Astrocytic cannabinoid receptor 1 promotes resilience by dampening stress-induced blood-brain barrier alterations

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Article

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Astrocytic cannabinoid receptor 1 promotes resilience by dampening stress-induced blood-brain barrier alterations.

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Abstract

Blood-brain barrier (BBB) alterations contribute to stress vulnerability and development of depressive behaviors. In contrast, neurovascular adaptations underlying stress resilience remain unexplored. Here, we report that high expression of astrocytic cannabinoid receptor 1 (CB1) in the nucleus accumbens (NAc) shell, particularly in the endfeet ensheathing blood vessels, is associated with resilience despite chronic social stress exposure. Viral-mediated overexpression of *Cnr1* in astrocytes of the NAc shell has baseline anxiolytic effects and dampened stress-induced anxiety- and depression-like behaviors. It also reduced astrocyte inflammatory response and morphological changes following an immune challenge with the cytokine interleukin-6, linked to stress susceptibility and mood disorders. At the preventive and therapeutic level, physical exercise and antidepressant treatment increased perivascular astrocytic *Cnr1* in mice. Loss of *CNR1* was confirmed in the NAc astrocytes of depressed individuals. These findings suggest a role for the astrocytic endocannabinoid system in stress responses and possibly, human depression, via BBB modulation.

Introduction

Major depressive disorder (MDD) is a leading cause of disabilities worldwide, with one out of five individuals affected throughout their lifetime[1-3]. MDD's increasing global burden is due to the low efficacy of current treatments, having only 30% success rate[3]. This highlights the need for better understanding of underlying causal biological factors, not addressed with traditional treatments. Chronic stress is the main environmental risk for MDD development, with its social aspect contributing to mood disorder prevalence and suicide attempts in victims of bullying[4]. We previously reported that both chronic social stress and MDD alter blood-brain barrier (BBB) integrity[5-7]. The BBB is a dynamic frontier responsible for regulation of molecular exchange between the periphery and the brain, critical for the maintenance of its homeostasis[8,9]. In mice, chronic social stress induces BBB disruption promoting depression-like behaviors, highlighting a link between neurovascular health and stress vulnerability[5-7,10]. Accordingly, mice resilient to stress exhibit molecular adaptations favoring BBB integrity, possibly contributing to proper coping strategies[6,7].

The endocannabinoid system (ECS) is a crucial regulator of stress responses, and its disruption is associated with depressive behaviors in both clinical and preclinical studies[11-13]. Cannabinoid receptor 1 (CB1, encoded by *Cnr1*) is the main ECS effector in the brain and CB1 downstream signaling has been implicated in stress resilience[12,14]. Astrocyte end-feet establish the link between endothelial cells and neurons, enabling neurovascular communication, crucial to BBB function[15]. Intriguingly, coverage of blood vessels by astrocyte end-feet interaction is reduced in postmortem brain samples from individuals with MDD[16]. Function of CB1 on the astrocyte membrane close to synaptic terminals has been widely investigated[17] and a recent study implicated mitochondrial CB1 in the regulation of glucose metabolism and behaviors[18]. However, perivascular astrocytic CB1 remain understudied[19] despite perfect positioning to modulate BBB properties during stress exposure and in mood disorders, or other conditions like vascular and neurodegenerative diseases, which are characterized by comorbidity with depressive symptoms and BBB alterations[1].

To address this knowledge gap, we first evaluated astrocytic *Cnr1* expression in the nucleus accumbens (NAc) and prefrontal cortex (PFC) of mice subjected to the chronic social defeat stress paradigm (CSDS), a mouse model of depression[20], with loss of BBB integrity being observed in these brain areas after chronic stress exposure[5,7]. The NAc is a forebrain nucleus playing key roles in reward and mood regulation while the PFC is involved in social behaviors, executive function and decision making[21]. Next, viral-mediated functional experiments were performed to manipulate expression of *Cnr1* in astrocytes and the impact thereof on anxiety- and depression-like behaviors was assessed. Morphological analysis of perivascular astrocytic CB1 with super-resolution microscopy was combined with cell-specific transcriptomic analysis and *in vitro* experiments to gain mechanistic insights on EC alterations underlying stress vulnerability vs resilience. Prevention and treatment of mood disorders remain a major challenge in psychiatry. Both physical exercise and positive antidepressant treatment response were associated with increased NAc *Cnr1* expression in mice. Translational value was validated on postmortem brain samples from individuals with MDD. This study is the first, to our knowledge, to link perivascular EC signaling at astrocytic CB1 with stress resilience and mood disorders.

Results

Increased astrocytic CB1 expression in the nucleus accumbens shell is associated with stress resilience.

CSDS induces a depression-like phenotype in a stress-susceptible (SS) subpopulation of mice, mimicking MDD symptoms such as social avoidance, anhedonia, and anxiety[20]. An advantage of this paradigm is the generation of a subpopulation of animals with behaviors comparable to unstressed controls (CTRL) and defined as resilient (RES) despite exposure to the same stressor, allowing to investigate the biology underlying resilience along with stress vulnerability. In the CSDS protocol, a male C57BL/6 mouse is subjected to 5 minutes of physical bouts of social defeat by a CD-1 mouse aggressor for a duration of 10 days (**Fig.1A**). Behavioral phenotype is assessed with the social interaction (SI) test performed 24 h after the last exposure to social stress (**Fig.1A**). Mice that display social avoidance are considered SS as opposed to RES animals with intact social behaviors (**Fig.1B**, **Supp.Fig.1A**). CSDS alters BBB integrity promoting depression-like behaviors in SS mice but the mechanism underlying neurovascular adaptations associated with stress resilience has yet to be determined. Considering its key role as mediator of stress responses[11-13], we first evaluated individual differences in the ECS potentially underlying stress responses in the NAc and PFC of SS vs RES males (**Fig.1C-D**). Indeed, we showed that chronic stress alters BBB integrity in a sex-specific manner with the BBB being more vulnerable in the female PFC[7] vs NAc for males[5]. An unbiased transcriptional profiling of >40 ECS targets with a Taqman array revealed increased *Cnr1* gene expression in the NAc of stressed males (**Fig.1D**), confirmed in RES only with more sensitive quantitative PCR analysis (**Fig.1C**). Notably, *Cnr1* mRNA levels positively correlated with social interactions (***p=0.0002) (**Fig.1C**). The changes were NAc specific, with no regulation observed in the PFC (**Fig 1D**, **Supp.Fig.1B**) in line with intact BBB integrity[5].

To identify whether RES-associated upregulation of NAc *Cnr1* is occurring in neurons or astrocytes, immunofluorescence analysis was performed with the neuronal marker microtubule-associated protein 2 (Map2) and S100 calcium-binding protein β (S100β), which is enriched in perivascular astrocytes[22]. In the NAc of male mice subjected to CSDS (**Supp.Fig.1C**) 71% of the CB1 protein signal colocalized with neurons and 23% with astrocytes (**Fig.1F**). The NAc is differentiated into at least two anatomically and functionally distinct regions[23,24]. The NAc shell has been implicated in the control of reward-seeking behavior by spatial/contextual information, whereas the core appears to be involved in learning and action during goal-directed behavior[25,26]. The CB1 increase was observed in S100β positive cells in the NAc shell of RES animals when compared to CTRL and SS animals, and significantly correlated with social interactions in stressed mice (**p=0.0012) (**Fig.1G-H**). CSDS led to an increase in CB1 in the NAc core neurons, but this was not correlated with behavioral outcome (**Fig.1I-J**). Altogether, these findings suggest that chronic stress resilience could be linked with NAc shell astrocyte specific CB1 upregulation.

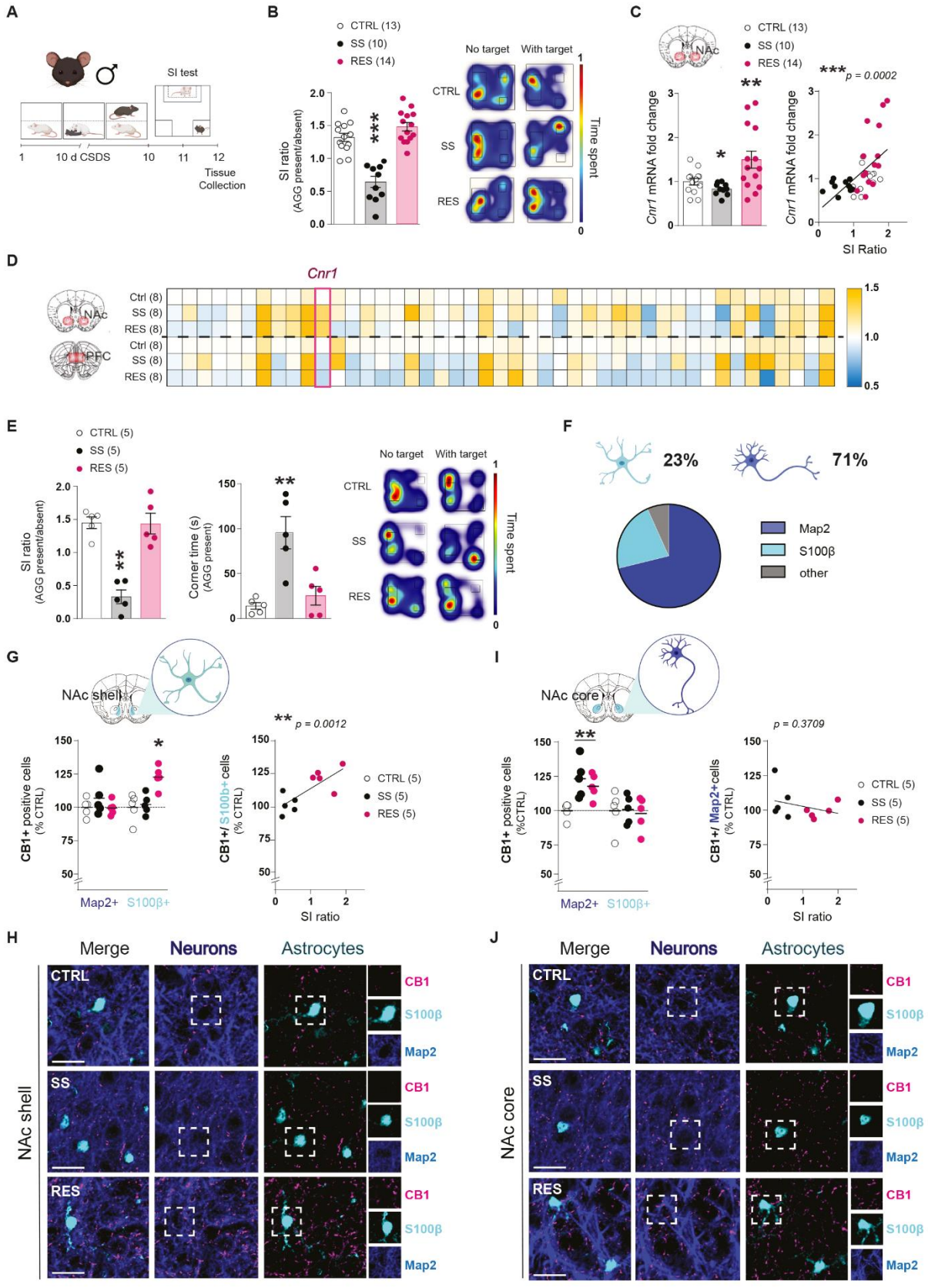
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Figure 1. Increased astrocytic CB1 expression in the nucleus accumbens shell is associated with stress resilience. **A**, Experimental timeline of 10-day chronic social defeat stress (CSDS), social interaction (SI) and tissue collection. **B**, Individual SI values (left) and representative heatmaps (right) of normalized time spent in the interaction zone during SI test for male CSDS. **C**, Quantitative PCR revealed upregulation of *Cannabinoid receptor 1* (*Cnr1*) gene expression in the nucleus accumbens (NAc) of stress resilient (RES) male mice when compared to unstressed control (CTRL) and susceptible (SS) animals. **D**, Endocannabinoid system Taqman array revealed higher *Cnr1* gene expression in stressed mice vs CTRL in the NAc but not prefrontal cortex (PFC), the range of color indicates individual differences within a group with yellow indicating increased expression and blue decreased as compared to CTRL. **E**, Individual SI values (left) and corner time (middle) with representative heatmaps (left). **F**, Cell type percentage colocalizing with CB1 protein detected by immunofluorescence. **G**, Increased number of S100β positive cells expressing CB1-encoded protein in RES males as compared to SS and CTRL in the NAc shell, but not the NAc core (**I**). Representative images of CB1, S100β and Map2 immunohistochemistry in the NAc after social defeat stress in shell (**H)** and core (**J**). Scale bars, 50 µm. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. One-way ANOVA or Brown-Forsythe ANOVA test followed by Holm-Šídák's or Tuckey's multiple comparison test was applied. For n lower than 8, Kruskal-Wallis test followed by Dunn's multiple comparisons evaluation was used; correlations were evaluated with Pearson's correlation coefficient; ***p≤0.001, **p≤0.01, *p≤0.05.

Astrocyte-specific increase of *Cnr1* expression in the NAc shell has anxiolytic effects.

Involvement of NAc astrocytic CB1 in the regulation of anxiety- and depression-like behaviors has yet to be investigated despite a key role for this brain area in emotion regulation and mood disorders[21]. Thus, we designed an adeno-associated virus (AAV) vector driving *Cnr1* expression in GFAP+ astrocytes that was first validated both *in vitro* and *in vivo* (**Fig.2A-B**, **Supp.Fig.2A-D**). This approach allows upregulation of *Cnr1* expression in a region- and cell-specific manner. Stereotactic injection of the AAV-GFAP-*Cnr1* in the NAc shell of naive male mice increased *Cnr1* expression in *GFAP* positive astrocytes when compared to a control AAV-GFAP-sham virus (**Fig.2A-B**). The NAc shell was targeted because stress resilience increases CB1 expression in this brain area (**Fig.1G-H**). Next, bilateral injection with either the AAV-GFAP-*Cnr1* or AAV-GFAP-sham virus was performed in the NAc shell on other cohorts of mice and a battery of behavioral tests was conducted 3 weeks later when viral expression is optimal (**Fig.2A, B**). Overexpression of astrocytic *Cnr1* led to a reduction of anxiety-related behaviors as indicated by increased time and number of entries in the center of the open field (OF) test arena when compared to AAV-GFAP-sham-injected controls (*p=0.0186) (**Fig.2E**, **Supp.Fig.2G**). In the elevated plus maze test (EPM), AAV-GFAP-*Cnr1*-injected mice did not spend more time in the open arms but entered them more often than their sham-injected counterparts (**Fig.2D**, **Supp.Fig.2F**). On the other hand, upregulation of *Cnr1* expression did not affect baseline social behaviors in the SI test (**Fig.2C**, **Supp.Fig.2E**). As for the splash test (ST), mice injected with AAV-GFAP-*Cnr1* in the NAc shell started grooming sooner vs the AAV-GFAP-sham controls despite being exposed to a new environment, suggesting decreased anxiety (*p=0.0500) (**Fig.2F**). Importantly, AAV-GFAP-*Cnr1* animals spending more time in the OF center also groomed earlier, linking anxiolytic effects across different behavioral domains (*p=0.0383) (**Fig.2H**). As for other depression-like behaviors, no effect was noted in the tail suspension test (TST) and forced swim test (FST) (**Fig.2G**). However, low basal anxiety correlated with reduced anhedonia in the sucrose preference test (SPT) and immobility in the FST (*p=0.0179) (**Fig.2H**). Taken together these results suggest that an increase in astrocytic *Cnr1* in the NAc shell has anxiolytic effects, even in unstressed animals. To our knowledge, this is the first evidence supporting a role for astrocytic *Cnr1* role in regulating anxiety-like behaviors.

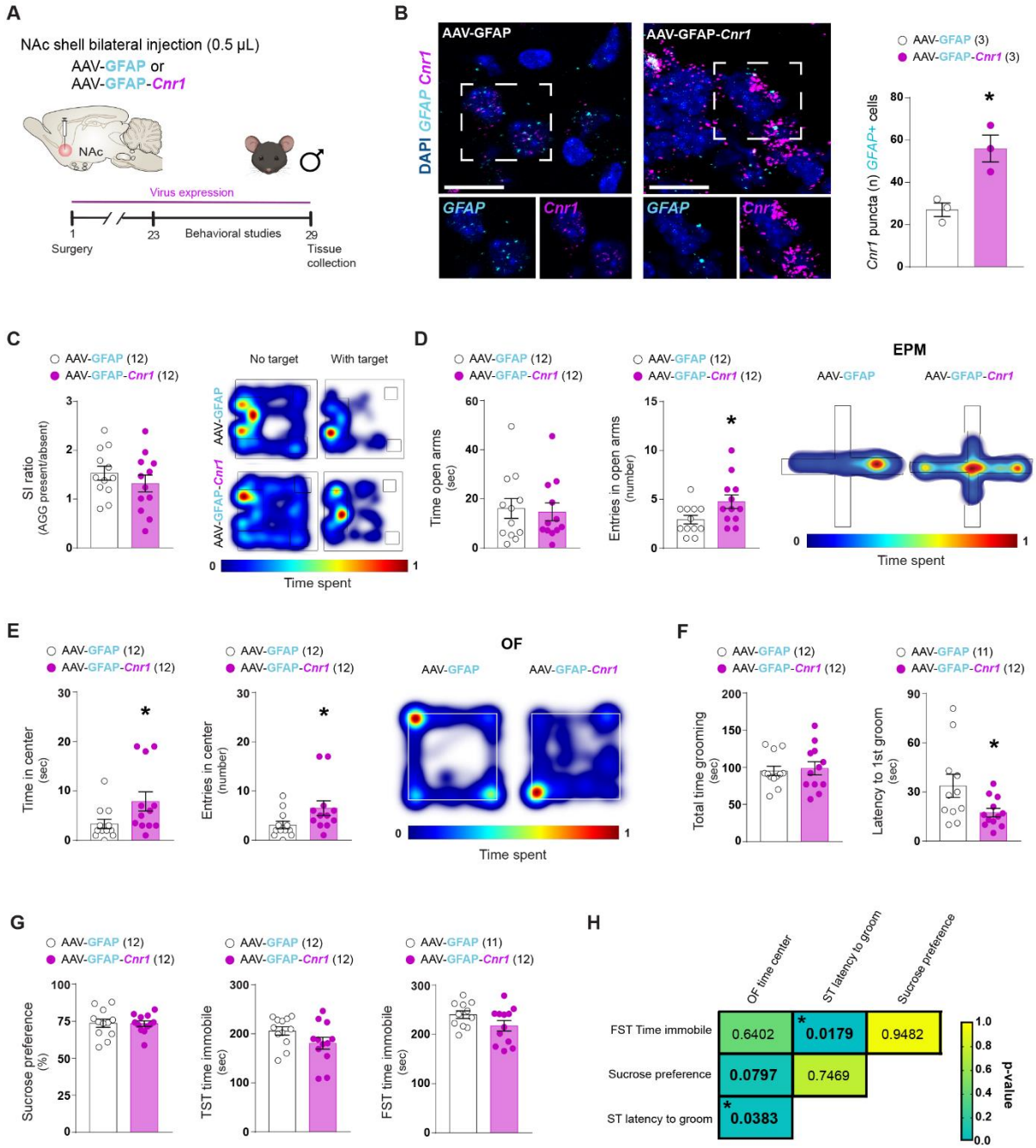


Figure 2. Overexpression of astrocytic *Cnr1* in the nucleus accumbens shell has anxiolytic effects but does not alter depression-like behaviors. A, Experimental timeline of nucleus accumbens (NAc) bilateral injection of AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses and following behavioral studies. **B**, Validation of AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses with RNA scope confirmed upregulation of *Cannabinoid receptor 1* (*Cnr1)* mRNA levels in AAV-GFAP-*Cnr1* infected astrocytes when compared to AAV-GFAP-sham-injected mice. RNA scope representative images of AAV-GFAP-sham (left) and AAV-GFAP-*Cnr1* (right) viruses, Scale bars, 20 µm. **C,** Viral manipulation does not alter social behaviors as measured with the social interaction (SI) test. Individual SI values (left) and representative heatmaps (right) of normalized time spent in the interaction zone during SI test. **D,** Bilateral injection with AAV-GFAP-*Cnr1* has anxiolytic effect in the elevated plus maze (EPM) as animals enter more times the open arms as compared to AAV-GFAP-sham injected animals (left). Representative heatmaps of time spent in the open arms during EPM for animals injected with AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses (right). **E,** Similarly,

following bilateral injection with AAV-GFAP-*Cnr1* mice spend more time in the center of the arena open field (OF) test when compared to AAV-GFAP-sham injected animals (left). Representative heatmaps of normalized time spent in the center during OF test for animals injected with AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses (right). **F,** Despite comparable latency of grooming behavior during the splash test (ST) (left), mice injected with the AAV-GFAP-*Cnr1* virus starts grooming sooner in a new environment (right), indicating decreased anxiety. **G,** No difference was observed in anhedonia (sucrose preference test, left) and other depression-like behaviors (tail suspension test, TST, middle and forced swim test, FST, right) following viral manipulation. **H,** Intra-individual correlation of different behavioral data points reveals correlations between anxiety and motivated behaviors. P values in the boxes refer to the strength of the correlation between behaviors. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. Two-tailed t-test or Mann-Whitney U test was applied; correlations were evaluated with Pearson's correlation coefficient; *p≤0.05.

Astrocyte-specific increase of *Cnr1* expression in the NAc shell promotes resilience following chronic social stress exposure.

Cnr1$^{-/-}$-deficient mice are highly sensitive to stress with increased mortality rate, altered hypothalamic-pituitary-adrenal axis activation and exacerbated microglia responses in the PFC, hippocampus, and amygdala[27]. Transgenic mice lacking *Cnr1* on specific cortical neuronal subpopulations are also more sensitive to the behavioral consequences of social stress exposure[28]. However, it is undetermined if *Cnr1* in the NAc astrocytes contributes to stress responses. Due to higher expression of *Cnr1* observed in the NAc shell of resilient mice (**Fig.1G-H**), we explored this hypothesis by injecting male mice with AAV-GFAP-*Cnr1* or the control AAV-GFAP-sham virus, followed by the 10-day CSDS paradigm 3 weeks later and then a battery of behavioral tests (**Fig.3A**). The detrimental effect of CSDS on social interactions was blunted in the AAV-GFAP-*Cnr1* group. Indeed, AAV-GFAP-sham-injected animals displayed social avoidance with less time spent in the interaction zone when the target is present when compared to unstressed AAV-GFAP-sham controls (stress effect: *p=0.0180), a phenomenon not observed in AAV-GFAP-*Cnr1* mice (**Fig.3B**, **Supp.Fig.3A**). Cell-specific expression of *Cnr1* in GFAP+ astrocytes was again validated with RNA scope *in vivo* (**Fig.3C**). Increasing *Cnr1* expression in the NAc shell astrocytes had anxiolytic effect not only in unstressed controls, confirming our previous observations (**Fig.2**), but also in animals subjected to CSDS (**Fig.3D-E**, **Supp.Fig.3B-C**). Mice injected with the AAV-GFAP-*Cnr1* in the NAc shell display decreased anxiety in EPM and OF tests when compared to AAV-GFAP-sham animals (EPM: virus effect *p=0.04, OF: virus effect **p=0.0089) (**Fig.3D-E**). Similarly, AAV-GFAP-*Cnr1* injected mice exhibited decreased latency to groom in ST test (virus effect *p=0.0496) (**Fig.3F**). Chronic stress induces grooming perturbation in the ST due to a decrease in motivated behaviors and hedonic inclination[29] and upregulation of astrocytic *Cnr1* in the NAc shell appears to protect against these behavioral deficits (**Fig.3F**). AAV-GFAP-*Cnr1*-injected mice were characterized by overall increased grooming behavior vs AAV-GFAP-sham animals following CSDS (virus effect: *p=0.0145) (**Fig.3F**). As for other depression-like behaviors induced by 10-day CSDS, no difference was observed for anhedonia or in the TST test (**Fig.3G**). However, higher mobility in the FST was observed in mice expressing AAV-GFAP-*Cnr1* in the NAc shell astrocytes when compared to animals injected with the AAV-GFAP-sham virus (virus effect: ****p<0.0001) (**Fig.3G**). Overall, these findings indicate that upregulation of *Cnr1* in the NAc shell astrocytes could represent a positive biological adaptation contributing to resilience in the context of chronic social stress exposure.

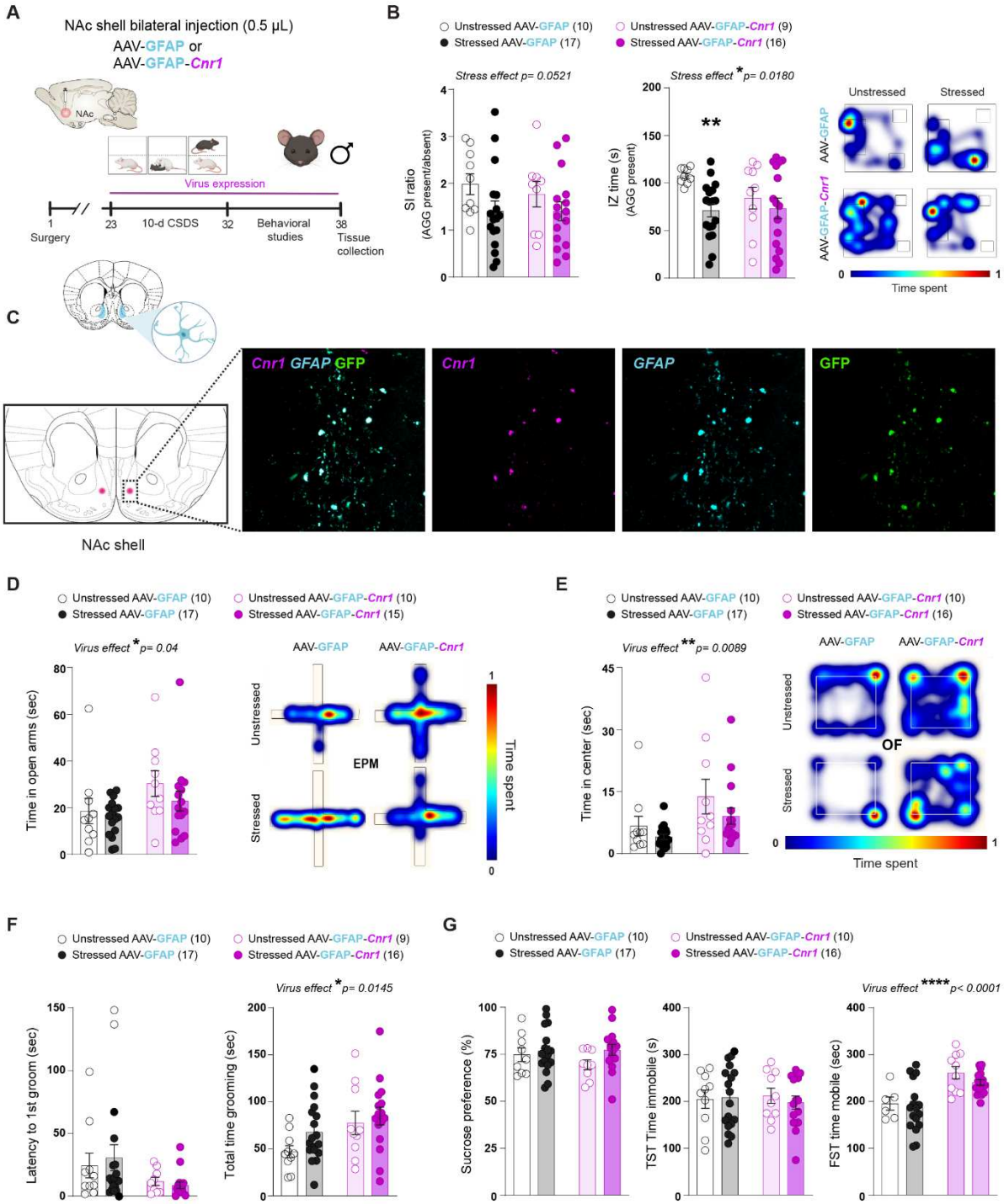


Figure 3. Overexpression of astrocytic *Cnr1* in the nucleus accumbens dampens anxiety- and depression-like behaviors induced by chronic social stress exposure. A, Experimental timeline of nucleus accumbens (NAc) bilateral injection of AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses followed by 10-day chronic social defeat stress (CSDS) and behavioral studies. **B**, Bilateral AAV-GFAP-*Cnr1*, but not AAV-GFAP-sham injection in the NAc shell prevents social deficits induced by CSDS in males as depicted by individual social interaction (SI) test values (left), time in the interaction zone (middle) and representative heatmaps (right). **C**, RNA scope *in vivo* validation of AAV-GFAP-*Cnr1* expression in the NAc shell. **D**, Following bilateral injection with AAV-GFAP-*Cnr1*, mice spend more time in the elevated plus maze (EPM) open arms as compared to AAV-GFAP-sham injected animals (left).

Representative heatmaps of normalized time spent in the open arms during EPM test for animals injected with AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses after CSDS (right). **E**, Decreased anxiety in animals treated with AAV-GFAP-*Cnr1* as measured by increased time spent in the center during the open field (OF) test (left) as compared to sham animals. Representative heatmaps of normalized time spent in OF arena for animals injected with AAV-GFAP-*Cnr1* or AAV-GFAP-sham viruses following CSDS (right). **F**, Bilateral AAV-GFAP-*Cnr1* injection in the NAc shell does not affect latency to start grooming (left) but increases grooming time in the splash test (ST) (right) as compared to AAV-GFAP-sham suggesting positive effect on motivated behaviors. **G**, Bilateral AAV-GFAP-*Cnr1* injection in the NAc shell does not affect anhedonia as evaluated by the sucrose preference test (left) but increases mobility in the forced swim test (FST) (right) as compared to AAV-GFAP-sham. No effects of stress or viral manipulations were noted for tail suspension (TST, middle). Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. Two-way ANOVA or Non-parametric Two-way ANOVA on Ranks followed by Holm-Šídák's or Wilcox multiple comparison test was applied; ****p< 0.0001, *p≤0.05.

Increased CB1 expression at the BBB interface in the NAc shell is associated with stress resilience.

Stress-induced BBB hyperpermeability mediated by loss of tight junction protein Cldn5 allowing passage of deleterious peripheral inflammatory mediators into the NAc is observed in SS, but not RES, animals[5,6] suggesting that protective neurovascular adaptations are present. Endocannabinoids can modulate BBB permeability *in vitro*[30] and have beneficial effects in the neuroinflammatory context of stroke[31] or traumatic brain injury[32], including via upregulation of Cldn5 expression. Astrocytic CB1 receptors are well positioned to react to stress-associated alterations in BBB endothelial cells. Thus, we evaluated if *Cldn5* loss in the male NAc affects expression of *Cnr1*/CB1 in astrocytes using a doxycycline-inducible Cldn5-targeting shRNA (AAV-shRNA-*Cldn5*)[5]. We chose this approach since *Cldn5*-deficient mice die within 10h of birth[33] and it allows downregulation of *Cldn5* in a region- and cell-specific manner, with this tight junction being expressed only in endothelial cells[34], leading to functional deficits with leakage of circulating dyes or proteins into the brain[5,35]. A cohort of mice was injected in the NAc shell with either the AAV-shRNA-Cldn5 or AAV-shRNA (control) virus (**Fig.4A**). Downregulation of Cldn5 expression resulted in a compensatory increase in *Cnr1* and astrocytic end-feet-related *aquaporin 4 (Aqp4)* expression when compared to AAV-shRNA-injected animals (**Fig.4B**, **Supp.Fig.4A-B**). Aqp4 is a water channel involved in BBB transport but also polarization of astrocyte end-feet[16]. Coverage of blood vessels by Aqp4+ astrocyte end-feet is reduced by 50% in PFC samples from individuals with MDD[16], supporting BBB alterations in this mood disorder. Intriguingly, *Cnr1* expression is enriched in perivascular astrocytes[36]. Morphological analysis of confocal microscopy images performed with the Imaris software revealed that, in AAV-shRNA-Cldn5-injected mice, high astrocyte Aqp4+ end-feet and endothelial CD31+ volume overlap is correlated with elevated level of perivascular CB1 proteins, suggesting an involvement in maintenance of BBB integrity (*p=0.0356) (**Fig.4C-D**).

Next, whether perivascular CB1 is linked or not to the resilient phenotype following CSDS was evaluated. Exposure to 10-day chronic social stress decreased *Aqp4* in the NAc of SS but not RES mice (**Fig.4E-G**). Importantly, a positive correlation between *Cldn5* and *Aqp4* expression was noted in the male NAc following CSDS (***p=0.0001) (**Fig.4G**). Decreased Aqp4 was reported in the cortex and hippocampus of rodents exposed to chronic unpredictable stress[37] or inflammation[38], but to our knowledge this is the first report of a similar effect of CSDS in the NAc. Aqp4+ end-feet and endothelial CD31+ volume overlap is reduced in the SS group when compared to unstressed controls and RES mice without reaching significance (**Fig.4H-J**, **Supp.Fig.4C-E**). However, a decrease in vasculature volume, as measured with endothelial CD31

immunostaining, was noted in SS animals (**Fig.4K**), while in the NAc of RES mice CB1/CD31 colocalization was increased when compared to both SS and unstressed control group and correlated with the level of social interactions (**p=0.0012) (**Fig.4L-M**). This observation was confirmed using super-resolution microscopy (**Fig. 4N**, **Supp.Fig.4F**). Altogether, these results suggest that astrocytic Cnr1 in the NAc dampens stress-induced neurovascular alterations promoting resilience.

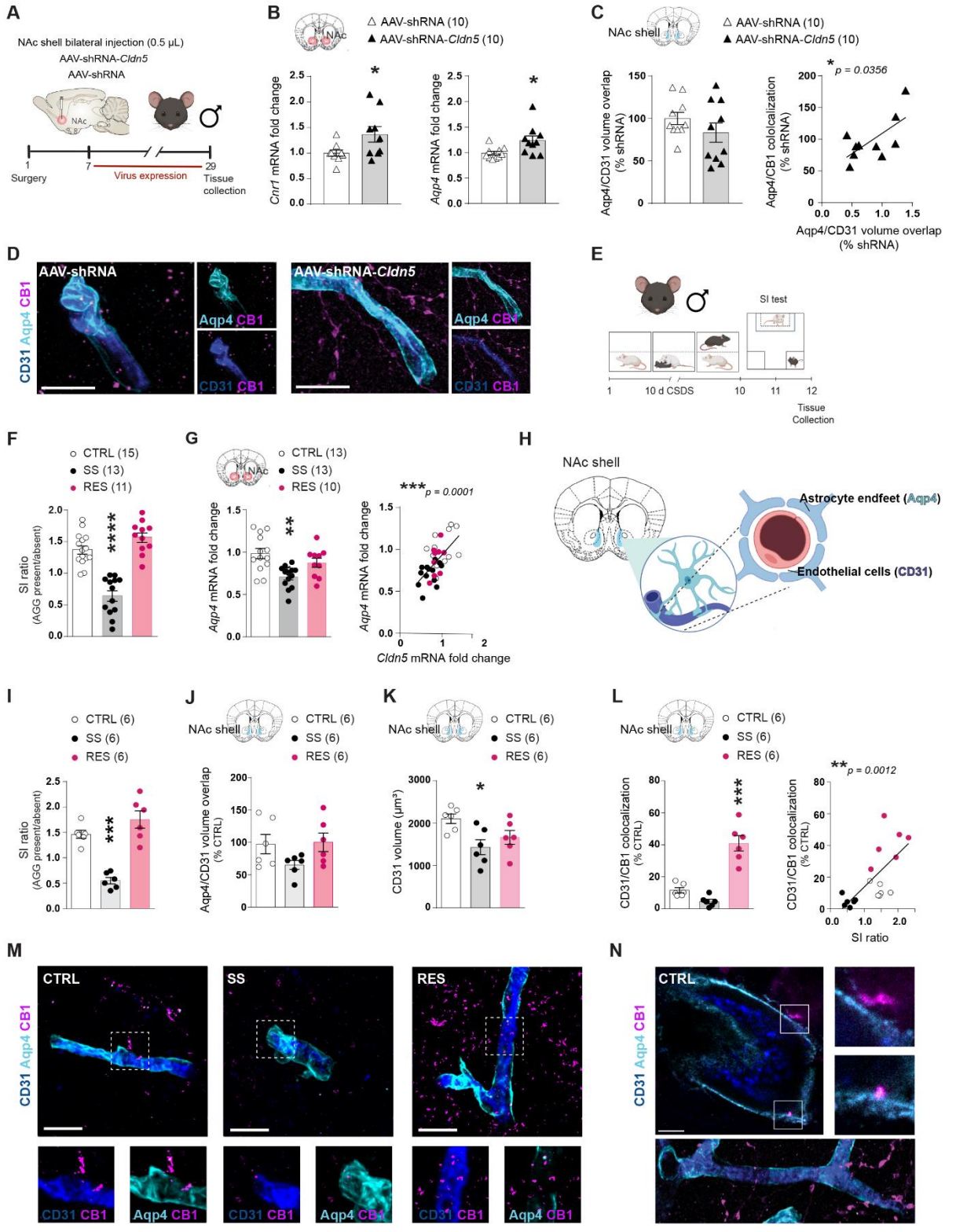


Figure 4. High perivascular CB1 expression is associated with stress resilience following CSDS. A, Experimental timeline of nucleus accumbens (NAc) bilateral virus injection of AAV-shRNA sham or AAV-shRNA-*Cldn5*, decreasing endothelial tight junction Claudin-5 (*Cldn5*) gene expression. **B**, Quantitative PCR revealed an increase in

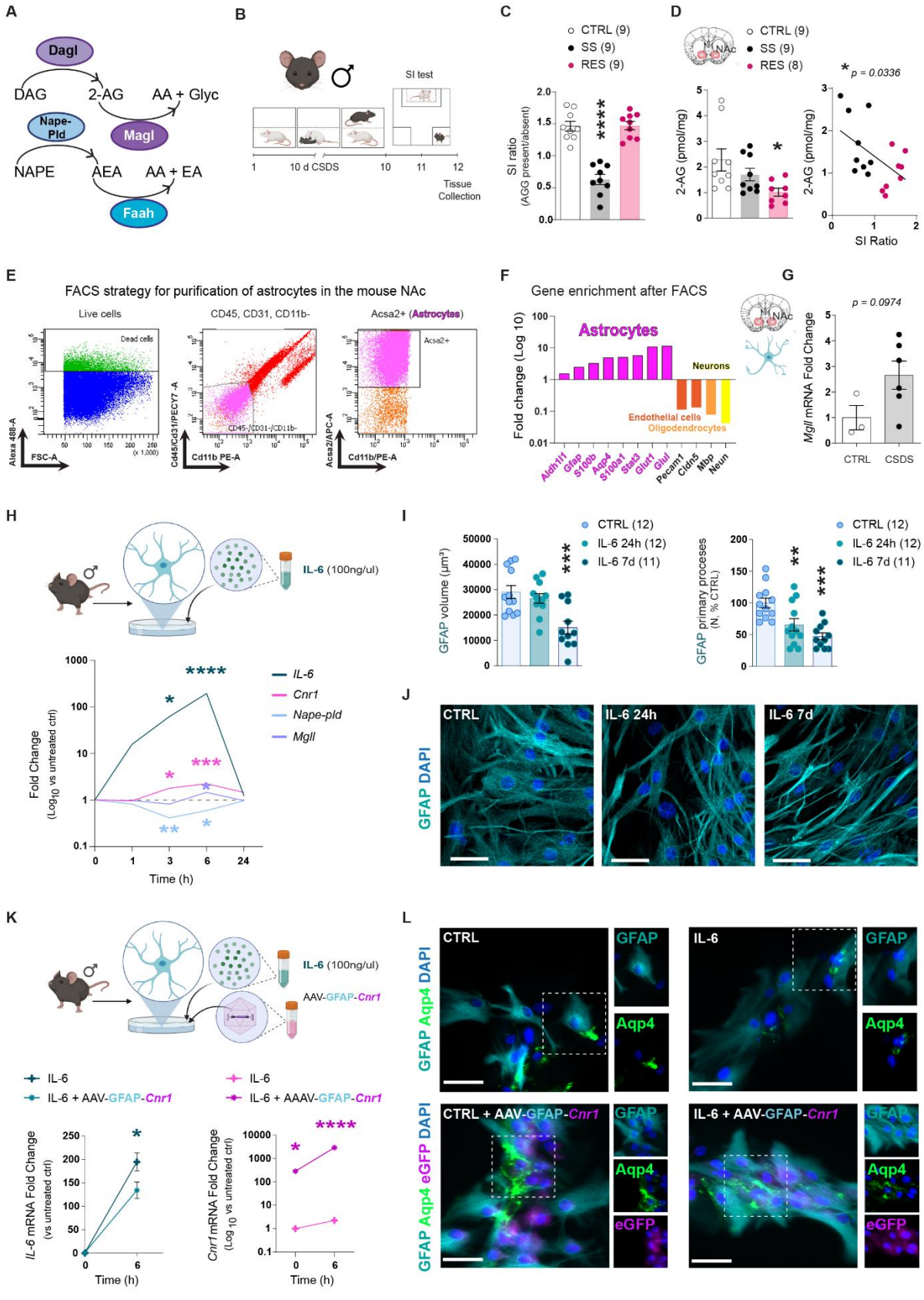
mRNA levels of *Cannabinoid receptor 1* (*Cnr1*, left) and astrocytic end-feet marker *Aquaporin-4* (*Aqp4*, right) in animals with BBB impairment induced by AAV-shRNA-*Cldn5* injection. **C**, Bilateral injection with AAV-shRNA-*Cldn5* in absence of stress does not alter astrocytic end-feet coverage of NAc shell vessels (left). Astrocyte end-feet expressing CB1 are more efficient in covering vessels in AAV-shRNA-*Cldn5* injected animals (right). **D**, Representative images of astrocytic end-feet coverage of NAc shell vessels in AAV-shRNA (left) and AAV-shRNA-*Cldn5*-injected animals (right). Scale bars 10 µm. **E**, Experimental timeline of 10-day chronic social defeat stress (CSDS), social interaction (SI) and tissue collection. **F**, Individual SI values after 10-day CSDS exposure. **G**, Quantitative PCR revealed significant decrease of mRNA levels for *Aqp4* gene in stress susceptible (SS) male mice when compared to unstressed control (CTRL) and resilient (RES) animals (left) which correlates with *Cldn5* stress-induced expression changes (right). **H**, Schematic of astrocytic end-feet and endothelial cells. **I**, Individual SI values for male CSDS. **J**, Astrocytic end-feet coverage of NAc shell vessels is lower following CSDS for SS mice without reaching significance. **K**, CSDS induces decreased vessel volume in SS mice not observed in RES and CTRL. **L**, RES mice are characterized by increased number of endothelial cells colocalizing with CB1 in the NAc shell as compared to SS and CTRL animals (left), and it correlates with social interactions (right). **M**, Representative images of CB1, Aqp4 and CD31 immunohistochemistry in the NAc shell after social defeat stress, scale bars, 20 µm, **N**, Stimulated emission depletion (STED) representative image of CB1, Aqp4 and Cd31 immunohistochemistry in the NAc shell of CTRL mice. Representative images intensity was adjusted with log scale, Scale bars, 2 µm. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. For 2 groups analysis two-tailed t-test was applied. In the case of 3 groups one-way ANOVA followed by Holm-Šídák's multiple comparison test was applied. For n lower than 8, a Kruskal-Wallis test followed by Dunn's multiple comparisons evaluation was used; ****p≤ 0.0001, ***p≤ 0.001, **p≤ 0.01, *p≤ 0.05.

The NAc astrocytic endocannabinoidome is modulated by stress exposure and associated inflammation.

Stress-induced alterations in CB1-related signaling modulates neuronal synaptic plasticity in the NAc leading to behavioral adaptations[12,39]. Contribution of astrocytic CB1-related signaling in this brain region and behaviors is undetermined, thus, we next aim to gain mechanistic insights. Acting on CB1 with different affinity are two main ECs: 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine or anandamide (AEA)[40] (**Fig.5A**). Basal brain levels of 2-AG are ~200-fold higher than those of AEA[41]. Mice were subjected to 10-day CSDS then the NAc was dissected from CTRL, SS and RES animals and 2-AG and AEA levels assessed using high-pressure liquid chromatography with mass spectrometry (HPLC-MS/MS) (**Fig.5B**). While no difference was noted for AEA (**Supp.Fig.5B**), 2-AG changes were linked with resilience (*p=0.0307) and correlated with social interactions following stress exposure (*p=0.0336) (**Fig.5C-D**). Astrocytes altogether with neurons regulate 2-AG content and endocannabinoid-dependent signalling[42] but astrocyte specific implication in the NAc stress responses remains unknown. AEA biosynthesis primarily rely on N-acylphosphatidylethanolamine (Nape)-specific phospholipase D-like hydrolase (Nape-Pld) whereas fatty acid amide hydrolase (Faah) is the main enzyme responsible for AEA hydrolysis[43,44]. As for 2-AG, diacylglycerol lipase α (Daglα) is the primary enzyme synthesizing it in both neurons and astrocytes[45]. Finally, 2-AG is primarily hydrolysed by monoacylglycerol lipase (Magl)[43] (**Fig.5A**). 10-day CSDS increased NAc expression of *Dagla* (**p=0.0018) and *Nape-Pld* (**p=0.0095) (**Supp.Fig.5C**) suggesting compensatory mechanisms in this brain area. We next assessed if stress alters endocannabinoid enzyme function in NAc astrocytes by taking advantage of fluorescence-activated cell sorting (FACS) for region- and cell-specific isolation following CSDS (**Fig.5E-F**, **Supp.Fig.5D**). CSDS seems to elevate astrocytic *Mgll* and *Dagla* expression in stressed mice while decreasing *Nape-Pld* (**Fig.5G**, **Supp.Fig.5F-G**). Due to technical limitations to characterize endocannabinoid signaling in a cell- and region-

specific manner with sufficient power samples for individual animals, we chose to complement our *in vivo* studies with *in vitro* experiments.

Reduced BBB integrity in the NAc of SS mice is associated with depression-like behaviors but also passage of peripheral interleukin-6 (IL-6)[5]. This proinflammatory cytokine has been linked to stress-related disorders and is elevated in the blood of individuals with MDD particularly those resistant to treatment[46,47]. Perivascular astrocytic CB1 receptors are well positioned to sense and react to stress-associated circulating inflammation possibly contributing to neurovascular adaptations leading to vulnerability vs resilience. To evaluate if CB1-dependent endocannabinoid signaling is altered by IL-6 in this cell population, mouse astrocytes were isolated and cultured *in vitro*[48] (**Fig.5H**, **Supp.Fig.5H-I**). Acute treatment with IL-6 (100ng/ul) led to an increase in astrocytic *IL-6* expression (3h: **p=0.0092; 6h: ****p<0.0001) in parallel with *Cnr1* (3h: *p=0.0173; 6h: ***p=0.0008). Conversely, *Nape-Pld* decreased at the same points (3h: **p=0.0039; 6h: *p=0.0361) with higher *Mgll* expression observed only after 6h (*p=0.0123) (**Fig.5H**). This is well in line with the changes observed following CSDS and reported above (**Fig.5G**, **Supp.Fig.5**). Importantly, alterations were not present anymore after 24h highlighting the dynamic relationship between the ECS and IL-6. Considering the chronic nature of our stress paradigm, cultured astrocytes were next treated for a longer period. Exposure to IL-6 (100ng/ul) for 7 days promoted morphological changes with a loss in GFAP volume (***p=0.0005) (**Fig.5I-J**). In fact, treatment with IL-6 for 24h was already sufficient to reduce astrocyte primary processes (**p=0.0083), which could be linked to the loss of astrocyte endfeet coverage reported in MDD[16]. Since overexpression of astrocytic *Cnr1* prevented stress-induced anxiety- and depression-like behaviors (**Fig.3**), we tested if it could dampen IL-6-associated changes in astrocytes. Astrocytes were transfected with either the AAV-GFAP-sham or AAV-GFAP-*Crn1* virus prior treatment with IL-6 (**Fig.5K**). High astrocytic *Cnr1* expression reduced IL-6-driven increase in *IL-6* (virus x treatment effect *p=0.0480) along with the expected elevation of IL-6-driven *Cnr1* production (virus effect: ****p<0.0001; virus x treatment effect ****p<0.0001) (**Fig.5K**, **Supp.Fig.5K-L**). It also prevented Aqp4 internalization caused by IL-6 treatment (**Fig.5L**) providing a mechanistic link between *Cnr1* in astrocytes, stress-induced inflammation, BBB alterations and behavioral responses.


A
Dagl
DAG
2-AG
AA + Glyc
Nape-Pld
Magl
NAPE
AEA
AA + EA
Faah
B
SI test
1
10 d CSDS
10
11
12
Tissue Collection
C
CTRL (9)
SS (9)
RES (9)
SI ratio (AGG present/absent)
****
D
NAc
CTRL (9)
SS (9)
RES (8)
2-AG (pmol/mg)
*
p = 0.0336
SI Ratio
E
FACS strategy for purification of astrocytes in the mouse NAc
Live cells
Dead cells
FSC-A
CD45, CD31, CD11b-
CD45-/CD31-/CD11b-
Acsa2+ (Astrocytes)
Acsa2+
Cd11b PE-A
F
Gene enrichment after FACS
Astrocytes
Neurons
Endothelial cells
Oligodendrocytes
Fold change (Log 10)
Aldh1l1
Gfap
S100b
Aqp4
S100a1
Stat3
Glut1
Glul
Pecam1
Cldn5
Mbp
Neun
G
NAc
p = 0.0974
Mgll mRNA Fold Change
CTRL
CSDS
H
IL-6 (100ng/ul)
IL-6
Cnr1
Nape-pld
Mgll
Fold Change (Log10 vs untreated ctrl)
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I
CTRL (12)
IL-6 24h (12)
IL-6 7d (11)
GFAP volume (µm³)
GFAP primary processes (N. % CTRL)
J
CTRL
IL-6 24h
IL-6 7d
GFAP DAPI
K
IL-6 (100ng/ul)
AAV-GFAP-Cnr1
IL-6
IL-6 + AAV-GFAP-Cnr1
IL-6 mRNA Fold Change (vs untreated ctrl)
Cnr1 mRNA Fold Change (Log 10 vs untreated ctrl)
Time (h)
L
CTRL
IL-6
CTRL + AAV-GFAP-Cnr1
IL-6 + AAV-GFAP-Cnr1
GFAP
Aqp4
eGFP
GFAP Aqp4 DAPI
GFAP Aqp4 eGFP DAPI

360

Figure 5. The astrocytic endocannabinoidome is modulated by stress exposure and associated inflammation. **A**, Schematic of endocannabinoid main enzymes responsible for their synthesis and degradation. Anandamide (AEA) is mainly synthetized by N-acylphosphatidylethanolamide-phospholipase D (Nape-Pld) and metabolized by fatty acid amidohydrolase (Faah). 2-arachidonoylglycerol (2-AG) is generated through the action of selective enzymes, including diacylglycerol lipase (Daglα) and is metabolized by both Faah and monoacylglycerol lipase (Magl). **B**, Experimental timeline of 10-day chronic social defeat stress (CSDS), social interaction (SI) test and tissue collection. **C**, Individual SI values following CSDS. **D**, CSDS decreases nucleus accumbens (NAc) 2-AG levels in resilient (RES) but not in stress-susceptible (SS) males (left). It correlates with social interaction levels (right). **E**, To assess NAc astrocyte-specific endocannabinoid changes induced by chronic stress, astrocytes were isolated using fluorescent activated cell sorting (FACS). **F**, Transcriptomic analysis confirmed enrichment of astrocytes after FACS. **G**, A trend for an increase in *Mgll* transcription was noted in stressed mice. **H**, *in vitro* experimental scheme for mouse primary cultured astrocytes treated with pro-inflammatory interleukin-6 (IL-6, 100 ng/ul). Acute treatment with IL-6 drives pro-inflammatory response in astrocytes leading to an increase of the endocannabinoidome gene transcription at 3h and 6h time points. **I**, Chronic (7-d) but not acute (24h) treatment with IL-6 results in decreased volume of GFAP + astrocytes (left). Both acute and chronic treatment led to decreased astrocyte morphology complexity (right). **J**, Representative images of astrocytes expressing GFAP untreated (left), acutely (middle) or chronically (right) treated with IL-6 (100 ng/ul). Scale bars, 20 µm. **K**, Infection of cultured astrocytes with AAV-GFAP-*Cnr1* but not AAV-GFAP-sham decreases pro-inflammatory response at the 6-h time point. IL-6 treatment increases expression of *Cnr1* gene in AAV-infected astrocytes. **L**, Acute IL-6 treatment leads to Aquaporin-4 (Aqp4) depolarization from the astrocyte end-feet to the nuclei, which is prevented by AAV-GFAP-*Cnr1* infection. Scale bars, 20 µm. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. For one factor analysis one-way ANOVA followed by Holm-Šídák's multiple comparison test was applied. For n lower than 8, the Kruskal-Wallis test followed by Dunn's multiple comparisons evaluation was used. For 2 groups analysis two-tailed t-test was applied. For analysis with two factors, two-way ANOVA followed by Holm-Šídák's multiple comparison test was utilized; correlations were evaluated with Pearson's correlation coefficient; ****p<0.0001, ***p≤0.001, **p≤0.01, *p≤ 0.05.

Stress-induced changes in *Cnr1* are sex-specific and observed in the female prefrontal cortex.

Stress-related disorders such as MDD are characterized by sex differences in prevalence, symptomatology, and treatment response[7]. Sex-specific MDD transcriptional signatures have been described in humans[49,50] and differences in the NAc transcriptome profiles of male vs female mice are associated with susceptibility vs resilience to stressors[51]. We recently reported that exposure to stress alters the BBB in the PFC, and not the NAc, of female mice[7]. Accordingly, CLDN5 loss noted in postmortem NAc samples from men with MDD[6] was instead observed in the PFC of women[7], supporting a sex-specific impact of stress and mood disorders on the neurovasculature. To evaluate if *Cnr1* could also be involved in female stress responses, female mice were subjected to a modified 10-day CSDS[7] as described by Harris et al[52]. Briefly, female C57Bl-6 mice were exposed daily (10 min/day) to bouts of social defeats by a larger, physically aggressive CD-1 male mouse after application of male CD-1 urine on the vagina, tail base, and upper back of the female (**Fig.6A**). Consistent with stress-induced changes in BBB integrity, an increase in *Cnr1* was measured in the PFC of RES females (*p=0.0153) with no change for the NAc (**Fig.6B-C**, **Supp.Fig.6**). Like in the NAc of SS males (**Fig.4G**), *Aqp4* expression was lower in the PFC of SS females (**p=0.0014) (**Fig.6D**). A baseline sex differences was noted in the unstressed control groups with lower *Aqp4* (*p=0.0319) in the female NAc when compared to males and a trend for *Cnr1* (p=0.0755) (**Fig.6E**). Regional brain difference was observed for *Cnr1* expression at baseline with higher expression in the PFC when compared to NAc for males (**p=0.0028), a difference

even greater for females (***p=0.0006) (**Fig.6F**). We next run a control experiment based on the previous observation that female rodents are generally considered more vulnerable to unpredictable stress[51]. Thus, other cohorts of mice were subjected to 28 days of chronic variable stress (CVS). This protocol consists in series of three different stressors namely foot shocks, tail suspension, or restraint stress (1/day) leading to the development of anxiety- and depression-like behavioral abnormalities to an equivalent degree in male and female mice[49]. Brain samples were collected 24h after the last stressor then *Aqp4* and *Cnr1* were compared vs unstressed controls (**Fig.6G**). CVS induces a similar loss of *Aqp4* in the male NAc (**p=0.0014) and female PFC (**p=0.0035) (**Fig.6H-I**). As expected, no significant difference was measured for *Cnr1* in the absence of resilient animals following exposure to this stress paradigm. Altogether, these data suggest that chronic stress induces sex-specific regional changes in *Cnr1* expression in line with BBB alterations and strengthen our hypothesis that upregulation of *Cnr1* is strictly associated with resilience to stress.

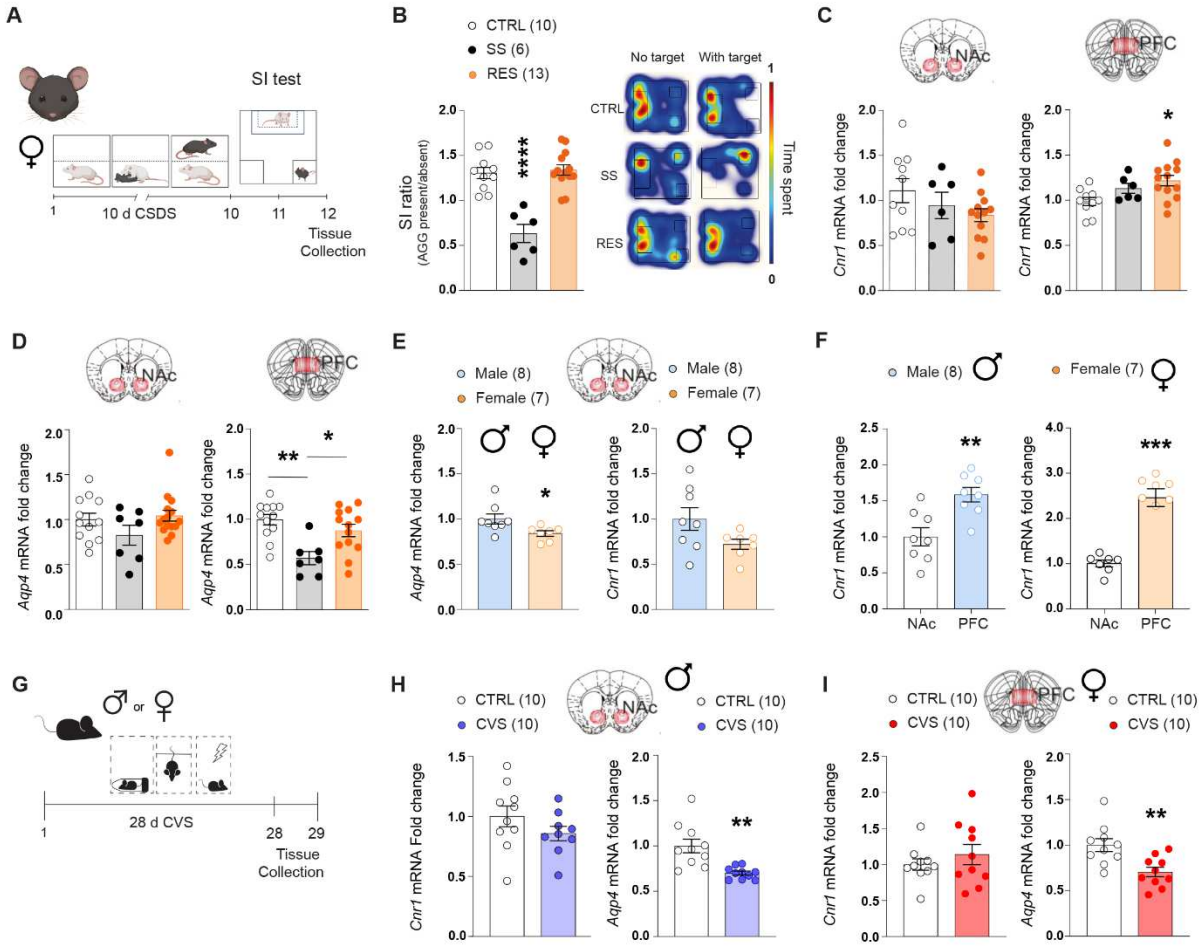

Figure 6. Stress-induced changes in *Cnr1* are sex-specific and observed in the female PFC. A, Experimental timeline of modified female10-day chronic social defeat stress (CSDS), social interaction (SI) and tissue collection. **B**, Individual SI values (left) and representative heatmaps (right) of SI test after CSDS. **C**, Quantitative PCR revealed upregulation of *Cannabinoid receptor 1* (*Cnr1*) gene expression in the prefrontal cortex (PFC, right) but not the nucleus accumbens (NAc, left) of resilient (RES) female mice when compared to unstressed control (CTRL). **D**, *Aquaporin-4* (*Aqp4*) gene expression is decreased in the PFC (right) but not NAc (left) of stress-susceptible (SS)

females when compared to RES and CTRL animals. **E**, Transcriptomic analysis highlighted sex differences in baseline gene expression of *Aqp4* (left) and *Cnr1* (right) in the NAc. **F**, *Cnr1* baseline mRNA levels exhibit regional differences both in male (left) and female (right) mice. **G**, Experimental timeline of 28-d chronic variable stress (CVS), and tissue collection. CVS induces transcriptional decrease for *Aqp4* (right) but not *Cnr1* (left) in (**H**) NAc of males and (**I**) PFC of females. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. For one factor analysis one-way ANOVA followed by Holm-Šídák's multiple comparison test was applied. For n lower than 8, the Kruskal-Wallis test followed by Dunn's multiple comparisons evaluation was used. For 2 groups analysis two-tailed t-test was used: ****p<0.0001, ***p<0.001, **p≤0.01, *p≤ 0.05.

Elevated astrocytic *Cnr1* expression in the NAc is associated with beneficial effects of physical exercise and antidepressant treatments.

Prevention and treatment of stress-related disorders remain challenging in psychiatry. Physical exercise has been associated with stress resilience[53] and neurovascular health[54], however, the biology underlying beneficial effects in the brain reward system is not completely understood. The ECS is activated by physical exercise as measured by high levels of endocannabinoids in the plasma[55], also in relation to stress and depression[56], leading us to hypothesize that it may influence the BBB via perivascular astrocytic CB1. Male mice were subjected to the classical 10-day CSDS paradigm but with access to a running wheel on their side of the cage (**Fig.7A**). The impact of chronic social stress exposure was still present with about 50% RES vs 50% SS animals (**Fig.7B**). However, decreased time spent in the interaction zone during the SI test remained significant only for mice without access to voluntary exercise (stress effect: ***p=0.0009; PE effect **p=0.0037) (**Fig.7C**, **Supp.Fig.7A**). Total running distance was similar between groups (**Supp.Fig.7B**) though RES mice spent more time running during daytime (***p=0.0009) mainly in the hour immediately following social defeat, which could represent a stress coping strategy (**Fig.7D, Supp.Fig.7C**). In fact, high daytime running was correlated with elevated *Cnr1* expression in the NAc (**p=0.0026) (**Fig.7E**). To confirm cell specificity of physical exercise effect on *Cnr1*, RNA scope was combined with immunofluorescence to identify endothelial cell (CD31) and astrocyte endfeet (Aqp4). Colocalization of *Cnr1* positive dots on the neurovasculature was more often observed in RES animals (**Fig.7F**). Our results indicate that physical exercise could modulate BBB properties via astrocytic perivascular *Cnr1* upregulation, thus promoting stress resilience.

Antidepressants are recommended for moderate to severe depression. However, 30-50% of individuals with MDD are not responsive to classical drugs targeting neurons reflecting that causal mechanisms, such as elevated circulating inflammation[46] or vascular dysfunction[9], remain untreated. To better understand the biology behind treatment response vs resistance and possible involvement of astrocytic CB1, male mice were subjected to 10-day CSDS then unstressed CTRL and SS animals treated i.p. for 2-3 weeks with either imipramine or fluoxetine, a tricyclic antidepressant and selective serotonin reuptake inhibitor, respectively (**Fig.7G**). Imipramine reverses transcriptional changes associated with stress susceptibility induced by CSDS, including in the NAc[57], while fluoxetine acts on astrocyte morphology and plasticity[58]. The 2nd SI test performed after treatment revealed a positive impact of treatment on social interactions (stress x treatment effect: *p=0.048) (**Fig.7H**, **Supp.Fig.7D**). Further analysis highlighted two groups of SS-treated mice – responder and non-responders – when SI test performance was compared for each individual. *Cnr1* expression was increased in the NAc of responders only (***p=0.001) and this elevation correlated with higher level of *Aqp4* in this brain area (**p=0.0011) (**Fig.7I**). Finally, the translational value of our mouse findings was assessed by measuring astrocytic *Cnr1* expression in postmortem NAc tissue from individuals with MDD using RNA scope, confirming

a loss of *Cnr1*+ astrocytes (Aqp4+) in the MDD brain (*p=0.0107). Conversely, antidepressant treatment was associated with a level of astrocytic *Cnr1* similar to controls (**Fig.7J-K**).

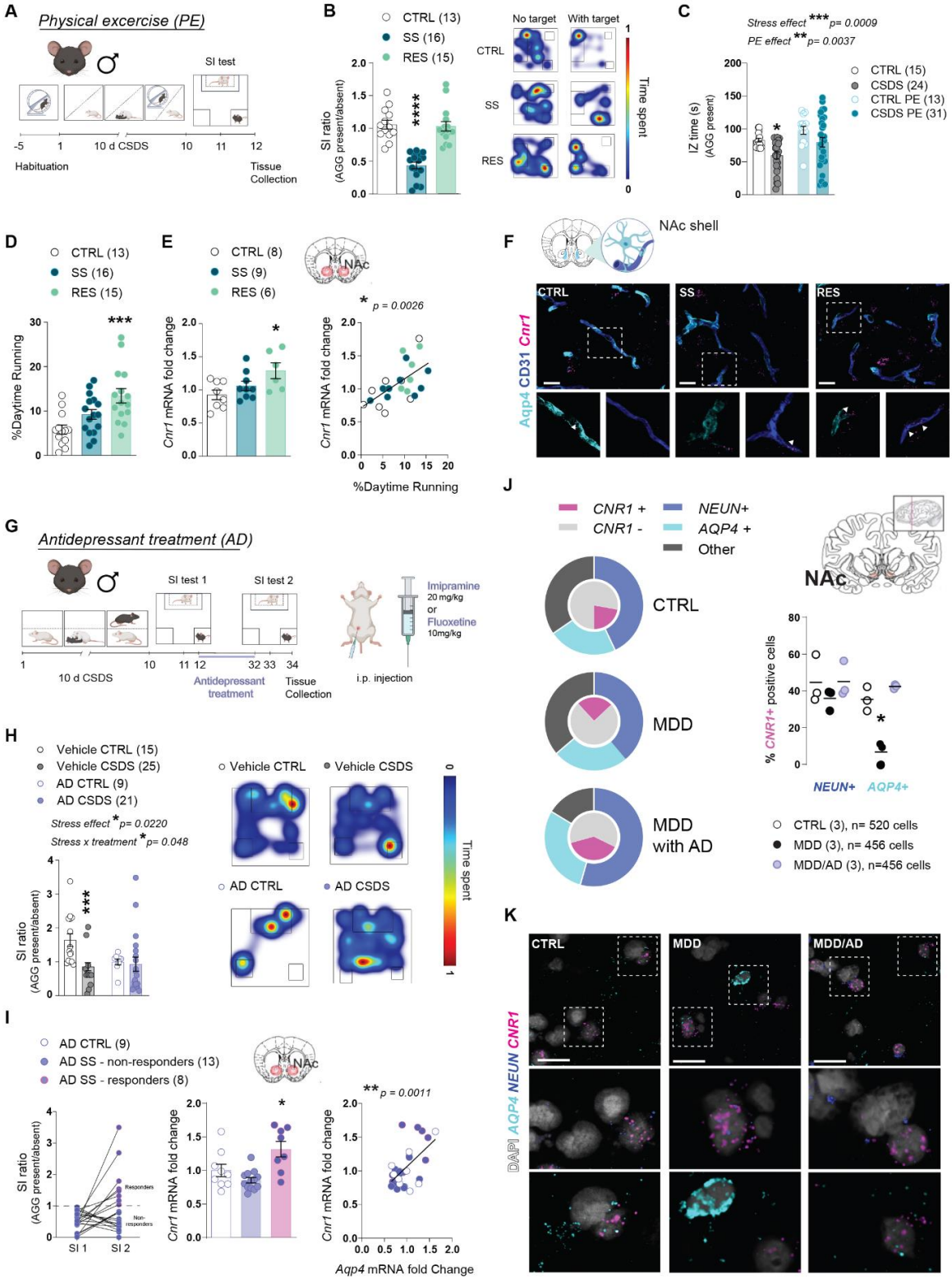

Figure 7. Physical exercise and antidepressant treatment promote stress resilience and increase *Cnr1* in the

nucleus accumbens. A, Experimental timeline of 10-day chronic social defeat stress (CSDS) with physical exercise, social interaction (SI) and tissue collection. **B**, Individual SI values (left) and representative heatmaps (right) after CSDS. **C**, Physical exercise prevents development of social deficits following CSDS. **D**, Resilient (RES) animals are characterized by increased daytime running suggesting a coping strategy when facing CSDS exposure. **E**, Increased transcription of *Cannabinoid receptor 1* (*Cnr1*) is observed in RES animals (left) and correlates with the level of physical exercise during daytime (right). **F**, Representative RNA scope and immunofluorescence images exhibiting increased *Cnr1* expression at the BBB (CD31 as endothelial cell marker) in the RES phenotype. Scale bar 10 µm. **G**, Experimental timeline of CSDS followed by an SI test to establish phenotype prior antidepressant treatment. A 2nd SI test was performed 24h before tissue collection. **H**, Individual SI values (left) and representative heatmaps (right) for these cohorts. **I**, Comparison of SI values (left) for treatment responders' vs non-responders. *Cnr1* mRNA level is increased in the nucleus accumbens (NAc) of treatment responders only (middle) and it correlates with *Aquaporin-4* (*Aqp4*) mRNA expression in this brain area (right). **J**, A loss in *Aqp4* positive cells expressing *Cnr1* is noted in men with MDD. This alteration was not observed for individuals undergoing treatment (right). **K**, Representative RNA scope images, scale bar 20 µm. Data represent mean ± s.e.m.; number of animals or subjects (n) is indicated on graphs. One-way ANOVA or Brown-Forsythe ANOVA test followed by Holm-Šídák's or Dunnett's multiple comparison test was applied. Two-way ANOVA or non-parametric Two-way ANOVA on Ranks followed by Holm-Šídák's or Wilcox multiple comparison test was applied; For N lower than 8, Kruskal-Wallis test followed by Dunn's multiple comparisons evaluation was used; correlations were evaluated with Pearson's correlation coefficient; ****p<0.0001, ***p≤0.001, **p≤ 0.01, *p≤0.05.

Discussion

CB1 signaling can promote positive adaptation following social stress exposure[12,27]. Indeed, striatal CB1 activation in neurons protects against CSDS-induced anxiety[12], while treatment with a CB1 agonist attenuates stress-induced neuroinflammation and anxiety-like behavior[59]. Thus, it has been suggested that CB1 receptors can facilitate the activation of resilience factors during and/or after stress exposure[11,60,61]. Nonetheless, the exact mechanisms remain elusive. An elegant study recently implicated astroglial mitochondria and glucose metabolism in endocannabinoid-related regulation of social behaviors[18]. However, perivascular astrocytic CB1 receptors are understudied[19] and their potential role in regulating chronic stress response had yet to be investigated. Here we show that stress resilience is linked to an increase in astrocytic *Cnr1* expression in the NAc shell, a subregion controlling reward-seeking behavior[62]. We localized these changes at the BBB interface by taking advantage of super resolution microscopy. Astrocytes are essential for BBB recovery after brain injury[63,64]. They are actively involved in maintaining BBB integrity by regulating tight junction formation[65,66] and thus may directly contribute to prevent stress-induced loss of Cldn5.

Additionally, we report that upregulation of astrocytic *Cnr1* in the NAc shell dampens inflammation and stress-associated neurovascular alterations promoting resilience. To bypass the limitations of CB1 antibodies specificity[67], we developed an astrocyte specific AAV driving *Cnr1* expression within this cell type, whereas previous cell-specific Cnr1 manipulations had mainly focused on neuronal populations[68]. To our knowledge this is the first viral strategy applied to increase *Cnr1* expression in astrocytes in a region-selective manner. CB1 receptors are important regulators of anxiety[69] with $Cnr1^{-/-}$ knockout animals exhibiting anxiolytic drug resistance and increased anxiety-like behavior under highly aversive conditions[69]. Following viral-mediated *Cnr1* overexpression in GFAP+ astrocytes of the NAc shell we observed baseline anxiolytic effects. Mutant mice lacking *Cnr1* are characterized by reduced social interactions[70] which has been linked with NAc shell function[71]. We expand these findings by highlighting a protective role for astrocytic CB1 in the context of chronic social stress exposure with reduced social avoidance, anxiety, and helplessness.

Not only CB1 itself but also its related signaling lipids are essential for positive stress adaptation[11]. 2-AG signaling within the NAc regulates anxiety and stress vulnerability as shown previously using pharmacological approaches[12,39]. Here, CSDS decreased 2-AG levels in NAc punches that negatively correlated with social behaviors. This finding encourages cell specific analysis as astrocytic 2-AG hydrolysis is mainly responsible for conversion to neuroinflammatory mediators[42]. When we analyzed astrocyte-specific expression of EC metabolic enzymes, we found that those regulating 2-AG levels tended to be increased in the NAc astrocytes of RES mice. Additionally, stress-induced alterations in EC levels are transient due to rapid hydrolysis[72]. In the future, therefore, it will be important to evaluate EC signaling dynamics at different time points in mice, ideally in a cell-specific manner, following stress exposure. Additionally, our *in vitro* findings indicated that both astrocytic *Cnr1* and *Mgll* are altered by an immune challenge after only a few hours. Change in 2-AG level via lower expression of its main degrading enzyme Magl has implication for astrocyte response to neuroinflammation[73,74]. In fact, viral-mediated increase of *Cnr1* in astrocytes reduced IL-6 driven deleterious inflammatory changes and morphological impairments including loss of endfeet-related Aqp4 which has been associated to depression pathogenesis[16,38]. Circulating inflammation is elevated in stressed animals and subpopulations of individuals with MDD particularly those resisting to treatments[46,47,75]. Astrocyte endfeet are perfectly positioned to sense circulating inflammation and react to it, particularly in the context of BBB breakdown and loss of tight junctions[9] as observed following CSDS and in the MDD brain.

Altogether, our findings support an active role of the BBB, via astrocyte endfeet, in stress resilience. Importantly, our results complement evidence suggesting that chronic stress affect the neurovasculature in a sex-specific manner[5-7] which may contribute to sex differences reported in MDD prevalence, symptomatology, and treatment response[76]. *Cnr1* upregulation was observed in the PFC but not NAc of female mice, highlighting the importance of studying sex differences in the context of stress-related disorders including for the neurovascular unit and ECS. Polymorphisms of the EC receptor genes (*CNR1* and *CNR2*) have been associated with MDD[77] and could influence vulnerability to psychosocial adversity[78]. Anti-obesity treatment with CB1 antagonist rimonabant increases the risk of anxiety and mood disorders[79]. In rodent models of depression, treatment with antidepressants imipramine or fluoxetine, alters striatal CB1 receptor density[80] and expression in neuronal cells[81]. In parallel, voluntary exercise enhances CB1 sensitivity in the striatum[82]. Here, we observed that both interventions increased expression of perivascular *Cnr1*.

To summarize, we propose that perivascular *Cnr1* plays an important role in modulating stress responses in mice and possibly MDD. Identification of beneficial EC-related adaptations within the BBB can represent a promising approach to develop innovative therapies for mood disorders.

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Author Contributions

K.A.D. and C.M. designed research, K.A.D., S.E.J.P., A.C., M.L., O.L., J.B., F.N.K., L.D.A., V.C.F., L.B.B., C.M. and N.F. performed research including behavioral experiments, stereotaxic surgeries, molecular, biochemical, and morphological analysis, M.G. and M.C. provided the constructs or AAVs for functional experiments. G.T. and N.M. obtained, characterized, and prepared the postmortem human samples and related data. K.A.D. and C.M. analyzed the data and wrote the manuscript which was edited by all authors.

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Ethics Declaration

The authors declare no competing financial or non-financial interests.

Data availability

All data supporting the findings of this study are available within the paper and Supplementary Information files.

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