QA ratio was especially evident in GD females. These findings are in keeping with those observed in patients with schizophrenia, where high levels of KYNA are associated with NMDA hypofunction, contributing to cognitive impairments and exacerbation of both positive and negative symptoms typical of this mental disorder (Adell, 2020; Comai et al., 2022). This pattern suggests possible overall

NMDA hypofunction occurring in GD animals, which exhibit increased KYNA and decreased QA levels.

Considering that QA is also an essential precursor in the de novo synthesis of NAD+, its reduction observed in GD rats might indicate a concurrent decrease in NAD + biosynthesis (Savitz, 2020). Given the critical role of NAD+ in cellular energy production, mitochondrial function, and redox homeostasis, such a reduction could have widespread implications beyond neurotransmission, potentially affecting neuronal metabolism and resilience to stress (Goeden et al., 2017; Katsyuba et al., 2020; Muneer, 2020). This metabolic shift might therefore reflect an adaptive response in GD, favoring the production of the neuroprotective KYNA over the synthesis of NAD+ precursors (J. H. Jang et al., 2022; Stone and Darlington, 2002).

Consistent with these results, there is also a reduction in 5-HT conversion from Trp in GD groups compared with CONs. The decrease in peripheral 5-HT conversion coincides with a reduction in the amount and number of 5-HT cells in the brains of GD rats. In addition, the GD groups show a similar diminution in DA and ORX. Another possible explanation could be increased QA at the central level, which, in addition to reducing the number and fibers of these neurotransmitters, could lead to altered cognitive processes and emotional state (Cummings and Walker, 1996; Katsuki and Akaike, 2004; Stepanova et al., 2020). Decrement of these neurotransmitters or their production predisposes subjects to the depressive symptoms, increased susceptibility to stress, and development of psychiatric disorders (Firk and Markus, 2007; Grafe and Bhatnagar, 2018; Moghaddam, 2002).

The development of harmful behaviors, such as compulsive behaviors, typical of addiction and mental disorders, chronically affects the reward and stress centers. A functional imbalance in these centers occurs as a consequence of a pathological adaptation of the brain to these abject behaviors, leading to reduced DA and 5-HT activity, and ultimately contributing to a state of anhedonia (Dresp-Langley, 2023; Prakash et al., 2020).

These two systems are mutually connected and exert inhibitory and excitatory interactions between them to balance their activity (Esposito et al., 2008). In the same way, both DA and 5-HT stimulate and are stimulated by ORX, which exerts an excitatory influence on both neurotransmitters (Calipari and España, 2012; Mavanji et al., 2022). In psychiatric diseases, this balance of interactions is disrupted, resulting in dysfunction of the network. One possible cause of this dysfunction is the direct action of the corticotropin-releasing hormone (CRH) system, which under chronic stress suppresses the release of DA, 5-HT, and ORX (Dedic et al., 2018; Fox and Lowry, 2013; Sargin, 2019).

Chronic stress is a common feature of many mental disorders and may predispose individuals to their development (Babenko et al., 2015; Cattaneo and Riva, 2016). For example, there is growing evidence indicating that stressogenic conditions can alter tryptophan metabolism, reducing its conversion to 5-HT and increasing the kynurenine metabolic pathway (Nazzari et al., 2020).

Thus, the reduction of these neurotransmitters in GD patients and in our rat model could be the result of the chronic action of the CRH system, which could exert its effects either directly on the neurotransmitter systems or indirectly by modulating the tryptophan metabolic pathway.

Animal models remain a fundamental tool in psychiatric research, enabling the study of neurobiological mechanisms underlying complex disorders. While models have inherent limitations, particularly in capturing the full clinical and subjective complexity of conditions like GD (Casile et al., 2024; Tricklebank and Garner, 2012), they nonetheless offer valuable insights. GD involves multifaceted genetic, environmental, cognitive, and social components that are difficult to fully replicate in animals. Behaviors such as compulsive gaming or social dysfunction are also challenging to translate due to species-specific differences (Hur, 2024; Jorgenson et al., 2016; Schou Andreassen et al., 2016).

Nevertheless, animal models, including the one developed in our laboratory, are instrumental in capturing core features of GD, including loss of control and compulsive-like behaviors (Casile et al., 2024). They provide a controlled framework to explore key neurotransmitter systems (e.g., dopamine, serotonin, kynurenine, orexin) involved in reward, motivation, and emotion, which are known to be dysregulated in GD (Kaasinen et al., 2023; Weinstein and Lejoyeux, 2020).

Pharmacological agents targeting these systems (e.g., bupropion, SSRIs, orexin receptor antagonists) have shown clinical efficacy (Ioannidis et al., 2025; Sá et al., 2023). Incorporating these treatments into preclinical models not only aids in identifying potential therapies but also strengthens the model's translational relevance and neurobiological validity.

5. Conclusions

GD is a mental disorder that is currently poorly understood. Current evidence is largely based on epidemiological and neuroimaging studies conducted in human patients. Disruptions in the dopaminergic, serotonergic, and orexinergic systems have been proposed as potential contributors to the onset and progression of this disorder. These three systems play essential roles in the proper functioning of brain pathways involved in the regulation of decision-making functions and in the perception and processing of emotional stimuli, including reward processing. The rat model developed in our laboratory exhibits these behavioral alterations consistent with those observed in GD patients, supporting the hypothesis of neurotransmitter impairment.

Taking advantage of our experimental model, we are able not only to investigate the neurobiological underpinnings of GD, but also to better understand common mechanisms shared with other psychiatric disorders. Widening the understanding of these overlapping pathways may ultimately facilitate the development and testing of more targeted and effective pharmacological therapies.

CRediT authorship contribution statement

Antonino Casile: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brigitta Bonaldo:** Writing – original draft, Methodology, Investigation, Conceptualization. **Martina Bettarelli:** Investigation. **Panagiotis Papadopoulos:** Investigation. **Maria Vittoria Micioni Di Bonaventura:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Sofia Nasini:** Investigation. **Stefano Comai:** Writing – review & editing, Writing – original draft, Validation, Investigation. **Stefania Sut:** Investigation. **Stefano Dall'Acqua:** Investigation. **Marilena Marraudino:** Investigation. **Stefano Gotti:** Validation, Supervision, Resources. **Carlo Cifani:** Validation, Supervision, Resources.

Ethical statement

Animals were housed according to general guidelines on protecting animals used for scientific purposes (EU Directives 201/63/EU). The Ethical Committee for Animal Experiments of Turin approved the animal experiments with Protocol number: 1035/2020-PR.

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Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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Glossary

+	positive
3-HK	3-Hydroxykynurenine
5-HT	Serotonine
AADC	Aromatic L-Amino Acid Decarboxylase
ACC	Anterior Cingulate Cortex
Aco	Anterior Cortical Amygdaloid Nucleus
AF	area fraction
ANOVA	Two-Way Analysis Of Variance
Arc	Arcuate Hypothalamic Nucleus
BLA	Basolateral Amygdaloid Nucleus
BMA	Basomedial Amygdaloid Nucleus, Anterior Part
BSTIA	Bed Nucleus Of The Stria Terminalis, Intra-amygdaloid Division
CeM	Central Amygdaloid Nucleus
Cg	Cingulate cortex
CON F	Control females
CON M	Control males
CRH	Corticotropin-Releasing Hormone
DA	Dopamine
DRD	Dorsal Raphe Nucleus, dorsal part
DRN	Dorsal Raphe Nucleus
DRV	Dorsal Raphe Nucleus, ventral part
DSM-5™	Diagnostic and statistical manual of mental disorders 5th edition
GD	Gaming Disorder
GD F	Females subjected to the GD protocol
GD M	Males subjected to the GD protocol
ICD-11	11th final revision of the International Classification of Diseases
IDO	Indoleamine 2,3-Dioxygenase
IPR	Interpeduncular Nucleus
ir	immunoreacivity
KAT	Kynurenine Aminotransferase
KMO	Kynurenine 3-Monooxygenase
KYN	Kynurenine
KYNA	Kynurenic Acid
LC-MS/MS	liquid chromatography-mass spectrometry
LH	Lateral Hypothalamus
MeAD	Medial Amygdaloid Nucleus, Anterodorsal Part
MLT	Melatonin
MnR	Median Raphe Nuclei
Nac	Nucleus Accumbens
NMDA	Neurotoxic Molecule N-Methyl-D-Aspartate
OFC	Orbitofrontal Cortex
ORX	orexin
PAG	Periaqueductal Gray
PBS	M phosphate-buffered saline
PFA	Paraformaldehyde
PRL	Prelimbic Cortex
PV	Paraventricular Thalamic
QA	Quinolinic Acid
ROI	region of interest
RRF	Retrorubral Field
Rt	Reticular thalamic
SN	Substantia Nigra
SNc	Substantia Nigra pars compacta
SNr	Substantia Nigra pars reticulata
TDO	Tryptophan 2,3-Dioxygenase
Trp	Tryptophan
VTA	Ventral Tegmental Area
WHO	World Health Organization

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2025.110703.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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