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Social stress under binge-like alcohol withdrawal in adolescence: evidence of cannabidiol effect on maladaptive plasticity in rats

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Original Article

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Abstract

Background. Alcohol binge drinking may compromise the functioning of the nucleus accumbens (NAc), i.e. the neural hub for processing reward and aversive responses.
Methods. As socially stressful events pose particular challenges at developmental stages, this research applied the resident–intruder paradigm as a model of social stress, to highlight behavioural neuroendocrine and molecular maladaptive plasticity in rats at withdrawal from binge-like alcohol exposure in adolescence. In search of a rescue agent, cannabidiol (CBD) was selected due to its favourable effects on alcohol- and stress-related harms.
Results. Binge-like alcohol exposed intruder rats displayed a compromised defensive behaviour against the resident and a blunted response of the stress system, in addition to indexes of abnormal dopamine (DA)/glutamate plasticity and dysfunctional spine dynamics in the NAc. CBD administration (60 mg/kg) was able to: (1) increase social exploration in the binge-like alcohol exposed intruder rats, at the expenses of freezing time, and in control rats, which received less aggressive attacks from the resident; (2) reduce corticosterone levels independently on alcohol previous exposure; (3) restore DA transmission and (4) facilitate excitatory postsynaptic strength and remodelling.
Conclusions. Overall, the maladaptive behavioural and synaptic plasticity promoted by the intersection between binge-like alcohol withdrawal and exposure to adverse social stress can be rescued by a CBD *détente* effect that results in a successful defensive strategy, supported by a functional endocrine and synaptic plasticity. The current data highlight CBD's relevant therapeutic potential in alcohol- and stress-related harms, and prompt further investigation on its molecular targets.

Introduction

Underage alcohol binge drinking has sharply increased and is a health and social concern (Halkjelsvik, Brunborg, & Bye, 2021). The intersection between intoxicating alcohol binges and the adolescent brain can trigger structural and functional impairment of critical neural pathways (Jones, Lueras, & Nagel, 2018) that can hinder the high-powered potential of the developing brain to maintain emotional control and cope with psychological threats (Sachser, Hennessy, & Kaiser, 2011). Adolescence indeed is sculpted with highly conserved characteristics to meet common evolutionary pressures and social stress, which may include confrontations among peers, and subordination when encountering intimidating older adults.

Both alcohol- and social stress-adaptation involve common neural circuitries (Bath et al., 2017; Brancato et al., 2018; Brancato, Plescia, Lavanco, Cavallaro, & Cannizzaro, 2016; Koob, 2013; Plescia et al., 2015) interconnected in the nucleus accumbens (NAc). Besides activating the neuroendocrine hypothalamic–pituitary–adrenal (HPA) axis, social stress exposure induces lasting changes in dopamine (DA) and excitatory plasticity-related signals in the reward circuitry (Baik, 2020; Deal, Konstantopoulos, Weiner, & Budygin, 2018) that may promote negative emotional states and abnormal coping.

Likewise, intoxicating alcohol binges induce hyper-responsiveness of the HPA axis, in terms of abnormal hypothalamic and extrahypothalamic corticotropin-releasing hormone (CRH) expression and serum glucocorticoid levels; in addition, metaplastic changes in the DA and glutamate signalling occur in the accumbal synaptic triad, that correlate with dysfunctional behaviour (Brancato et al., 2021; Retson, Sterling, & Van Bockstaele, 2016). Previous

work evidenced a considerable susceptibility of NAc morpho-functional architecture to acute and prolonged alcohol withdrawal resulting in altered synaptic plasticity and dendritic remodelling (Cannizzaro et al., 2019; Spiga et al., 2014).

While several reports describe how the adolescent brain is particularly vulnerable to alcohol as a result of stress-related changes (Boutros et al., 2018; Burke & Miczek, 2014), little evidence can be found on the interference intoxicating alcohol binges have on specific abilities to adapt to psychosocial stress. Indeed, adolescents can easily engage both in alcohol binge drinking and in challenging social threats, putting at risk the development of appropriate adaptive responses.

Face and construct validity support the use of the resident–intruder paradigm (RIP), as a tool for studying applicable aspects of social stress response in rodents: isolated, older aggressive resident rats are confronted in their home cage with grouped young intruders that implement – and adjust – their defensive behaviour to receive fewer attacks (Burke & Miczek, 2015; Koolhaas et al., 2013).

Hence, the current research addresses the perplexing issue namely if coping strategy to social stress might be jeopardized in young rats following repeated intermittent exposure to intoxicating alcohol levels during adolescence. Stress-related HPA axis response, in terms of corticosterone plasma levels, is also investigated.

Once we assessed the expression of abnormal defensive behaviour in the RIP, we explored different levels of neuroplasticity in the rat NAc. Here, alcohol abuse and withdrawal can disarrange the subtle balance between DA levels and glutamate release that critically regulates synaptic plasticity and affect spine shape and stability in the medium spiny neurons (MSNs) (Cannizzaro et al., 2019; Dani & Zhou, 2004; Fasano et al., 2013; Paillé et al., 2010; Spiga et al., 2014).

Indeed, our working hypothesis is that the NAc represents the intersection point in which maladaptive plasticity induced by binge-alcohol withdrawal combines – and impacts – neuroadaptation that occurs as a consequence of social stress exposure. In detail, we investigated DA mesolimbic signalling by measuring presynaptic tyrosine hydroxylase (TH) and DA transporter (DAT) density, as a measure of DA synthesis and reuptake, and postsynaptic D1- and D2-receptor mRNA expression; MSN excitatory plasticity through post-synaptic density protein 95 (PSD95) and Homer 1 (HOM1) expression (Brancato et al., 2021; Brancato, Castelli, Lavanco, Marino, & Cannizzaro, 2020); spine dynamics and activation, by quantifying the expression of the activity-regulated cytoskeleton-associated protein (ARC) (Guzowski et al., 2006), and experience-dependent structural and functional adaptation in MSNs, through the expression of forkhead box P1 (FOXP1) (Anderson, Kulkarni, Harper, & Konopka, 2020).

At last, we hypothesized a counteractive strategy based on the favourable effects of the largest non-psychotomimetic (Viudez-Martínez et al., 2019) phytochemical component of cannabis, cannabidiol (CBD), on alcohol-related harms in preclinical models and stress-induced anxiety and discomfort in humans (Elsaid, Kloiber, & Le Foll, 2019; Viudez-Martínez et al., 2018b). CBD can functionally interact with the mesolimbic DA system in the NAc, where it exerts modulatory effects on various cognitive and emotional processes via a multimodal pharmacological profile that involves not exclusively the endocannabinoid transmission (Bhattacharyya et al., 2010; Guimarães, Zuardi, Del Bel, & Guimarães, 2004; Melas, Scherma, Fratta, Cifani, & Fadda, 2021). Accordingly, we administered sub-chronic doses of CBD during the RIP to test its potential in offsetting the behavioural and molecular changes associated with binge-like alcohol exposure during adolescence.

Materials and methods

For a complete description of the experimental methods, refer to the online Supplementary material.

Animals

Adolescent male Wistar rats and retired breeder male Wistar rats (Envigo, Italy) were housed in standard polycarbonate cages with bedding (in pairs and single, respectively), maintained at 22 ± 2°C, with 55 ± 5% humidity, on a 12 h light/dark cycle (lights on at 08:00 AM), with ad libitum food (Mucedola, Italy) and water. Procedures were approved by the Italian Ministry of Health (1119/2016-PR) in adherence with Italian (D.L.26/2014) and European (2010/63/EU) legislation on laboratory animals' use. Every effort was made to minimize the number of animals used and their suffering.

Drugs

Alcohol (96%; Carlo Erba, Italy) was diluted with tap water at 25% v/v. Cannabidiol (2-[(1*R*,6*R*)-6-isopropenyl-3-methylcyclohex-2-en-1-yl]-5pentylbenzene-1,3-diol, CBD) was isolated in the Forensic Laboratory of Biologically Active Substances of the University of Chemistry and Technology of Prague from supercritical CO_2 extracts obtained from technical cannabis, with purity (NMR) >99% (Nemeškalová, Hájková, Mikulů, Sýkora, & Kuchař, 2020). Binge-like alcohol withdrawn (BAW) and control (CTRL) rats were sub-chronically administered with CBD at 60 mg/kg, or vehicle (1% ethanol, 1% Tween 80, saline) (i.p.) (Brancato et al., 2021), 90 min before the experimental sessions (Deiana et al., 2012). This dose, devoid of suppressive motor activity (online Supplementary material), was shown to reduce alcohol reinforcing properties, motivation and relapse, together with specific related gene expression (Viudez-Martínez et al., 2018b).

Binge-like alcohol exposure

Rats were exposed to an intermittent binge-like alcohol paradigm during adolescence (PND 35–54, Spear, 2000) at the dosage of 3.5 g/kg (Varlinskaya, Truxell, & Spear, 2014), every other day, for nine exposures (Fig. 1*a*). The 25% alcohol solution was daily prepared and administered *per os* as previously described (Turner, Brabb, Pekow, & Vasbinder, 2011), resulting in binge-like blood alcohol concentrations (193 ± 19 mg/dl) (Brancato et al., 2021). CTRL rats were given an isovolumetric amount of water on the same exposure days.

Resident–intruder paradigm

Twenty-four hours after the last alcohol administration, BAW and CTRL rats were tested in the RIP (Burke & Miczek, 2015) at PND 55 (session 1), 57 (session 2), 61 (session 3) and 64 (session 4) (Fig. 1*a*). Briefly, the intruder rat was introduced into the resident's home cage for the confrontation, which terminated 5 min after the first attack bite, and exposed to further sensory contact for 10 min. RIP occurred between 9:00 AM and 1:00 PM under dim light (15–20 lux). The sessions were recorded and intruder- and resident behaviours during the confrontation were quantified by a trained observer, blind to the treatment, using Boris v. 7.9.4 (Friard & Gamba, 2016). They were expressed as a percentage of total confrontation time. Resident aggressions were expressed in

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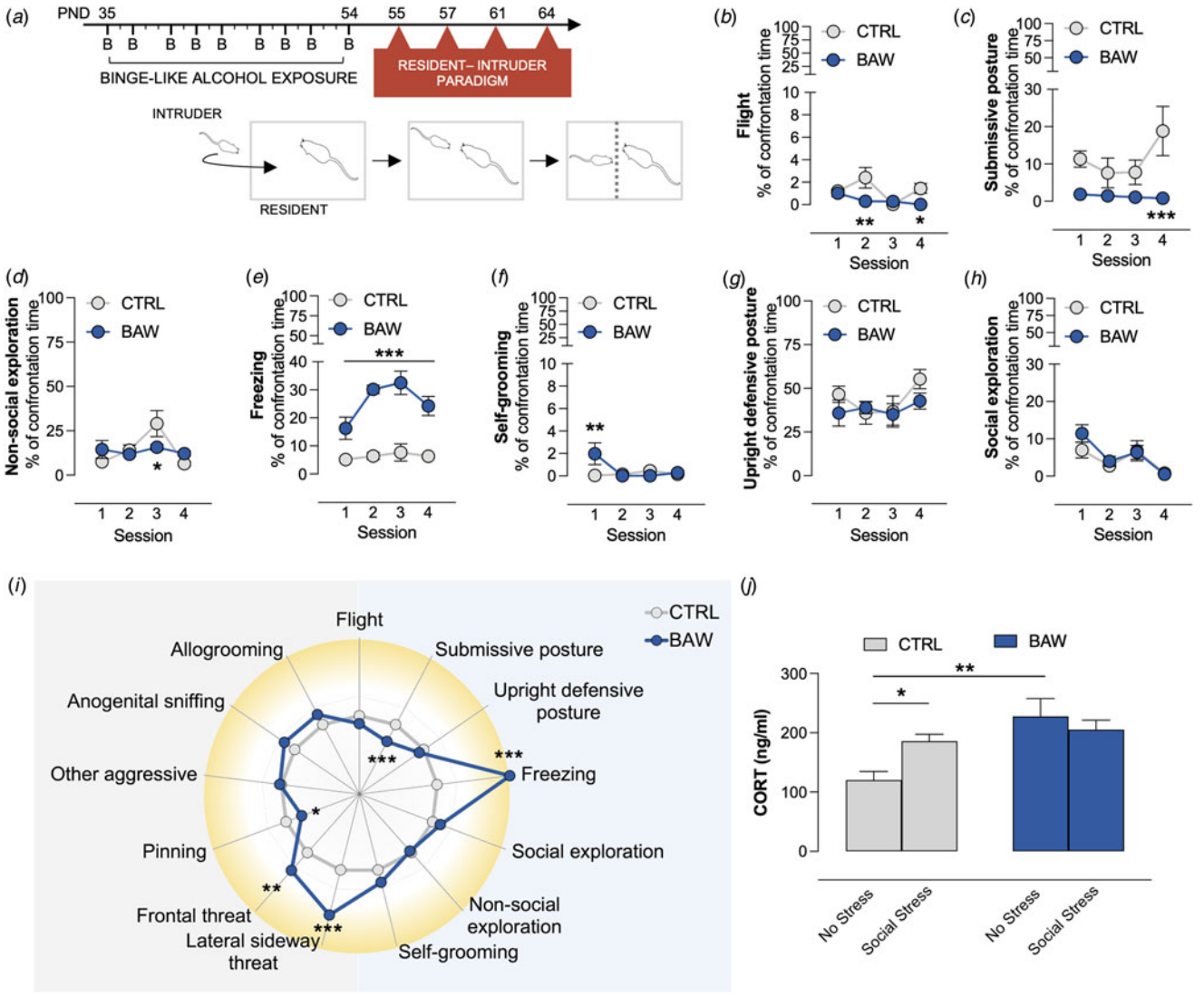

Fig. 1. BAW impairs behavioural and neuroendocrine social stress coping. (*a*) Rats were exposed to binge-like alcohol during adolescence and evaluated for coping abilities in four sessions of the RIP during withdrawal, in comparison with CTRL counterparts. (*b*) BAW rats displayed decreased flight, (*c*) submissive postures and (*d*) non-social exploration when compared with CTRL rats. On the other hand, (*e*) BAW rats showed increased freezing over the sessions, and (*f*) self-grooming in comparison with CTRLs. (*g*) Upright defensive posture and (*h*) social exploration were not affected by BAW. (*i*) Overall, the analysis of cumulated percentages of intruder behaviour indicated a different social stress-coping strategy in BAW rats with respect to CTRLs (two-way ANOVA, BAW: $F_{(1,84)} = 0.3543$, $p = 0.5533$; behaviour: $F_{(6,84)} = 131.2$, $p < 0.0001$; interaction: $F_{(6,84)} = 13.99$, $p < 0.0001$). In detail, BAW intruders displayed decreased submissive postures ($t = 4.087$, df = 84.00, $p = 0.0007$) and increased freezing ($t = 7.884$, df = 84.00, $p < 0.0001$) when compared with CTRL, while no significant differences were highlighted in cumulated percentages of flight ($t = 0.3508$, df = 84, $p > 0.999$); upright defensive posture ($t = 2.225$; df = 84; $p = 0.2014$); social exploration ($t = 0.4957$, df = 84, $p > 0.999$); non-social exploration ($t = 0.2913$, df = 84, $p > 0.999$) and self-grooming ($t = 0.1493$, df = 84, $p > 0.999$). Moreover, the analysis highlighted a higher resident aggressive behaviour towards BAW rats than CTRLs. Indeed, although no different mean rate of bites was observed (CTRL = 0.324 ± 0.122 attack/min; BAW = 0.546 ± 0.481 attack/min; Mann–Whitney test: $U = 17$, $p = 0.3666$), the analysis of cumulated percentages of aggressive behaviour the intruders showed that BAW rats underwent higher level of aggressive behaviour (two-way ANOVA, binge-like alcohol exposure: $F_{(1,72)} = 18.79$, $p < 0.0001$; behaviour: $F_{(5,72)} = 28.85$, $p < 0.0001$; interaction: $F_{(5,72)} = 15.38$, $p < 0.0001$), with increased lateral sideways threats ($t = 8.508$, df = 72, $p < 0.0001$) and frontal threat posture ($t = 3.821$, df = 72, $p = 0.0017$) with respect to CTRL rats. In addition, post hoc analysis showed a decrease in cumulated pinning in BAW rats ($t = 2.819$, df = 72, $p = 0.0373$) when compared to CTRLs, and no significant difference in cumulated percentages of other aggressive behaviour ($t = 0.0665$, df = 72, $p > 0.999$); anogenital sniffing ($t = 0.2139$, df = 72, $p > 0.999$) and allogrooming ($t = 0.8259$, df = 72, $p > 0.999$). (*j*) In addition, neuroendocrine response to the RIP revealed a blunted neuroendocrine response to social stress in BAW rats. Each circle and each bar represent the mean of $n = 7$ rats, while error bars indicate S.E.M. Dots in radar graph represent standardized values, cantered at zero for the CTRL group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. PND, postnatal day; CTRL, control; BAW, binge alcohol withdrawal.

number of attacks per minute. No-stress exposed groups (NS-CTRL; NS-BAW) remained undisturbed in their home cage.

Corticosterone determination

Twenty-four hours after the last RIP session, rats were anesthetized and sacrificed (1:00 and 3:00 PM), trunk blood samples were collected for serum preparation and kept at −20°C. Serum corticosterone levels (CORT, ng/ml) were measured using a commercially available ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany), according to the manufacturers' instructions.

Gene expression analysis

Brains were rapidly removed, divided into two sagittal halves in a brain matrix on ice, for NAc dissection (Paxinos & Watson,

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1986). Tissue was flash frozen, and stored at −80°C until gene expression analysis. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) were conducted as previously described (Brancato et al., 2021). Primers employed in qRT-PCR experiments are provided in the online Supplementary material. Analysis was performed using the ΔΔC(*t*) method, using glyceraldehyde 3-phosphate dehydrogenase as a reference gene. Data are expressed as fold change relative to relevant control group (NS-CTRL; CTRL).

Immunofluorescence experiments

Free-floating immunofluorescent staining was performed as previously described (Brancato et al., 2020, 2021). Details, including primary and secondary antibodies employed, are provided in the online Supplementary material. For each NAc section, images of core and shell were acquired at 100× magnification (U plan 100×/1.25 oil, Meji Techno, Japan), and deconvolved using Deltapix Insight, Denmark. Immunofluorescence was quantified as to integrated density over the threshold (ImageJ, NIH). Shell and core values from each NAc section were averaged and expressed as relative immunofluorescence percentages, with reference to controls levels (NS-CTRL; CTRL).

Data analysis

Behavioural data were analysed by repeated measure (RM) two-way analysis of variance (ANOVA), or RM three-way ANOVA with Geisser–Greenhouse correction, performed on the percentage of the duration of each behavioural category over the four RIP sessions. Two-way or three-way ANOVA were employed for the analysis of cumulated percentages over the four sessions. Mann–Whitney test was performed, when appropriate. Neuroendocrine and neurobiological data were analysed by two-way ANOVA. Bonferroni post-hoc test was employed, when necessary. Grubb's test was employed to identify outliers and one sample, out of seven, was excluded from the statistical analysis of D2R expression data. Data are reported as mean ± S.E.M. Radar graphs represent standardized measures to the reference condition. Statistical analysis was performed using Prism v.9.3.1 (GraphPad). Significance was set at alpha = 0.05.

Results

BAW alters social stress response

BAW intruder rats' defensive behaviour displayed a significant decrease in flight (Fig. 1*b*), submissive postures (Fig. 1*c*), non-social exploration (Fig. 1*d*); a significant increase in freezing (Fig. 1*e*) and self-grooming (Fig. 1*f*), compared with CTRLs. No difference was observed in upright defensive postures and social exploration (online Supplementary material). Complete statistical analysis is reported in online Supplementary Table S1.

Overall, the analysis of cumulated percentages of behaviour indicated different social stress coping in BAW rats with respect to CTRLs: BAW rats displayed lower submissive postures and higher freezing than CTRL rats; moreover, BAW intruders underwent increased lateral sideways and frontal threats, and decreased pinning, compared to CTRLs (Fig. 1*i*).

In addition, different serum CORT levels were observed in response to the RIP (BAW: $F_{(1,24)} = 10.74$, $p = 0.0032$; stress: $F_{(1,24)} = 1.248$, $p = 0.2750$; interaction: $F_{(1,24)} = 5.167$, $p = 0.0323$). NS-BAW rats showed higher CORT levels than NS-CTRL rats ($t = 3.924$, df = 24, $p = 0.0013$); however, while RIP exposure was associated with higher CORT levels in CTRLs ($t = 2.397$, df = 24, $p = 0.0493$), no difference was observed in BAW rats ($t = 0.8174$, df = 24, $p = 0.8435$) (Fig. 1*j*).

BAW modifies neuroplasticity in the NAc

BAW ($F_{(1,24)} = 4.526$, $p = 0.0439$) and its interaction with social stress exposure ($F_{(1,24)} = 35.37$, $p < 0.0001$) significantly altered TH-positive immunofluorescence (main effect of stress: $F_{(1,24)} = 1.662$, $p = 0.2096$). In detail, NS-BAW rats showed higher TH levels than NS-CTRLs ($t = 5.71$, df = 24, $p < 0.001$); however, social stress exposure was associated with increased TH levels in CTRL intruder rats ($t = 117$, df = 24, $p = 0.002$), and to decreased TH immunofluorescence in BAW intruder rats ($t = 3.294$, df = 24, $p = 0.0184$), compared with respective NS counterparts (Fig. 2*b*, *c*).

In addition, DAT-positive immunofluorescence was higher in BAW rats than in CTRLs, irrespective of RIP exposure (BAW: $F_{(1,24)} = 26.36$, $p < 0.0001$; stress: $F_{(1,24)} = 1.398$, $p = 0.2486$; interaction: $F_{(1,24)} = 0.7994$, $p = 0.3802$) (Fig. 2*b*, *d*).

When DA-related postsynaptic markers were evaluated, we found altered D1R expression (BAW: $F_{(1,24)} = 4.807$, $p = 0.0383$; stress: $F_{(1,24)} = 6.654$, $p = 0.0164$; interaction: $F_{(1,24)} = 9.966$, $p = 0.0043$): BAW intruder rats displayed increased D1R expression levels in comparison with NS counterpart ($t = 4.056$, df = 24, $p = 0.0027$) and CTRLs ($t = 3.783$, df = 24, $p = 0.0055$) (Fig. 2*e*). Likewise, differences in D2R expression were highlighted (BAW: $F_{(1,23)} = 6.421$, $p = 0.0185$; stress: $F_{(1,23)} = 34.47$, $p < 0.0001$; interaction: $F_{(1,23)} = 4.464$, $p = 0.0457$). In detail, BAW intruders showed increased D2R expression in comparison with NS-BAW rats ($t = 5.536$, df = 23, $p < 0.001$) and CTRLs ($t = 3.222$, df = 23, $p = 0.0226$) (Fig. 2*f*).

As to excitatory neuroplasticity in MSNs, in the face of no significant difference in the number of 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei ($p > 0.999$), we observed differences in PSD95-positive levels (BAW: $F_{(1,24)} = 0.4774$, $p = 0.4962$; stress: $F_{(1,24)} = 0.2571$, $p = 0.6167$; interaction: $F_{(1,24)} = 17.77$, $p = 0.0003$); in particular CTRL intruder rats displayed increased PSD95 levels with respect to NS-CTRLs ($t = 3.339$, df = 24, $p = 0.0164$); BAW intruders showed a trending decrease compared with NS-BAW ($t = 2.622$, df = 24, $p = 0.0896$) and lower PSD95 levels than CTRLs ($t = 3.469$, df = 24, $p = 0.0119$) (Fig. 2*g*, *h*).

Gene expression analysis revealed significant differences in HOM1 expression (BAW: $F_{(1,24)} = 9.541$, $p = 0.0050$; stress: $F_{(1,24)} = 38.01$, $p < 0.001$; interaction: $F_{(1,24)} = 6.033$, $p = 0.0217$). In detail, NS-BAW rats showed higher HOM1 levels than the NS-CTRL group ($t = 3.921$, df = 24, $p = 0.0039$); social stress did not affect HOM1 levels in CTRL rats ($t = 2.623$, df = 24, $p = 0.0895$), while decreased its expression in BAW rats ($t = 6.097$, df = 24, $p < 0.001$) (Fig. 2*i*). Significant differences were highlighted in ARC expression (BAW: $F_{(1,24)} = 8.579$, $p = 0.0073$; stress: $F_{(1,24)} = 28.61$, $p < 0.0001$; interaction: $F_{(1,24)} = 17.26$, $p = 0.0004$). In detail, NS-BAW rats showed higher ARC levels than NS-CTRLs ($t = 5.009$, df = 24, $p = 0.0002$); social stress did not affect ARC levels in CTRL rats ($t = 0.8445$, df = 24, $p > 0.999$), whereas it decreased ARC expression in BAW rats ($t = 6.720$, df = 24, $p < 0.0001$) (Fig. 2*j*).

In addition, two-way ANOVA indicated significant differences in FOXP1 expression (BAW: $F_{(1,24)} = 27.58$, $p < 0.0001$; stress: $F_{(1,24)} = 63.41$, $p < 0.0001$; interaction: $F_{(1,24)} = 25.56$, $p < 0.0001$). NS-BAW rats displayed higher FOXP1 levels than NS-CTRLs

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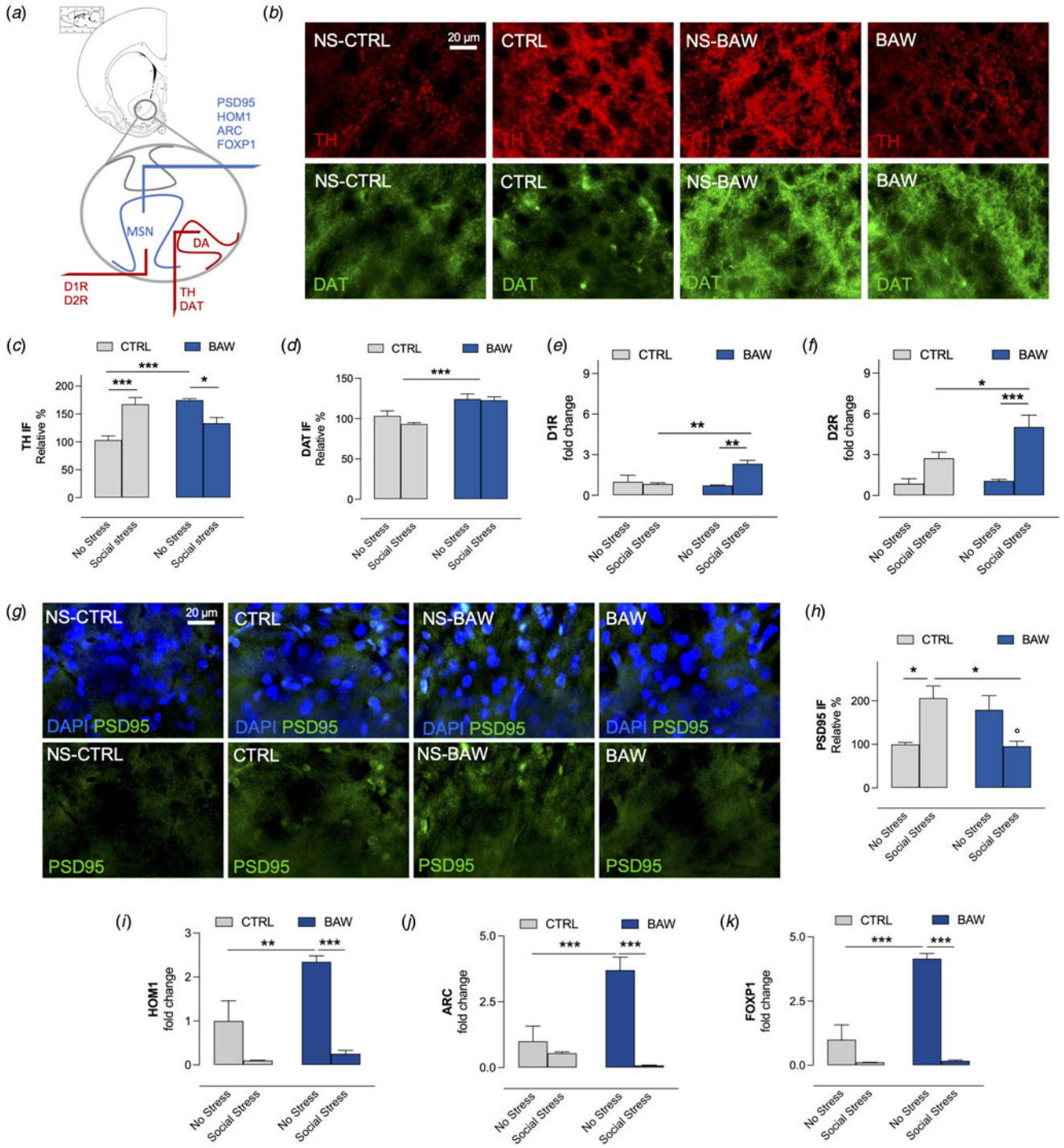

Fig. 2. BAW disrupts social stress-related DA and glutamate neuroplasticity in the NAc. (*a*) Relevant markers of DA- and glutamate-related neuroplasticity in the NAc following the RIP were evaluated in BAW and CTRL rats and compared with NS-counterparts. (*b*) DA presynaptic markers were assessed by immunofluorescence, as shown in representative pictures of TH (red) and DAT (green) staining. We observed: (*c*) increased TH levels in CTRL rats compared to NS-CTRL, and decreased levels in BAW rats with respect to NS-BAW; moreover (*d*) higher DAT-positive immunofluorescence in BAW rats than in CTRLs. As to DA-related postsynaptic markers, RIP-exposed BAW rats displayed (*e*) increased D1R expression, and (*f*) increased D2R levels, in comparison with NS-BAW rats and CTRLs. (*g*) PSD95 (green) and nuclear (DAPI, blue) immunofluorescence staining was employed for assessing excitatory neuroplasticity. (*h*) While CTRL rats showed increased PSD95 levels with respect to NS-CTRLs, BAW rats displayed a decreasing trend compared to NS-BAW, and lower PSD95 levels than respective CTRLs. In addition, we observed (*i*) decreased HOM1-, (*j*) ARC- and (*k*) FOXP1-levels in BAW rats, compared to the NS-BAW group. Each bar represents the mean of $n = 6$–7 rats, while error bars indicate SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{\circ}p < 0.1$. PND, postnatal day; CTRL, control; BAW, binge alcohol withdrawal; DA, dopamine; MSN, medium spiny neuron; TH, tyrosine hydroxylase; DAT, dopamine transporter; D1R, type 1 dopamine receptor; D2R, type 2 dopamine receptor; PSD95, post-synaptic density protein 95; HOM1, Homer 1 protein; ARC, activity-regulated cytoskeleton-associated protein; FOXP1, Forkhead Box P1; IF, immunofluorescence; NS, no exposure to social stress in the RIP; DAPI, 4′,6-diamidino-2-phenylindole.

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($t = 7.289$, df = 24, $p < 0.0001$); social stress did not modify FOXP1 levels in CTRL rats ($t = 2.056$, df = 24, $p = 0.3051$), while it significantly decreased FOXP1 expression in BAW rats ($t = 9.206$, df = 24, $p < 0.0001$) (Fig. 2*k*).

Sub-chronic CBD administration modifies social stress coping in the RIP

CBD at 60 mg/kg did not alter rats' locomotor activity and behavioural reactivity in the open-field test (online Supplementary material). CBD effects on CTRL and BAW intruder rats were evaluated on each behavioural category over the RIP sessions (Fig. 3*a*). We observed that CBD decreased flight and submissive posture in CTRLs and not in BAW rats (Fig. 3*b*, *c*). Upright defensive postures significantly increased in CBD-administered CTRL rats, in session 1 (Fig. 3*d*). CBD did not affect freezing in CTRL rats while a significant decrease was observed in BAW rats (Fig. 3*e*). Interestingly, CBD exerted a significant increase in social interaction in both CTRL and BAW rats, from session 2 onwards (Fig. 3*f*). On the other hand, CBD decreased non-social exploration in CTRL rats but not in BAW rats (Fig. 3*g*). At last, the evaluation of data from self-grooming indicated a significant three-way interaction among session, BAW and CBD (Fig. 3*h*). Complete statistical results are reported in online Supplementary Table S2. Overall, CBD discretely affected cumulated defensive behaviour of CTRL and BAW rats, exerting a significant increase in social exploration in both CTRL and BAW rats. Moreover, CBD administration decreased the cumulate aggressive behaviour towards BAW and CTRL intruders (Fig. 3*i*).

As to the neuroendocrine response to social stress, CBD administration decreased serum CORT levels (BAW: $F_{(1,24)} = 4.036$, $p = 0.0559$; CBD: $F_{(1,24)} = 5.493$, $p = 0.0277$; interaction: $F_{(1,24)} = 0.7644$, $p = 0.3906$) (Fig. 3*j*).

Sub-chronic CBD administration affects BAW-induced maladaptive plasticity in the NAc

With reference to DA-related signalling in the NAc (Fig. 4*a*), TH levels were significantly different (BAW: $F_{(1,24)} = 0.0164$, $p = 0.8991$; CBD: $F_{(1,24)} = 18.12$, $p = 0.0003$; interaction: $F_{(1,24)} = 14.88$, $p = 0.0008$). In detail, BAW rats displayed a decreasing trend in TH levels when compared with the CTRL group ($t = 2.819$, df = 24, $p = 0.0571$); CBD administration decreased TH levels in CTRL rats ($t = 5.738$, df = 24.00, $p < 0.001$) (Fig. 4*b*, *c*).

In addition, DAT-positive immunofluorescence was affected (BAW: $F_{(1,24)} = 15.85$, $p = 0.0006$; CBD: $F_{(1,24)} = 10.84$, $p = 0.0031$; interaction: $F_{(1,24)} = 11.28$, $p = 0.0026$). BAW rats showed higher DAT levels than CTRLs ($t = 5.19$, df = 24, $p = 0.0002$). CBD administration did not modify DAT levels in CTRL rats ($t = 0.046$, df = 24.00, $p > 0.999$) while it decreased DAT immunofluorescence in the BAW group ($t = 4.703$, df = 24, $p = 0.0005$), with no difference between CTRL-CBD and BD-CBD rats ($t = 0.4402$, df = 24, $p > 0.999$) (Fig. 4*b*, *d*).

Significant differences were highlighted in D1R expression (BAW: $F_{(1,24)} = 6.549$, $p = 0.0172$; CBD: $F_{(1,24)} = 0.8612$, $p = 0.3626$; interaction: $F_{(1,24)} = 12.71$, $p = 0.0016$). The significant increase in D1R expression that BAW rats displayed in comparison with CTRLs ($t = 4.33$, df = 24, $p = 0.0014$), was normalized by CBD administration ($t = 3.177$, df = 24, $p = 0.0244$) (Fig. 4*e*).

When D2R expression was evaluated, two-way ANOVA revealed a significant main effect of CBD administration in decreasing D2R expression in both CTRL and BAW rats ($F_{(1,24)} = 17.79$, $p = 0.0003$) (BAW: $F_{(1,24)} = 3.830$, $p = 0.0621$; interaction: $F_{(1,24)} = 3.161$, $p = 0.0881$) (Fig. 4*f*).

When excitatory neuroplasticity was considered (Fig. 4*a*), in the face of no significant difference in the number of DAPI-stained nuclei ($p > 0.999$), we observed significant PSD95 levels (BAW: $F_{(1,24)} = 4.954$, $p = 0.0357$; CBD: $F_{(1,24)} = 2.537$, $p = 0.143$; interaction: $F_{(1,24)} = 354$, $p < 0.001$). In detail, BAW rats displayed decreased PSD95 levels with respect to CTRLs ($t = 5.669$, df = 24, $p < 0.001$). CBD decreased PSD95 levels in CTRLs ($t = 5.222$, df = 24, $p = 0.001$), whereas it increased PSD95 immunofluorescence in BAW rats ($t = 2.969$, df = 24.00, $p = 0.0401$) (Fig. 4*g*, *h*).

In addition, CBD administration increased HOM1 expression in CTRL and BAW rats (BAW: $F_{(1,24)} = 3.414$, $p = 0.0770$; CBD: $F_{(1,24)} = 18.75$, $p = 0.0002$; interaction: $F_{(1,24)} = 0.1392$, $p = 0.7123$) (Fig. 4*i*), while ARC expression was discretely affected (BAW: $F_{(1,24)} = 4.918$, $p = 0.0363$; CBD: $F_{(1,24)} = 12.45$, $p = 0.0017$; interaction: $F_{(1,24)} = 4.756$, $p = 0.0392$). In detail, the significant decrease in ARC expression that we observed in BAW rats in comparison with CTRLs ($t = 3.11$, df = 24, $p = 0.0286$) was reversed by CBD administration ($t = 4.037$, df = 24, $p = 0.0029$) (Fig. 4*j*).

Moreover, significant differences were observed in FOXP1 expression (BAW: $F_{(1,24)} = 0.02471$, $p = 0.8764$; CBD: $F_{(1,24)} = 2.855$, $p = 0.1040$; interaction: $F_{(1,24)} = 5.369$, $p = 0.0294$); in detail, CBD administration increased FOXP1 expression level in CTRL rats ($t = 2.833$, df = 24, $p = 0.0184$) but not in BAW rats ($t = 0.4436$, df = 24, $p > 0.999$) (Fig. 4*k*).

Discussion

The current investigation looked at the effects of binge-like alcohol administration during adolescence on defensive behaviour and neurobiological correlates in rats exposed to repeated social stress during withdrawal. The working hypothesis was that in the NAc alcohol withdrawal-induced aberrant plasticity combines with – and impacts – neuroadaptation due to social stress, entailing alterations in stress coping, and in significant components of synaptic plasticity.

Indeed, BAW intruder rats displayed a deteriorated pattern of adaptive behaviour when exposed to the RIP, a test which allows the spontaneous and natural expression of both offensive and defensive behaviour in a semi-natural laboratory setting. In response to aggression, defeat and subjugation trigger an adaptive behavioural and physiological response aimed at reducing the offensive attacks. The 'predatory imminence theory' posits that defensive behaviour resizes with threat proximity on a spatio-temporal scale, such that freezing is observed in post-encounter modes whereas flight occurs when the threat is proximal (Fanselow & Lester, 1988). Freezing is not a passive state but rather a brake on the motor system, relevant to perception and preparation of appropriate defensive action, i.e. fight-or-flight reaction (Roelofs, 2017; Tringali, Greco, Lisi, Pozzoli, & Navarra, 2012). Accordingly, in our study, CTRL did not increase their freezing behaviour throughout the interactive sessions once the threat had been realized and shifted towards a proactive strategy, i.e. flight and submissive postures, to optimize their response capacity. On the other hand, BAW rats did not display an evolution in their defensive behaviour, maintaining higher freezing levels than controls, at the expenses of flight and submissive postures. The level and intensity of offensive aggression follow from, at least partially, the defensive strategy of the intruder. Indeed, we found that BAW intruder rats received a higher degree of

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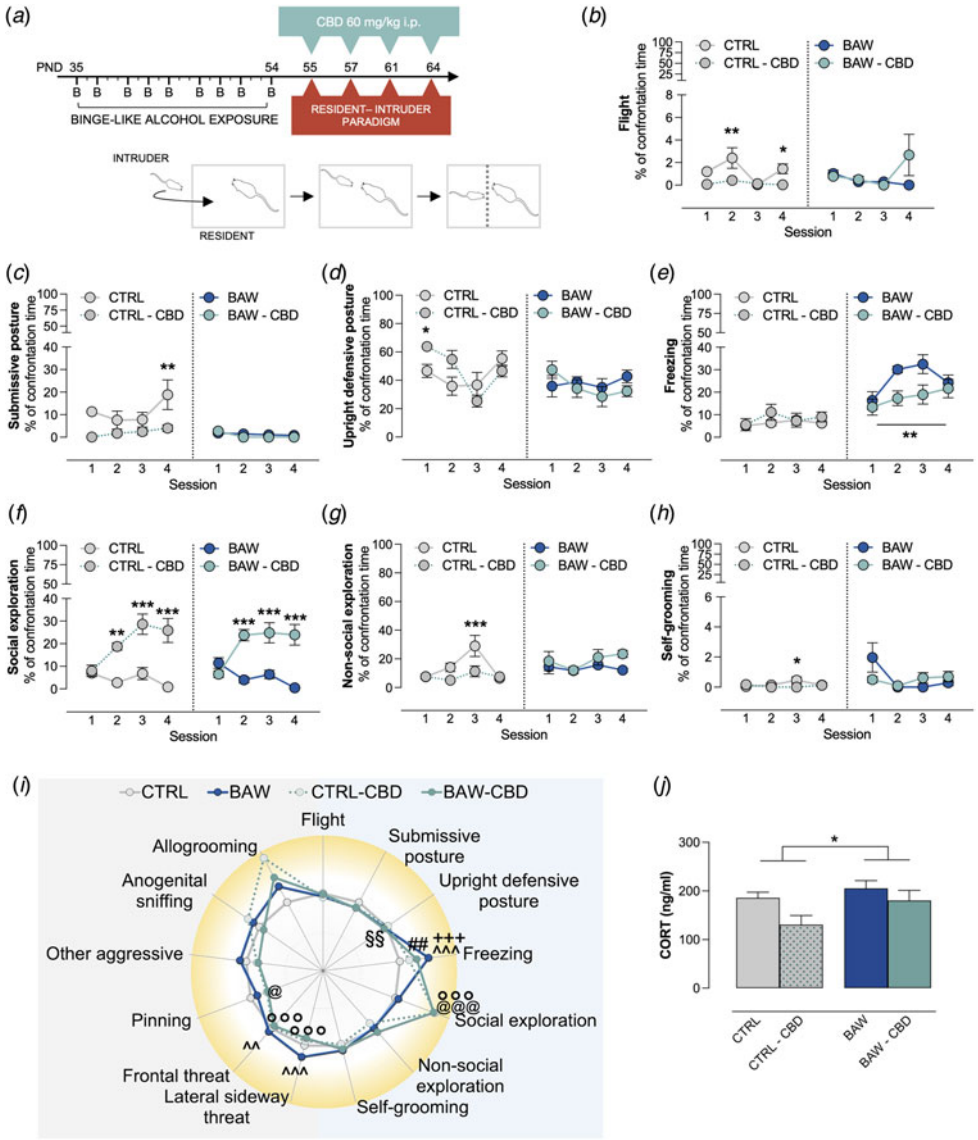

Fig. 3. CBD ameliorates social stress coping deficits in BAW rats. (*a*) CBD was administered before each session of the RIP and its effects were evaluated on each behavioural category. CBD induced a decrease in (*b*) flight and (*c*) submissive postures in CTRL rats, but not in BAW rats. (*d*) CBD altered upright defensive postures in CTRL rats but not in BAW rats. However, (*e*) CBD decreased freezing in BAW rats and (*f*) increased social exploration in both CTRL and BAW groups. In addition, (*g*) CBD administration decreased non-social exploration and (*h*) self-grooming in CTRL rats. (*i*) Overall, the analysis of cumulated behaviour indicated a different social stress coping strategy in CBD-administered rats. Indeed, when CBD effect was evaluated on cumulated defensive behaviour of CTRL and BAW rats, we observed a three-way interaction among behavioural category, CBD and BAW ($F_{(6,168)} = 4.539$, $p = 0.0003$). In addition, significant two-way interactions between behavioural category and CBD ($F_{(6,168)} = 13.11$, $p < 0.0001$), and between behavioural category and BAW ($F_{(6,168)} = 17.50$, $p < 0.0001$) were observed. On the other hand, the two-way interaction between CBD and BAW was not significant ($F_{(1,168)} = 0.1666$, $p = 0.6836$). In addition, a significant main effect of behavioural category ($F_{(6,168)} = 245.3$, $p < 0.0001$), but not of CBD ($F_{(1,168)} = 2.118$, $p = 0.1474$) and BAW ($F_{(1,168)=}1.494$, $p = 0.2233$) were highlighted. Post hoc analysis indicated that CBD did not affect flight ($p > 0.999$) and submissive posture ($p > 0.999$); BAW rats administered with CBD showed a significant decrease in upright defensive posture with respect to the CTRL-CBD group ($t = 4.705$, df = 168, $p = 0.0020$). In addition, BAW rats showed increased freezing when compared to CTRL rats ($t = 7.625$, df = 168, $p < 0.001$); BAW-CBD rats displayed no significant difference when compared to CBD-CTRL rats ($t = 3.758$, df = 168, $p = 0.0891$), but higher freezing than CTRLs ($t = 4.512$, df = 168, $p = 0.0045$). Interestingly, CBD significantly increased social exploration in both CTRL ($t = 6.272$, df = 168, $p < 0.001$) and BAW rats ($t = 5.575$, df = 168, $p < 0.001$). No differences were observed in non-social exploration ($p > 0.999$) and self-grooming ($p > 0.999$). Moreover, the statistical analysis highlighted a lower resident aggressive behaviour towards CBD-administered intruders. Indeed, when cumulate aggressive behaviour towards BAW and CTRL intruders was analyses, we observed a significant three-way interaction among behavioural category, CBD and BAW ($F_{(5,144)} = 17.55$, $p < 0.0001$). Moreover, significant two-way interactions between behavioural category and CBD ($F_{(5,144)} = 14.67$, $p < 0.0001$), behavioural category and BAW ($F_{(5,144)} = 6.524$, $p < 0.0001$) and CBD and BAW ($F_{(1,144)} = 41.59$, $p < 0.0001$) were highlighted. In addition a significant main effect of behavioural category ($F_{(5,144)} = 30.62$, $p < 0.0001$) and CBD ($F_{(1,144)} = 37.40$, $p < 0.0001$) was revealed. BAW rats received higher lateral sideways threats ($t = 10.04$, df = 144, $p < 0.001$) and frontal threats ($t = 4.508$, df = 144, $p = 0.0037$) than CTRL intruders. However, when BAW rats were administered with CBD, we observed a significant reduction in lateral sideway threats ($t = 13.11$, df = 144, $p < 0.001$) and frontal threats ($t = 6.138$, df = 144, $p < 0.001$) with respect to BAW. A significant decrease in pinning was also observed in CBD-administered CTRL intruders with respect to CTRL ($t = 6.138$, df = 144, $p = 0.0110$). Overall, both CTRL and BAW intruders administered with CBD underwent a decreased mean rate of attack bites (two-way ANOVA, CBD: $F_{(1,24)} = 4.51$, $p = 0.0442$; BAW: $F_{(1,24)} = 0.866$, $p = 0.3614$; interaction: $F_{(1,24)} = 1.22$, $p = 0.2798$). (*j*) Moreover, CBD administration decreased serum CORT levels in both groups. Each circle and each bar represent the mean of $n = 7$ rats, while error bars indicate S.E.M. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Dots in radar graph represent standardized values, cantered at zero for the CTRL group. $^{*}p < 0.05$; $^{***}p < 0.001$ *v.* CTRL. $^{\S\S}p < 0.01$ CBD-BAW *v.* CBD-CTRL; $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ BAW *v.* CTRL; $^{+++}p < 0.001$ BAW *v.* CBD-CTRL; $^{\#\#}p < 0.01$ CBD-BAW *v.* CTRL; °°°CBD-BAW *v.* BAW; $^{@}p < 0.05$, $^{@@@}p < 0.001$ CBD-CTRL *v.* CTRL. PND, postnatal day; CTRL, control; BAW, binge alcohol withdrawal; CBD, cannabidiol.

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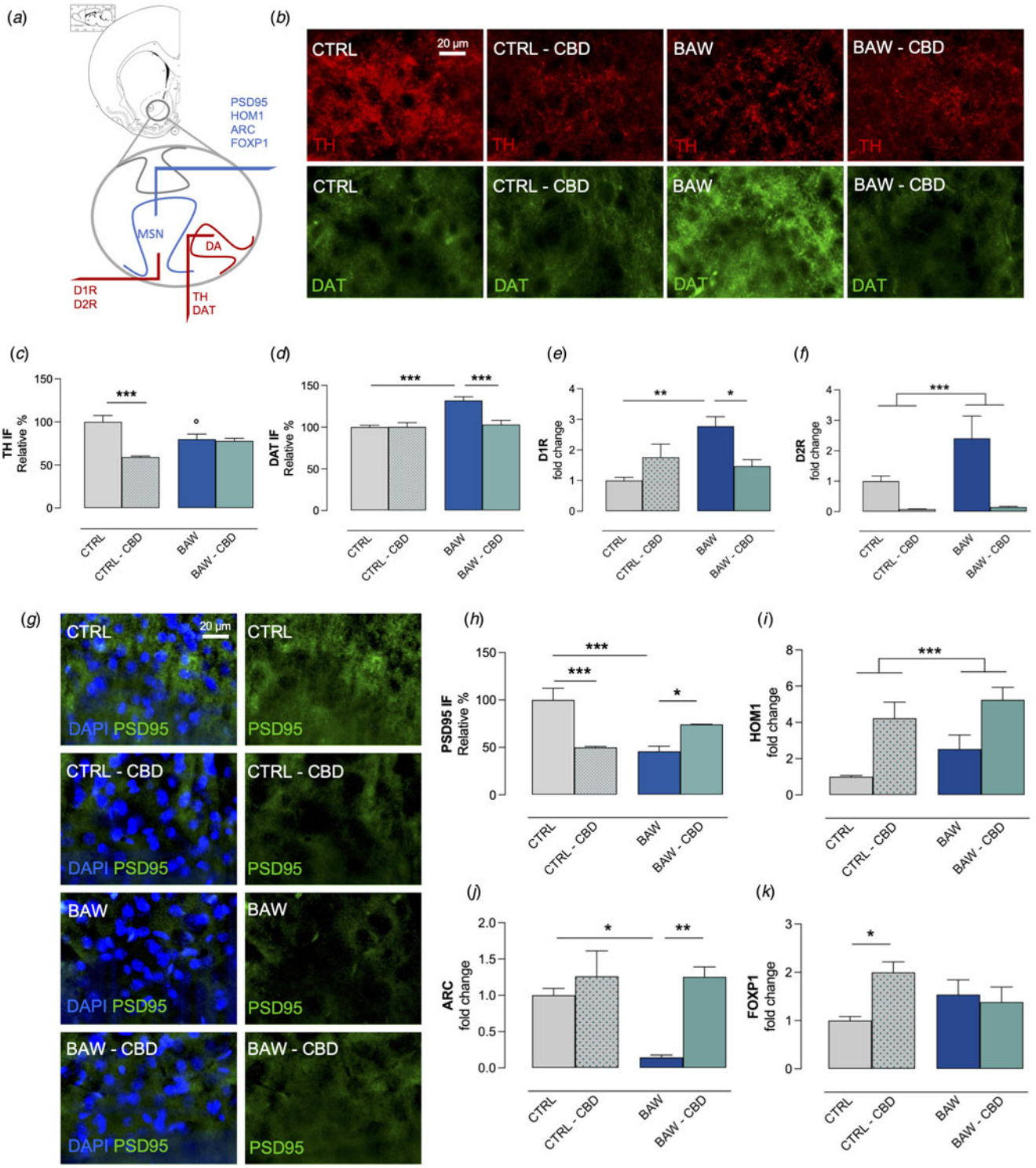

Fig. 4. CBD promotes a stress-adaptive synaptic plasticity in the NAc of BAW rats. (*a*) The effects of CBD administrations during the RIP were evaluated on the markers of DA- and glutamate-related neuroplasticity in the NAc. (*b*) Immunofluorescence assessment of DA presynaptic markers, as shown in representative pictures of TH (red) and DAT (green) staining, showed that (*c*) CBD administration decreased TH levels in CTRL rats and (*d*) decreased DAT levels in BAW rats. (*e*) In addition, CBD administration normalized the D1R overexpression in BAW rats and (*f*) and abolished D2R overexpression in both groups. The evaluation of excitatory neuroplasticity in immunofluorescent experiments, with (*g*) representative pictures of nuclei (DAPI, blue), and PSD95 (green) staining, indicated that (*h*) CBD reduced PSD95-positive immunofluorescence in CTRL rats, whereas increased PSD95 levels in BAW rats. Gene expression analysis of relevant markers of glutamate-related neuroplasticity revealed that CBD administration (*i*) increased HOM1 expression in CTRL and BAW rats, (*j*) increased ARC levels in BAW rats and (*k*) increased FOXP1 levels in CTRL rats. Each bar represents the mean of $n = 7$ rats, error bars indicate S.E.M. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. DA, dopamine; MSN, medium spiny neuron; TH, tyrosine hydroxylase; DAT, dopamine transporter; DAPI, 4′,6-diamidino-2-phenylindole; D1R, type 1 dopamine receptor; D2R, type 2 dopamine receptor; PSD95, post-synaptic density protein 95; HOM1, Homer 1 protein; ARC, activity-regulated cytoskeleton-associated protein; FOXP1, Forkhead box P1; IF, immunofluorescence; CTRL, control; BAW, binge-like alcohol withdrawal; CBD, cannabidiol.

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aggressive behaviour from the resident than CTRLs, suggesting that binge-like alcohol exposure in adolescence can result in a vulnerable phenotype characterized by deficits in coping with dominance.

Our evidence is consistent with studies showing that male rats exposed to chronic alcohol displayed heightened freezing in context-induced memory tasks indicating the occurrence of a maladaptive coping to adverse stimuli (Rorick, Finn, & Steinmetz, 2003; Staples et al., 2021). Notably, the only report available, to our knowledge, on the response to social stress in a human population exposed to alcohol during adolescence highlighted a significant association between early alcohol use and impaired stress perception and HPA axis reactivity during a social stress procedure (Evans, Greaves-Lord, Euser, Franken, & Huizink, 2012). Besides, adolescent binge drinking has been also associated with increased rate and severity of stress-related psychopathologies (Fortier et al., 2021). Current research is ongoing to examine reliable sex-specific paradigms of social stress, since female rats do not typically display territorial aggression unless subjected to irreversible hypothalamic lesions (Solomon, 2017). Social stress coping combines a proper emotional control and the selection of an adaptive natural defensive repertoire. Consistent behavioural findings from our recent experiments, in rats withdrawn from binge-like alcohol during adolescence, show the occurrence of an emotional dysregulation in paradigms such as the social interaction test and the novelty-suppressed feeding test, and decreased coping with the inescapable swim stress (Brancato et al., 2021).

HPA axis activation is an initial step in an integrated neuroendocrine–neurochemical–behavioural response when the organism evaluates a threat and triggers defence reactions to cope with it. Our data show an altered neuroendocrine stress response in BAW rats in terms of higher basal CORT levels than in CTRLs and a dampened response to the RIP. High levels of circulating glucocorticoids have been described in alcohol withdrawal, together with increased extrahypothalamic CRH levels (Brancato et al., 2021). Heavy drinkers also display a blunted stress-induced HPA axis response, suggesting neuroendocrine tolerance and impaired inhibitory HPA axis control (Blaine & Sinha, 2017; Koob, 2010; Thayer, Hall, Sollers, & Fischer, 2006). In our model, withdrawal after chronic binge-like alcohol exposure in adolescence is associated with HPA axis basal hyperactivation, a blunted stress-related response, and a disruption in defensive behaviour organization. The prolongation and repetition of freezing in BAW intruders, therefore, can be interpreted as an impaired behavioural flexibility to the social threat that likely mirrors an anomaly in social behaviour-related brain areas.

Among them the NAc is essential for driving reward and aversion-related behaviour. Indeed, the emotional ambience of external environments retunes the valence of the functions there generated (Reynolds & Berridge, 2008) and allows the expression of both appetitive and fearful responses (Faure, Reynolds, Richard, & Berridge, 2008). This group has previously reported that DA- and glutamate-related plasticity in the NAc during alcohol withdrawal results in aberrant processing of aversive stimuli (Cannizzaro et al., 2019). Consistently now we show that the interplay between BAW-induced abnormal plasticity and RIP-induced neuroadaptation results in relevant markers of DA and glutamate aberrant signalling. This is in accordance with other groups' evidence of metaplastic changes in striatal microcircuits during alcohol withdrawal (Ostroumov & Dani, 2018). Indeed, our data suggest a higher DA synthesis in RIP-exposed CTRL, while DA reuptake is not affected. On the other hand, the evidence of a reduced TH expression and increased DAT levels in BAW rats suggests a decreased DA synthesis and higher DA removal from the synaptic cleft. This is highly consistent with reports from other groups showing that social defeat stress can increase burst activity in the ventral tegmental area and phasic DA release in the NAc in freely moving rats (Deal et al., 2018). However, a history of alcohol self-administration can increase the rate of DA uptake and blunt the effects of social stress on NAc DA dynamics (Karkhanis, Rose, Huggins, Konstantopoulos, & Jones, 2015). These alterations can correlate with impairment in aversive limbic memory and behavioural flexibility, as reported by this and other research groups (Cannizzaro et al., 2019; Kern, Stanwood, & Smith, 2010; Korn et al., 2021). Intriguingly, we report a prominent D2R mRNA expression relative to DR1 in the NAc of RIP-exposed BAW rats, compared to non-stressed counterparts and CTRLs, which suggests a function-specific vulnerability of this pathway in BAW rats. Notably, the NAc drives distinct processes through the recruitment of discrete subpopulations of cells. Generally, NAc D1R-expressing MSNs are approach-promoting, whereas NAc D2R-positive MSNs are aversion-promoting and essential for aversive learning (Hikida et al., 2013). D2Rs play a major role in the expression of defensive behaviour in rodents and alterations in D2 receptor signalling may produce hyperdefensiveness and altered environmental processing (Tang, Yang, Shi, & Chen, 2022). Therefore, the altered presynaptic markers of DA-synthesis and reuptake, in association with the prominent activity of avoidance-driving D2R signalling of BAW intruder rats may contribute to the dysfunctional elaboration of the social threat, and the generation of the aberrant behavioural output, thus contravening the 'practice makes perfect' rule of complex-learned social motor actions. It is worth noting, however, that the NAc is a downstream projection area of brain regions which drive defeat-induced active social avoidance, including excitatory inputs from the basolateral amygdala (Diaz & Lin, 2020).

As to MSN excitatory plasticity, the downstream pathway of glutamatergic signalling in BAW intruders displayed a decrease in synaptic strength, opposite to what was observed in CTRLs. The alterations in synaptic plasticity in response to stress are crucially orchestrated by the postsynaptic-density proteins, which include receptor complexes, scaffold proteins and adaptor proteins that are located predominantly in glutamatergic synapses. A reduction in PSD95 expression could indicate a deficit in assembling clusters of glutamate *N*-methyl-D-aspartate (NMDA) receptors in the postsynaptic membrane that is correlated with a decrease in synaptic strength (Prybylowski et al., 2002). In addition, PSD95 also interacts with the DA D1 receptor, shaping up a functional PSD95-D1R-NMDARs multiprotein complex at the MSN spine (Kruusmägi et al., 2009). On this basis, it could be speculated that the decrease in PSD95 observed in stress-exposed BAW rats may also affect the multiprotein complex functionality, and thus weaken the D1R-related approach-driven signalling.

On the other hand, HOM1 is critical in postsynaptic density remodelling, by affecting synaptic architecture and facilitating glutamate signal transduction (Yoon et al., 2021). Fluctuations in HOM1 are observed in animals after restraint-, social defeat-, prenatal-stress and alcohol consumption (Brancato et al., 2021; Castelli, Brancato, Cavallaro, Lavanco, & Cannizzaro, 2017; Reshetnikov & Bondar, 2021). Interestingly, deletion of HOM1 has been correlated with stress susceptibility and HPA

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dysfunction (Reshetnikov & Bondar, 2021) indicating a prominent role in the feedback regulation of the HPA axis and stress coping. In line with our previous data on alterations of the effector systems regulating the expression of specific markers of synaptic remodelling in the NAc of BAW rats (Brancato et al., 2021), the consistent reduction of PSD95 and HOM1 scaffolding currently observed is associated with the suppression of both ARC and FOXP1 expression. ARC is a downstream protein of the metabotropic glutamate receptor–Homer pathway, which has been implicated in alcohol-related synaptic remodelling (Dong, Guidotti, Zhang, & Pandey, 2018). On the other hand, FOXP1 is a key transcription factor that controls the signalling pathways of MSNs and related behaviours (Anderson et al., 2020). Reduction of FOXP1 correlates with defects in social behaviour in mice and humans (Araujo et al., 2015); besides, FOXP1 knockdown impairs behavioural learning and inhibits the experience-dependent reorganization of network-level activity and remodelling (Garcia-Oscos et al., 2021). Overall, binge-like alcohol withdrawal seems to disrupt cellular mechanisms that enable the onset of experience-dependent changes in cellular and behavioural functional plasticity, likely contributing to the maladaptive coping to social stress here observed.

The next aim of this research was to identify a counterbalancing factor able to rescue, at least in part, those abnormalities. Our data show a relevant broad-spectrum activity of CBD that imparted a homogeneous significant twist in rat defensive strategy of both rat groups. Indeed, we observed a social approach-oriented behaviour rather than freezing in BAW rats, and flight or submission in CTRLs. Noteworthily, at the first confrontations, CTRL rats stood up facing the aggressor, actively defending themselves, while afterwards they opted in favour of social interaction. This evidence is consistent with other reports on a pro-social effect after repeated CBD administration (Mastinu et al., 2022) and anti-stress properties in fear-induced paradigms of anxiety-like behaviour (Melas et al., 2021). Notably, freezing levels remained higher in CBD-treated BAW rats than in CTRLs, highlighting a certain degree of rigidity in this behavioural pattern. CBD-induced *détente* strategy resulted in reduced general aggressive actions from the residents highlighting the occurrence of a more convenient defensive coping to dominance.

It is already known that CBD, at different doses, decreases HPA axis response under stress conditions (Viudez-Martínez, García-Gutiérrez, & Manzanares, 2018a), including cortisol levels and anxiety in drug-abstinent patients (Hurd et al., 2019). Accordingly, CBD was able to attenuate HPA axis dysregulation produced by the interception of BAW and psychosocial stress, *sub conditionibus*, although dose, mode, gender and task-related differences might affect CBD-induced outcomes (Viudez-Martínez, García-Gutiérrez, & Manzanares, 2020). Overall, given the connection between HPA axis dysregulation and alcohol-related brain dysfunction in pathways involved in biobehavioural emotional regulation and stress response, CBD effect on HPA axis activity plays a key role in the restoration of emotional control (Brancato et al., 2021) and the exploitation of an appropriate behavioural coping strategy. Accordingly, compounds able to reduce HPA axis over-reactivity in the basal states, while also normalizing blunted HPA phasic responses such as the neurosteroids, improve alcohol-induced maladaptive stress coping (Blaine & Sinha, 2017), through a positive allosteric modulation of GABAergic pathways.

Interestingly, CBD acts as a positive allosteric modulator of GABA-A receptor, which is a primary target of alcohol, increasing GABA-evoked currents amplitude in a benzodiazepine-like manner (Bakas et al., 2017).

However, as opposite to benzodiazepines, CBD acts also on γ subunit-lacking receptors, thus suggesting that CBD reversing effect can overcome repeated alcohol-induced GABA-A receptor subunit rearrangements (Ruffolo et al., 2018).

In addition, previous reports reveal that behaviourally effective CBD doses elicit a predominant decrease in spontaneous DA neuronal frequency and bursting activity in the ventral tegmental area, which is correlated with a decrease in aversion-like and freezing behaviour (Norris et al., 2016), whereas repeated CBD administrations at the same doses used in this study can reduce TH expression (Viudez-Martínez et al., 2020).

Although we observed consistent results in CBD-treated CTRLs, the overall CBD effect upon DA metaplasticity in the NAc of BAW intruder rats is suggestive of a balancing effect resulting in decreased DA reuptake and blunted D2R expression. Interestingly, ablation of D2R-mediated signal in the NAc has been related to a decrease in avoidance and facilitation in approach-behaviour in a conflicting environment, suggesting that defeat-induced avoidance is dependent on the relative contribution of DA D1/D2 receptor signalling in the NAc. Accordingly, we tested the effect of the D2R antagonist sulpiride on RIP-exposed BAW rats' defensive behaviour and measured a reduction in freezing time (online Supplementary material) similar to CBD.

Seminal works report CBD-induced reduction in aversive emotional learning and consolidation (Norris et al., 2016) and the contribution of relieved plasticity (Maggio, Shavit Stein, & Segal, 2018). Moreover, the neuroprotective role of CBD has been associated with region-specific increased expression of synaptophysin, PSD95 and spine density (Sales et al., 2019). In accordance, we report a selective increase in players of neuronal activity-dependent postsynaptic plasticity, such as PSD95, HOM1 and ARC, which are evocative of an enhancement in synaptic strength. Our data on increased indexes of excitatory plasticity and markers of spine remodelling substantiate CBD-driven restoring of synaptic architecture, that can contribute to the expression of convenient, functional behavioural responses. The lack of a rescue effect on FOXP1 in BAW rats, contrarily to CTRLs, might be interpreted as the endurance of a certain rigidity as regards both behaviour and NAc metaplasticity.

Overall, it is tempting to speculate that the behavioural *détente* effect of CBD in the RIP involves neuroadaptation in the NAc where a functional dynamic of dopaminergic transmission is associated with functional strength in the excitatory synapse, so that fear-related aversive behaviour shifts towards a pro-social successful strategy towards the psychosocial threat. Currently, CBD's mechanisms of action have not been disentangled. Indeed, although the pharmacological effects of CBD in different *in vitro* biological systems have been extensively investigated, the mechanisms responsible for its therapeutic potential are not univocal but rather depend on the behavioural outcome being measured (Campos, Moreira, Gomes, Del Bel, & Guimarães, 2012). A further integrated multidisciplinary investigation is currently ongoing.

The overall layout of the multidimensional abnormalities observed in BAW rats exposed to repeated social stress reflects a disarrangement in the physiological response to adverse social stimuli as a consequence of withdrawal from adolescent binge-like alcohol exposure. Although we do not rule out the occurrence of similar impairment in older rats, these findings pose a further

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warning towards the early and protracted abuse of alcohol at vulnerable ages, as a relevant cause of susceptibility to psychosocial threats and maladaptive coping. The current data highlight the potential of CBD in attenuating the complex vulnerable phenotype observed and further promote interest in the understanding of its complex pharmacological profile and clinical application in adolescent populations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291722002744.

Data. Data supporting reported results are available on request from the corresponding author.

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Conflict of interest. The authors declare that there are no competing financial interests in relation to the work described.

References

Anderson, A. G., Kulkarni, A., Harper, M., & Konopka, G. (2020). Single-cell analysis of Foxp1-driven mechanisms essential for striatal development. *Cell Reports*, *30*(9), 3051–3066.e7. https://doi.org/10.1016/j.celrep.2020.02.030.

Araujo, D. J., Anderson, A. G., Berto, S., Runnels, W., Harper, M., Ammanuel, S., … Konopka, G. (2015). FoxP1 orchestration of ASD-relevant signaling pathways in the striatum. *Genes & Development*, *29*(20), 2081–2096. https://doi.org/10.1101/gad.267989.115.

Baik, J. H. (2020). Stress and the dopaminergic reward system. *Experimental & Molecular Medicine*, *52*(12), 1879–1890. https://doi.org/10.1038/s12276-020-00532-4.

Bakas, T., van Nieuwenhuijzen, P. S., Devenish, S. O., McGregor, I. S., Arnold, J. C., & Chebib, M. (2017). The direct actions of cannabidiol and 2-arachidonoyl glycerol at $GABA_A$ receptors. *Pharmacological Research*, *119*, 358–370.

Bath, K. G., Russo, S. J., Pleil, K. E., Wohleb, E. S., Duman, R. S., & Radley, J. J. (2017). Circuit and synaptic mechanisms of repeated stress: Perspectives from differing contexts, duration, and development. *Neurobiology of Stress*, *7*, 137–151. https://doi.org/10.1016/j.ynstr.2017.05.001.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., … McGuire, P. K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*(3), 764–774. https://doi.org/10.1038/npp.2009.184.

Blaine, S. K., & Sinha, R. (2017). Alcohol, stress, and glucocorticoids: From risk to dependence and relapse in alcohol use disorders. *Neuropharmacology*, *122*, 136–147. https://doi.org/10.1016/j.neuropharm.2017.01.037.

Boutros, N., Der-Avakian, A., Kesby, J. P., Lee, S., Markou, A., & Semenova, S. (2018). Effects of adolescent alcohol exposure on stress-induced reward deficits, brain CRF, monoamines and glutamate in adult rats. *Psychopharmacology*, *235*(3), 737–747. https://doi.org/10.1007/s00213-017-4789-0.

Brancato, A., Castelli, V., Cavallaro, A., Lavanco, G., Plescia, F., & Cannizzaro, C. (2018). Pre-conceptional and peri-gestational maternal binge alcohol drinking produces inheritance of mood disturbances and alcohol vulnerability in the adolescent offspring. *Frontiers in Psychiatry*, *9*, 150. https://doi.org/10.3389/fpsyt.2018.00150.

Brancato, A., Castelli, V., Lavanco, G., Marino, R., & Cannizzaro, C. (2020). In utero Δ9-tetrahydrocannabinol exposure confers vulnerability towards cognitive impairments and alcohol drinking in the adolescent offspring: Is there a role for neuropeptide Y? *Journal of Psychopharmacology*, *34*(6), 663–679. https://doi.org/10.1177/0269881120916135.

Brancato, A., Castelli, V., Lavanco, G., Tringali, G., Micale, V., Kuchar, M., … Cannizzaro, C. (2021). Binge-like alcohol exposure in adolescence: Behavioural, neuroendocrine and molecular evidence of abnormal neuroplasticity… and return. *Biomedicines*, *9*(9), 1161. https://doi.org/10.3390/biomedicines9091161.

Brancato, A., Plescia, F., Lavanco, G., Cavallaro, A., & Cannizzaro, C. (2016). Continuous and intermittent alcohol free-choice from pre-gestational time to lactation: Focus on drinking trajectories and maternal behavior. *Frontiers in Behavioral Neuroscience*, *10*, 31. https://doi.org/10.3389/fnbeh.2016.00031.

Burke, A. R., & Miczek, K. A. (2014). Stress in adolescence and drugs of abuse in rodent models: Role of dopamine, CRF, and HPA axis. *Psychopharmacology*, *231*(8), 1557–1580. https://doi.org/10.1007/s00213-013-3369-1.

Burke, A. R., & Miczek, K. A. (2015). Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: Role of social experience and adaptive coping behavior. *Psychopharmacology*, *232*(16), 3067–3079. https://doi.org/10.1007/s00213-015-3947-5.

Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimarães, F. S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *367*(1607), 3364–3378. https://doi.org/10.1098/rstb.2011.0389.

Cannizzaro, C., Talani, G., Brancato, A., Mulas, G., Spiga, S., De Luca, M. A., … Diana, M. (2019). Dopamine restores limbic memory loss, dendritic spine structure, and NMDAR-dependent LTD in the nucleus accumbens of alcohol-withdrawn rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *39*(5), 929–943. https://doi.org/10.1523/JNEUROSCI.1377-18.2018.

Castelli, V., Brancato, A., Cavallaro, A., Lavanco, G., & Cannizzaro, C. (2017). Homer2 and alcohol: A mutual interaction. *Frontiers in Psychiatry*, *8*, 268. https://doi.org/10.3389/fpsyt.2017.00268.

Dani, J. A., & Zhou, F. M. (2004). Selective dopamine filter of glutamate striatal afferents. *Neuron*, *42*(4), 522–524. https://doi.org/10.1016/j.neuron.2004.05.008.

Deal, A. L., Konstantopoulos, J. K., Weiner, J. L., & Budygin, E. A. (2018). Exploring the consequences of social defeat stress and intermittent ethanol drinking on dopamine dynamics in the rat nucleus accumbens. *Scientific Reports*, *8*(1), 332. https://doi.org/10.1038/s41598-017-18706-y.

Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., … Riedel, G. (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology*, *219*(3), 859–873. https://doi.org/10.1007/s00213-011-2415-0.

Diaz, V., & Lin, D. (2020). Neural circuits for coping with social defeat. *Current Opinion in Neurobiology*, *60*, 99–107. https://doi.org/10.1016/j.conb.2019.11.016.

Dong, E., Guidotti, A., Zhang, H., & Pandey, S. C. (2018). Prenatal stress leads to chromatin and synaptic remodeling and excessive alcohol intake comorbid with anxiety-like behaviors in adult offspring. *Neuropharmacology*, *140*, 76–85. https://doi.org/10.1016/j.neuropharm.2018.07.010.

Elsaid, S., Kloiber, S., & Le Foll, B. (2019). Effects of cannabidiol (CBD) in neuropsychiatric disorders: A review of pre-clinical and clinical findings. *Progress in Molecular Biology and Translational Science*, *167*, 25–75. https://doi.org/10.1016/bs.pmbts.2019.06.005.

Evans, B. E., Greaves-Lord, K., Euser, A. S., Franken, I. H., & Huizink, A. C. (2012). The relation between hypothalamic–pituitary–adrenal (HPA) axis activity and age of onset of alcohol use. *Addiction (Abingdon, England)*, *107*(2), 312–322. https://doi.org/10.1111/j.1360-0443.2011.03568.x.

Fanselow, M. S., & Lester, L. S. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In R. C. Bolles & M. D. Beecher (Eds.), *Evolution and learning* (pp. 185–212). New York: Lawrence Erlbaum Associates, Inc.

Fasano, C., Bourque, M. J., Lapointe, G., Leo, D., Thibault, D., Haber, M., … Trudeau, LÉ (2013). Dopamine facilitates dendritic spine formation by

cultured striatal medium spiny neurons through both D1 and D2 dopamine receptors. *Neuropharmacology*, *67*, 432–443. https://doi.org/10.1016/j.neuropharm.2012.11.030.

Faure, A., Reynolds, S. M., Richard, J. M., & Berridge, K. C. (2008). Mesolimbic dopamine in desire and dread: Enabling motivation to be generated by localized glutamate disruptions in nucleus accumbens. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(28), 7184–7192. https://doi.org/10.1523/JNEUROSCI.4961-07.2008.

Fortier, C. B., Whitworth, J. W., Fonda, J. R., Currao, A., Beck, B. M., Levin, L., … McGlinchey, R. E. (2021). Early adolescent binge drinking increases risk of psychopathology in post-9/11 veterans and mild traumatic brain injury exacerbates symptom severity. *Alcohol and Alcoholism*, *56*(1), 116–124. https://doi.org/10.1093/alcalc/agaa075.

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330.

Garcia-Oscos, F., Koch, T., Pancholi, H., Trusel, M., Daliparthi, V., Co, M., … Roberts, T. F. (2021). Autism-linked gene FoxP1 selectively regulates the cultural transmission of learned vocalizations. *Science Advances*, *7*(6), eabd2827. https://doi.org/10.1126/sciadv.abd2827.

Guimarães, V. M., Zuardi, A. W., Del Bel, E. A., & Guimarães, F. S. (2004). Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. *Life Sciences*, *75*(5), 633–638. https://doi.org/10.1016/j.lfs.2004.01.015.

Guzowski, J. F., Miyashita, T., Chawla, M. K., Sanderson, J., Maes, L. I., Houston, F. P., … Barnes, C. A. (2006). Recent behavioral history modifies coupling between cell activity and Arc gene transcription in hippocampal CA1 neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(4), 1077–1082. https://doi.org/10.1073/pnas.0505519103.

Halkjelsvik, T., Brunborg, G. S., & Bye, E. K. (2021). Are changes in binge drinking among European adolescents driven by changes in computer gaming? *Drug and Alcohol Review*, *40*(5), 808–816. https://doi.org/10.1111/dar.13226.

Hikida, T., Yawata, S., Yamaguchi, T., Danjo, T., Sasaoka, T., Wang, Y., & Nakanishi, S. (2013). Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(1), 342–347. https://doi.org/10.1073/pnas.1220358110.

Hurd, Y. L., Spriggs, S., Alishayev, J., Winkel, G., Gurgov, K., Kudrich, C., … Salsitz, E. (2019). Cannabidiol for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder: A double-blind randomized placebo-controlled trial. *The American Journal of Psychiatry*, *176*(11), 911–922. https://doi.org/10.1176/appi.ajp.2019.18101191.

Jones, S. A., Lueras, J. M., & Nagel, B. J. (2018). Effects of binge drinking on the developing brain. *Alcohol Research: Current Reviews*, *39*(1), 87–96.

Karkhanis, A. N., Rose, J. H., Huggins, K. N., Konstantopoulos, J. K., & Jones, S. R. (2015). Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens. *Drug and Alcohol Dependence*, *150*, 24–30. https://doi.org/10.1016/j.drugalcdep.2015.01.019.

Kern, C. H., Stanwood, G. D., & Smith, D. R. (2010). Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. *Synapse* (*New York, N.Y.*), *64*(5), 363–378. https://doi.org/10.1002/syn.20736.

Koob, G. F. (2010). The role of CRF and CRF-related peptides in the dark side of addiction. *Brain Research*, *1314*, 3–14. https://doi.org/10.1016/j.brainres.2009.11.008.

Koob, G. F. (2013). Theoretical frameworks and mechanistic aspects of alcohol addiction: Alcohol addiction as a reward deficit disorder. *Current Topics in Behavioral Neurosciences*, *13*, 3–30. https://doi.org/10.1007/7854_2011_129.

Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. (2013). The resident–intruder paradigm: A standardized test for aggression, violence and social stress. *Journal of Visualized Experiments: JoVE* (77), e4367. https://doi.org/10.3791/4367.

Korn, C., Akam, T., Jensen, K., Vagnoni, C., Huber, A., Tunbridge, E. M., & Walton, M. E. (2021). Distinct roles for dopamine clearance mechanisms in regulating behavioral flexibility. *Molecular Psychiatry*, *26*(12), 7188–7199. https://doi.org/10.1038/s41380-021-01194-y.

Kruusmägi, M., Kumar, S., Zelenin, S., Brismar, H., Aperia, A., & Scott, L. (2009). Functional differences between D(1) and D(5) revealed by high resolution imaging on live neurons. *Neuroscience*, *164*(2), 463–469.

Maggio, N., Shavit Stein, E., & Segal, M. (2018). Cannabidiol regulates long term potentiation following status epilepticus: Mediation by calcium stores and serotonin. *Frontiers in Molecular Neuroscience*, *11*, 32. https://doi.org/10.3389/fnmol.2018.00032.

Mastinu, A., Ascrizzi, R., Ribaudo, G., Bonini, S. A., Premoli, M., Aria, F., … Memo, M. (2022). Prosocial effects of nonpsychotropic *Cannabis sativa* in mice. *Cannabis and Cannabinoid Research*, *7*(2), 170–178. https://doi.org/10.1089/can.2021.0017.

Melas, P. A., Scherma, M., Fratta, W., Cifani, C., & Fadda, P. (2021). Cannabidiol as a potential treatment for anxiety and mood disorders: Molecular targets and epigenetic insights from preclinical research. *International Journal of Molecular Sciences*, *22*(4), 1863. https://doi.org/10.3390/ijms22041863.

Nemeškalová, A., Hájková, K., Mikulů, L., Sýkora, D., & Kuchař, M. (2020). Combination of UV and MS/MS detection for the LC analysis of cannabidiol-rich products. *Talanta*, *219*, 121250. https://doi.org/10.1016/j.talanta.2020.121250.

Norris, C., Loureiro, M., Kramar, C., Zunder, J., Renard, J., Rushlow, W., & Laviolette, S. R. (2016). Cannabidiol modulates fear memory formation through interactions with serotonergic transmission in the mesolimbic system. *Neuropsychopharmacology*, *41*(12), 2839–2850. https://doi.org/10.1038/npp.2016.93.

Ostroumov, A., & Dani, J. A. (2018). Convergent neuronal plasticity and metaplasticity mechanisms of stress, nicotine, and alcohol. *Annual Review of Pharmacology and Toxicology*, *58*, 547–566. https://doi.org/10.1146/annurev-pharmtox-010617-052735.

Paillé, V., Picconi, B., Bagetta, V., Ghiglieri, V., Sgobio, C., Di Filippo, M., … Calabresi, P. (2010). Distinct levels of dopamine denervation differentially alter striatal synaptic plasticity and NMDA receptor subunit composition. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*(42), 14182–14193. https://doi.org/10.1523/JNEUROSCI.2149-10.2010.

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates*. New York: Academic Press.

Plescia, F., Cannizzaro, E., Brancato, A., Martines, F., Di Naro, A., Mucia, M., … Cannizzaro, C. (2015). Acetaldehyde effects in the brain. *Acta Medica Mediterranea*, *31*(4), 813–817.

Prybylowski, K., Fu, Z., Losi, G., Hawkins, L. M., Luo, J., Chang, K., … Vicini, S. (2002). Relationship between availability of NMDA receptor subunits and their expression at the synapse. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *22*(20), 8902–8910. https://doi.org/10.1523/JNEUROSCI.22-20-08902.2002.

Reshetnikov, V. V., & Bondar, N. P. (2021). The role of stress-induced changes of Homer1 expression in stress susceptibility. *Biochemistry. Biokhimiia*, *86*(6), 613–626. https://doi.org/10.1134/S0006297921060018.

Retson, T. A., Sterling, R. C., & Van Bockstaele, E. J. (2016). Alcohol-induced dysregulation of stress-related circuitry: The search for novel targets and implications for interventions across the sexes. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *65*, 252–259. https://doi.org/10.1016/j.pnpbp.2015.05.009.

Reynolds, S. M., & Berridge, K. C. (2008). Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. *Nature Neuroscience*, *11*(4), 423–425. https://doi.org/10.1038/nn2061.

Roelofs, K. (2017). Freeze for action: Neurobiological mechanisms in animal and human freezing. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1718), 20160206. https://doi.org/10.1098/rstb.2016.0206.

Rorick, L. M., Finn, P. R., & Steinmetz, J. E. (2003). High-alcohol-drinking rats exhibit persistent freezing responses to discrete cues following Pavlovian fear conditioning. *Pharmacology, Biochemistry, and Behavior*, *76*(2), 223–230. https://doi.org/10.1016/j.pbb.2003.07.001.

Ruffolo, G., Cifelli, P., Roseti, C., Thom, M., van Vliet, E. A., Limatola, C., … Palma, E. (2018). A novel GABAergic dysfunction in human Dravet syndrome. *Epilepsia*, *59*(11), 2106–2117. https://doi.org/10.1111/epi.14574.

https://doi.org/10.1017/S0033291722002744 Published online by Cambridge University Press

Sachser, N., Hennessy, M. B., & Kaiser, S. (2011). Adaptive modulation of behavioural profiles by social stress during early phases of life and adolescence. *Neuroscience and Biobehavioral Reviews*, *35*(7), 1518–1533. https://doi.org/10.1016/j.neubiorev.2010.09.002.

Sales, A. J., Fogaça, M. V., Sartim, A. G., Pereira, V. S., Wegener, G., Guimarães, F. S., & Joca, S. (2019). Cannabidiol induces rapid and sustained antidepressant-like effects through increased BDNF signaling and synaptogenesis in the prefrontal cortex. *Molecular Neurobiology*, *56*(2), 1070–1081. https://doi.org/10.1007/s12035-018-1143-4.

Solomon, M. B. (2017). Evaluating social defeat as a model for psychopathology in adult female rodents. *Journal of Neuroscience Research*, *95*(1-2), 763–776. https://doi.org/10.1002/jnr.23971.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*(4), 417–463. https://doi.org/10.1016/s0149-7634(00)00014-2.

Spiga, S., Talani, G., Mulas, G., Licheri, V., Fois, G. R., Muggironi, G., … Diana, M. (2014). Hampered long-term depression and thin spine loss in the nucleus accumbens of ethanol-dependent rats. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(35), E3745–E3754. https://doi.org/10.1073/pnas.1406768111.

Staples, M. C., Herman, M. A., Lockner, J. W., Avchalumov, Y., Kharidia, K. M., Janda, K. D., … Mandyam, C. D. (2021). Isoxazole-9 reduces enhanced fear responses and retrieval in ethanol-dependent male rats. *Journal of Neuroscience Research*, *99*(11), 3047–3065. https://doi.org/10.1002/jnr.24932.

Tang, J., Yang, C., Shi, M., & Chen, W. (2022). Activation of dopamine D_2 receptors in the shell of nucleus accumbens triggers conditioned avoidance responses in rats. *Behavioural Brain Research*, *422*, 113759. https://doi.org/10.1016/j.bbr.2022.113759.

Thayer, J. F., Hall, M., Sollers, J. J. 3rd, & Fischer, J. E. (2006). Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, *59*(3), 244–250. https://doi.org/10.1016/j.ijpsycho.2005.10.013.

Tringali, G., Greco, M. C., Lisi, L., Pozzoli, G., & Navarra, P. (2012). Cortistatin modulates the expression and release of corticotrophin releasing hormone in rat brain. Comparison with somatostatin and octreotide. *Peptides*, *34*(2), 353–359. https://doi.org/10.1016/j.peptides.2012.02.004.

Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: Routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science: JAALAS*, *50*(5), 600–613.

Varlinskaya, E. I., Truxell, E., & Spear, L. P. (2014). Chronic intermittent ethanol exposure during adolescence: Effects on social behavior and ethanol sensitivity in adulthood. *Alcohol* (*Fayetteville, N.Y.*), *48*(5), 433–444. https://doi.org/10.1016/j.alcohol.2014.01.012.

Viudez-Martínez, A., García-Gutiérrez, M. S., & Manzanares, J. (2018a). Cannabidiol regulates the expression of hypothalamus–pituitary–adrenal axis-related genes in response to acute restraint stress. *Journal of Psychopharmacology*, *32*(12), 1379–1384. https://doi.org/10.1177/0269881118805495.

Viudez-Martínez, A., García-Gutiérrez, M. S., & Manzanares, J. (2020). Gender differences in the effects of cannabidiol on ethanol binge drinking in mice. *Addiction Biology*, *25*(3), e12765. https://doi.org/10.1111/adb.12765.

Viudez-Martínez, A., García-Gutiérrez, M. S., Medrano-Relinque, J., Navarrón, C. M., Navarrete, F., & Manzanares, J. (2019). Cannabidiol does not display drug abuse potential in mice behavior. *Acta Pharmacologica Sinica*, *40*(3), 358–364. https://doi.org/10.1038/s41401-018-0032-8.

Viudez-Martínez, A., García-Gutiérrez, M. S., Navarrón, C. M., Morales-Calero, M. I., Navarrete, F., Torres-Suárez, A. I., & Manzanares, J. (2018b). Cannabidiol reduces ethanol consumption, motivation and relapse in mice. *Addiction Biology*, *23*(1), 154–164. https://doi.org/10.1111/adb.12495.

Yoon, S., Piguel, N. H., Khalatyan, N., Dionisio, L. E., Savas, J. N., & Penzes, P. (2021). Homer1 promotes dendritic spine growth through ankyrin-G and its loss reshapes the synaptic proteome. *Molecular Psychiatry*, *26*(6), 1775–1789. https://doi.org/10.1038/s41380-020-00991-1.

https://doi.org/10.1017/S0033291722002744 Published online by Cambridge University Press