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Degradation of Perineuronal Nets in the Ventral Hippocampus of Adult Rats Recreates an Adolescent-Like Phenotype of Stress Susceptibility

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ABSTRACT

BACKGROUND: Psychiatric disorders often emerge during late adolescence/early adulthood, a period with increased susceptibility to socioenvironmental factors that coincides with incomplete parvalbumin interneuron (PVI) development. Stress during this period causes functional loss of PVIs in the ventral hippocampus (vHip), which has been associated with dopamine system overdrive. This vulnerability persists until the appearance of perineuronal nets (PNNs) around PVIs. We assessed the long-lasting effects of adolescent or adult stress on behavior, ventral tegmental area dopamine neuron activity, and the number of PVIs and their associated PNNs in the vHip. Additionally, we tested whether PNN removal in the vHip of adult rats, proposed to reset PVIs to a juvenile-like state, would recreate an adolescent-like phenotype of stress susceptibility.

METHODS: Male rats underwent a 10-day stress protocol during adolescence or adulthood. Three to 4 weeks poststress, we evaluated behaviors related to anxiety, sociability, and cognition, ventral tegmental area dopamine neuron activity, and the number of PV^+ and PNN^+ cells in the vHip. Furthermore, adult animals received intra-vHip infusion of ChABC (chondroitinase ABC) to degrade PNNs before undergoing stress.

RESULTS: Unlike adult stress, adolescent stress induced anxiety responses, reduced sociability, cognitive deficits, ventral tegmental area dopamine system overdrive, and decreased PV^+ and PNN^+ cells in the vHip. However, intra-vHip ChABC infusion caused the adult stress to produce changes similar to the ones observed after adolescent stress.

CONCLUSIONS: Our findings underscore adolescence as a period of heightened vulnerability to the long-lasting impact of stress and highlight the protective role of PNNs against stress-induced damage in PVIs.

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Adolescence is a period of heightened susceptibility to the development of psychiatric disorders (1,2). During this period, neural structures are highly plastic and particularly sensitive to adverse socioenvironmental factors (3). Clinical evidence indicates that there is an association between psychosocial adversities and trauma during adolescence with the onset of mental illnesses, such as depression, anxiety disorders, and schizophrenia (4–8).

Adolescent stress affects brain regions that are essential for regulating stress response, such as the prefrontal cortex (PFC), basolateral amygdala, and ventral hippocampus (vHip) (9–11). Alterations in the activity of these regions caused by adolescent stress have been linked to impairments in parvalbumin-expressing GABAergic (gamma-aminobutyric acidergic) interneurons (PVIs) (2,12), which play a critical role in synchronizing the activity of glutamatergic pyramidal neurons and, consequently, in maintaining an excitatory-inhibitory balance (13,14).

During adolescence, PVIs exhibit higher plasticity in terms of excitatory drive and functional activity (3,15), which renders them particularly vulnerable to stress-induced damage (2). The period of vulnerability for PVIs persists until adulthood and the full emergence of perineuronal nets (PNNs), a glycosaminoglycan extracellular matrix (16,17) surrounding the PVIs, which coincides with their maturation (18). PNNs act as a physical barrier that offers stability to glutamatergic inputs and shields PVIs from oxidative and metabolic damage (19,20). Therefore, during adolescence, when PNNs are not entirely formed, PVIs are more susceptible to stress-induced damage, which could contribute to the development of psychiatric disorders (2,18). We previously found that, unlike stress applied to adult animals, stress during adolescence caused PVI deficits in the vHip, which were associated with behavioral abnormalities, increased activity of pyramidal neurons in the vHip, and ventral tegmental area (VTA) dopamine system overdrive (18,21,22).

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Here, we evaluated the impact of stress during adolescence and adulthood on behavior, including responses related to anxiety, sociability, cognitive function, VTA dopamine system activity, and the number of PV^+ and PNN^+ cells in the vHip. In addition, because PNNs have a protective role against damage to PVIs, we tested whether the chemical removal of PNNs in the vHip through the local infusion of ChABC (chondroitinase ABC), an enzyme that degrades PNNs (23), in adulthood (a period during which PNNs are fully formed around PVIs) would recreate an adolescent-like phenotype of stress susceptibility.

METHODS AND MATERIALS

Animals

Male Sprague Dawley rats obtained from Central Animal House of the University of São Paulo, Ribeirão Prêto Campus, were housed in groups of 2 to 3 per microisolated cage, with water and food available ad libitum, and under standard laboratory conditions. Animals from the same experimental group were housed together. All procedures followed Brazilian and international regulations for the care and use of laboratory animals and were approved by the Ribeirão Prêto Medical School Ethical Committee (#155/2018).

Stress Protocol

The animals were subjected to the following combination of footshock and restraint stress, as previously described (18,21,22,24). Briefly, rats were exposed to daily footshocks for 10 days during adolescence (between postnatal day [PND] 31 and 40) or adulthood (between PND 61 and 70 or PND 64 and 73 in animals subjected to surgery). During each session, 25 randomized footshocks (1.0 mA, 2 seconds) were delivered every 60 $\pm$ 20 seconds. Rats were also subjected to 3 restraint stress sessions (days 1, 2, and 10) for 1 hour during which they were placed inside an acrylic tube of an age-adjustable size immediately after the footshock session. Naïve animals were left undisturbed in their home cages in the animal room.

Between 3 and 4 weeks after the stress exposure, animals were tested for anxiety-related responses (light-dark box [LDB]), sociability (social interaction), and cognitive function (novel object recognition [NOR] test). After that, some animals were subjected to VTA dopamine system activity evaluation through in vivo electrophysiology, and others were assessed for PV^+ and PNN^+ cells in the vHip.

Only male rats were used in this study (a total of 163) because we previously found that female adolescent rats were resistant to presenting behavioral and electrophysiological changes after exposure to the same stress protocol (25).

Behavioral Tests

Animals were tested in LDB, social interaction, and NOR tests. Please see the Supplement for a detailed description of these behavioral tests.

In Vivo Electrophysiological Activity of VTA Dopamine Neurons

Extracellular single-cell electrophysiological recordings were performed in animals anesthetized with 8% chloral hydrate (400 mg/kg, intraperitoneally). After positioning the animal in a stereotaxic frame, the coordinates used to record the activity of VTA dopamine neurons were 5.3 mm posterior to bregma, 0.6 mm lateral to the midline, and 6.5 to 9.0 mm ventral from the brain surface. Glass electrodes filled with 2% Chicago Sky Blue in 2M NaCl were lowered through 6 to 9 vertical tracks in a predetermined pattern (26). The number of spontaneously active dopamine neurons identified according to established criteria (27), their firing rate, and the percentage of spikes in burst were recorded. At the end of the recording, Chicago Sky Blue dye from the electrode was infused via iontophoresis (20 μA constant negative current, 20 minutes) for histological confirmation of the electrode sites.

Immunofluorescence for PV/PNN Expression in the vHip

After the behavioral tests, animals not subjected to VTA dopamine neuron recordings were anesthetized and perfused with 0.1M phosphate-buffered saline and 4% paraformaldehyde. Brains were removed and kept in 4% paraformaldehyde for 2 hours and then placed in 30% sucrose solution for 30 hours. For each animal, 5 to 6 tissue sections (30 μm thick) covering the rostrocaudal axis were collected and subjected to the free-floating immunofluorescence method to evaluate the expression of PV and PNNs in the vHip, more specifically in the ventral subiculum subregion of the vHip. Sections were incubated with a combination of 1% fetal bovine serum, goat anti-PV antibody (1:150) (#ab32895; Abcam), and biotinylated *Wisteria floribunda* agglutinin (1:500) (#FL-1351-2; Vector Laboratories) for 18 hours at 4 °C. *Wisteria floribunda* agglutinin is currently the most often used marker for PNNs, binding to N-acetylgalactosamine residues in CSPGs (chondroitin sulfate proteoglycans) and enabling their visualization in imaging studies (28). Next, the sections were incubated with a solution containing 1% fetal bovine serum, donkey anti-goat AlexaFluor 594 (1:300) (#A-11058; Invitrogen), and AlexaFluor 488 conjugated to streptavidin (1:1000) (#S11223; Invitrogen) for 2 hours at room temperature. The slides were mounted with a mounting medium containing DAPI (#ab104139; Abcam) to visualize the borders of the ventral subiculum. Image acquisition was conducted using the Leica TCS SP5 confocal microscope and the Leica Application Suite software (see the Supplement for details of image acquisition and analysis).

Degradation of PNNs in the vHip Through Chondroitinase Infusion

Rats (PND 55–58) were anesthetized with isoflurane and subjected to stereotaxic surgery for bilateral infusion of ChABC from *Proteus vulgaris* (0.05 U/μL in 0.75 μL) (Sigma) or penicillinase from *Bacillus cereus* (an inert enzyme in mammals used as a control; 0.05 U/μL in 0.75 μL) (Sigma) into 2 vHip regions. The coordinates were 5.3 mm posterior to bregma, 4.4 mm lateral to the midline, and 6.8 and 7.3 mm ventral to the brain surface. The animals were subjected to a combination of stressors 1 week after surgery. Subsequently (3–4 weeks after the end of stress), the behavioral changes and VTA dopamine neuron activity or PV/PNN expression in the vHip were measured.

Statistical Analyses

Data were presented as the mean ± SEM. We verified whether all data followed the homogeneity of variances (Bartlett's test) and the normal distribution (Shapiro-Wilk's test). Data that met these parameters were subjected to parametric analysis [2-way analysis of variance (ANOVA) followed by Tukey's post-test or Student's *t* test]. Otherwise, they were subjected to nonparametric analysis (Kruskal-Wallis followed by Dunn's test). For the 2-way ANOVA, condition (naïve and stressed) and stress period (adolescence and adulthood) or treatment (penicillinase and ChABC) were considered as the main factors. At the end of the behavioral tests, we calculated a behavioral *z* score (29) to reduce the intrinsic variability of single behavioral tests and provide a more reliable characterization of the impact of stress on each animal's behavior (see the Supplement for details). Pearson correlation analyses were performed to examine correlations between the behavioral *z* score and changes in PV^+, PNN^+, and PV^+/PNN^+ cells in the vHip after adolescent and adult stress. The significance level was set at $p < .05$.

RESULTS

Adolescent Stress Causes Long-Term Behavioral Impairments and Increased VTA Dopamine System Activity in Adult Animals

The animals (n = 12/group) were subjected to stress during adolescence (PND 31–40) or adulthood (PND 61–70). Between 3 and 4 weeks later, animals were tested for anxiety (LDB), sociability (social interaction test), and cognitive function (NOR test). After the behavior tests, animals underwent VTA dopamine neuron recordings or evaluation of PV^+ and PNN^+ cells in the vHip (Figure 1A).

Adolescent stress causes the adult animal to spend less time in the light compartment of the LDB, indicating a preference for dark spaces, which may suggest anxiety-like behavior (Figure 1B). The Kruskal-Wallis test indicated a significant effect (H = 14.86, $p < .05$). Dunn's posttest showed that the exploration time of the light zone in animals exposed to adolescent stress was significantly lower ($p < .05$ vs. naïve animals from the adolescent stress protocol). Stress also caused the animals to

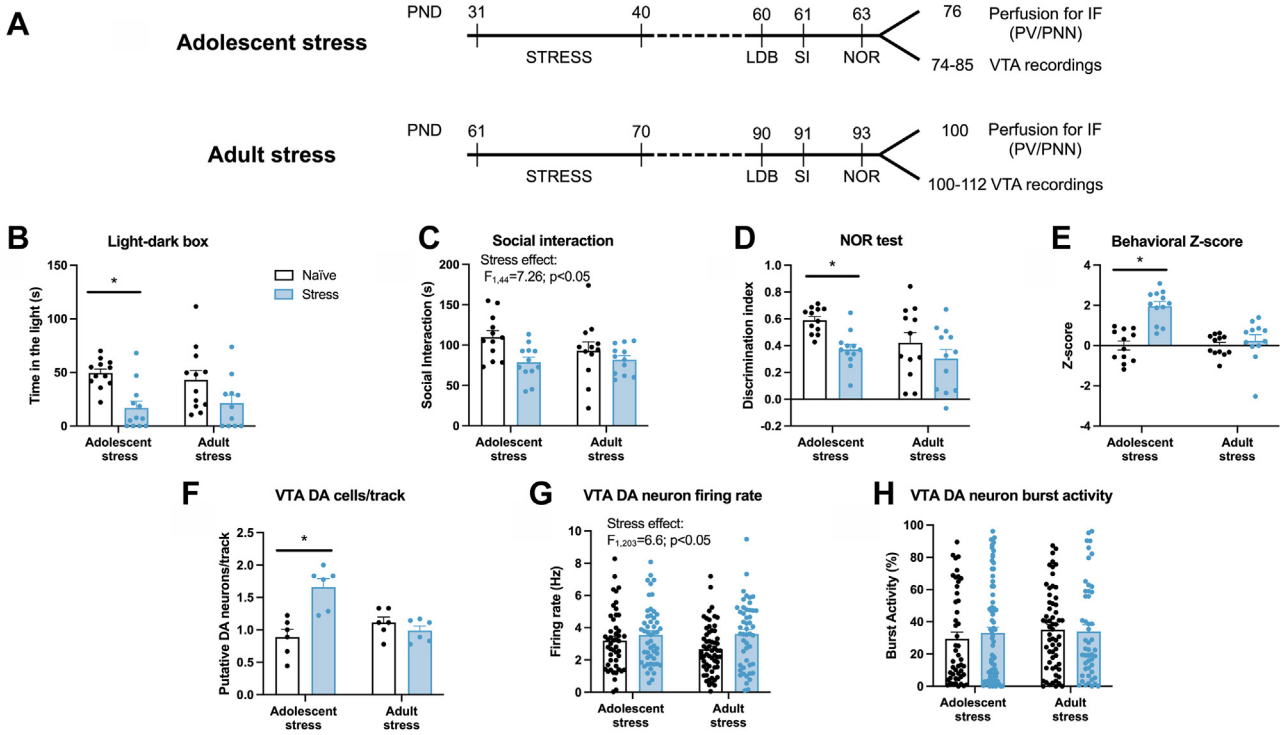

Figure 1. Long-term impact of adolescent and adult stress on behavioral and VTA dopamine system activity in adult animals. **(A)** Experimental design of procedures carried out to evaluate the impact of stress during adolescence (PND 31–40) and adulthood (PND 61–70) on anxiety (LDB), sociability (SI), cognition (NOR test; n = 12/group), and VTA dopamine system activity through in vivo electrophysiology (n = 6/group). Animals that did not undergo VTA recordings were perfused to evaluate PV and PNN expression in the ventral hippocampus (Figure 2). **(B)** Adolescent stress-induced anxiety-like response in the LDB test indicated by a decrease in light zone exploration (1 outlier from the adult stress group identified by the ROUT test was removed from the analysis). **(C)** Adolescent and adult stress decreased SI. **(D)** Adolescent stress also impaired discrimination memory in the NOR test. **(E)** A higher behavioral *z* score was observed for animals that were exposed to adolescent stress, suggesting a higher deleterious impact of an adolescent than adult stress on behavioral performance. In addition, **(F)** adolescent stress increased the number of spontaneously active dopamine neurons in the VTA. There was also **(G)** a stress effect in the firing rate, **(H)** but with no changes in the burst activity (adolescent stress—naïve: 48 cells, stress: 77 cells; adult stress—naïve: 60 cells, stress: 49 cells). Data represent mean ± SEM. For the exploration of the light compartment in the LDB, the discrimination index in the NOR test, and the behavioral *z* score, $^*p < .05$ after the Kruskal-Wallis test followed by Dunn's posttest. For the other parameters, $^*p < .05$ after 2-way ANOVA followed by Tukey's posttest. DA, dopamine; IF, immunofluorescence; LDB, light-dark box; NOR, novel object recognition; PND, postnatal day; PNN, perineuronal net; PV, parvalbumin; SI, social interaction; VTA, ventral tegmental area.

show lower sociability (Figure 1C). A 2-way ANOVA revealed an effect of stress ($F_{1,44}$ = 7.26, $p < .05$) but no effect of stress period and no interaction. Regarding cognitive function, the Kruskal-Wallis test indicated a significant effect in the NOR test (H = 12.94, $p < .05$). Dunn's posttest indicated that animals exposed to adolescent stress presented a lower discrimination index ($p < .05$ vs. naïve animals from the adolescent stress protocol) (Figure 1D).

Using the behavioral z score approach, we found that adolescent stress caused more marked, long-lasting behavioral deficits than adult stress (Figure 1E). The Kruskal-Wallis test indicated a significant effect (H = 23.19, $p < .05$). Dunn's posttest revealed a significantly higher behavioral z score caused by adolescent stress ($p < .05$ vs. all groups), suggesting a more pronounced impact of stress on the animals' overall performance.

In addition, similar to our previous findings (18,21,22), adolescent stress caused the animals to present a significantly higher VTA dopamine neuron population activity in adulthood than naïve animals (n = 6/group) (Figure 1F). A 2-way ANOVA indicated an effect of stress ($F_{1,20}$ = 9.51, $p < .05$), stress period ($F_{1,20}$ = 4.64, $p < .05$), and the interaction between them ($F_{1,20}$ = 18.29, $p < .05$). Tukey's posttest showed that the number of spontaneously active VTA dopamine neurons was significantly higher in adulthood after adolescent stress ($p < .05$ vs. naïve animals from the adolescent stress protocol), which was not observed after adult stress. Following stress exposure, there was also higher firing of VTA dopamine neurons (stress effect: $F_{1,203}$ = 6.6, $p < .05$) (Figure 1G) but with no effect of stress period and no interaction. However, no change was observed in the burst activity after adolescent or adult stress (Figure 1H).

Adolescent Stress Decreases PV$^+$ and PNN$^+$ Cells in the vHip in Adult Animals

Animals not subjected to electrophysiology (n = 6/group) were perfused for subsequent immunofluorescence to investigate the long-term impact of adolescent and adult stress on the expression of PV and PNN in the vHip (Figure 2A, B). Overall, animals exposed to adolescent but not adult stress had significantly lower numbers of PV$^+$ cells, PNN content, and PV$^+$ cells surrounded by PNNs, which correlated with behavioral deficits. Regarding the number of PV$^+$ cells, a 2-way ANOVA indicated an effect of stress ($F_{1,20}$ = 12.88, $p < .05$) and interaction between stress and stress period ($F_{1,20}$ = 23.74, $p < .05$), with no effect of the stress period. As for the number of PNN$^+$ cells, there was an interaction between stress and stress period ($F_{1,20}$ = 22.13, $p < .05$), with no effect of stress or stress period. Concerning PV$^+$/PNN$^+$ cells, there was an effect of stress ($F_{1,20}$ = 11.39, $p < .05$) and interaction between stress and stress period ($F_{1,20}$ = 5.69, $p < .05$) but no impact of stress period. Tukey's posttest indicated a significant reduction in PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells caused by adolescent stress ($p < .05$ vs. naïve animals from the adolescent stress protocol). In magnitude, adolescent stress decreased the number of PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells by 36%, 27%, and 29%, respectively. In contrast, adult stress did not induce such changes.

Furthermore, correlation analysis indicated that the higher the detrimental impact of adolescent stress on behavioral performance (behavioral z score), the lower the number of PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells in the vHip (Figure 2C). This was not observed after adult stress (Figure S1).

PNN Degradation in the vHip Recreates an Adolescent-Like Phenotype of Stress Susceptibility in Adult Animals

To evaluate the effectiveness of ChABC infusion on PNN degradation, an independent group of nonstressed animals received a bilateral injection of ChABC or penicillinase into the vHip at PND 56–58 (n = 4–5/group) (Figure 3A). Their brains were collected on what would be equivalent to the first (PND

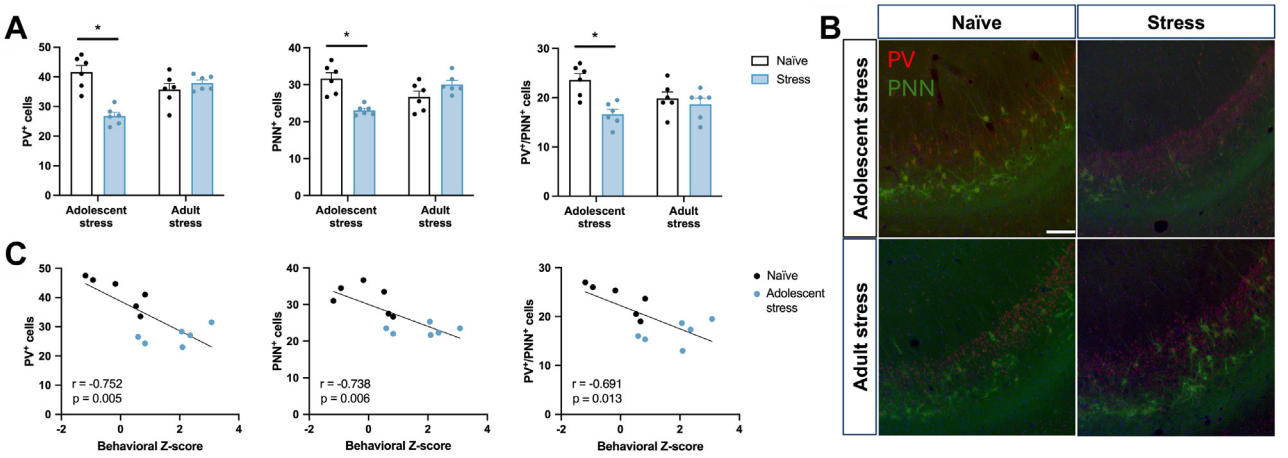

Figure 2. Impact of adolescent and adult stress on PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells in the vHip in adulthood. **(A)** Adolescent stress decreased the number of PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells in the vHip of adult animals (n = 6/group). Data represent mean ± SEM. *$p < .05$, 2-way ANOVA followed by Tukey's posttest. **(B)** Representative images of the vHip showing lower expression of PV/PNNs after adolescent stress (green: PNNs; red: PV; blue: cell nucleus). Image acquired on the Leica TCS SP5 confocal microscope at 20× magnitude. Scale bar = 100 μm. **(C)** Pearson's correlation analysis indicated a significant negative correlation between the behavioral z score and the number of PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells in the vHip, indicating that the lower the number of PV$^+$, PNN$^+$, and PV$^+$/PNN$^+$ cells in the vHip, the higher the detrimental impact of adolescent stress on behavior was. PNN, perineuronal net; PV, parvalbumin; vHip, ventral hippocampus.

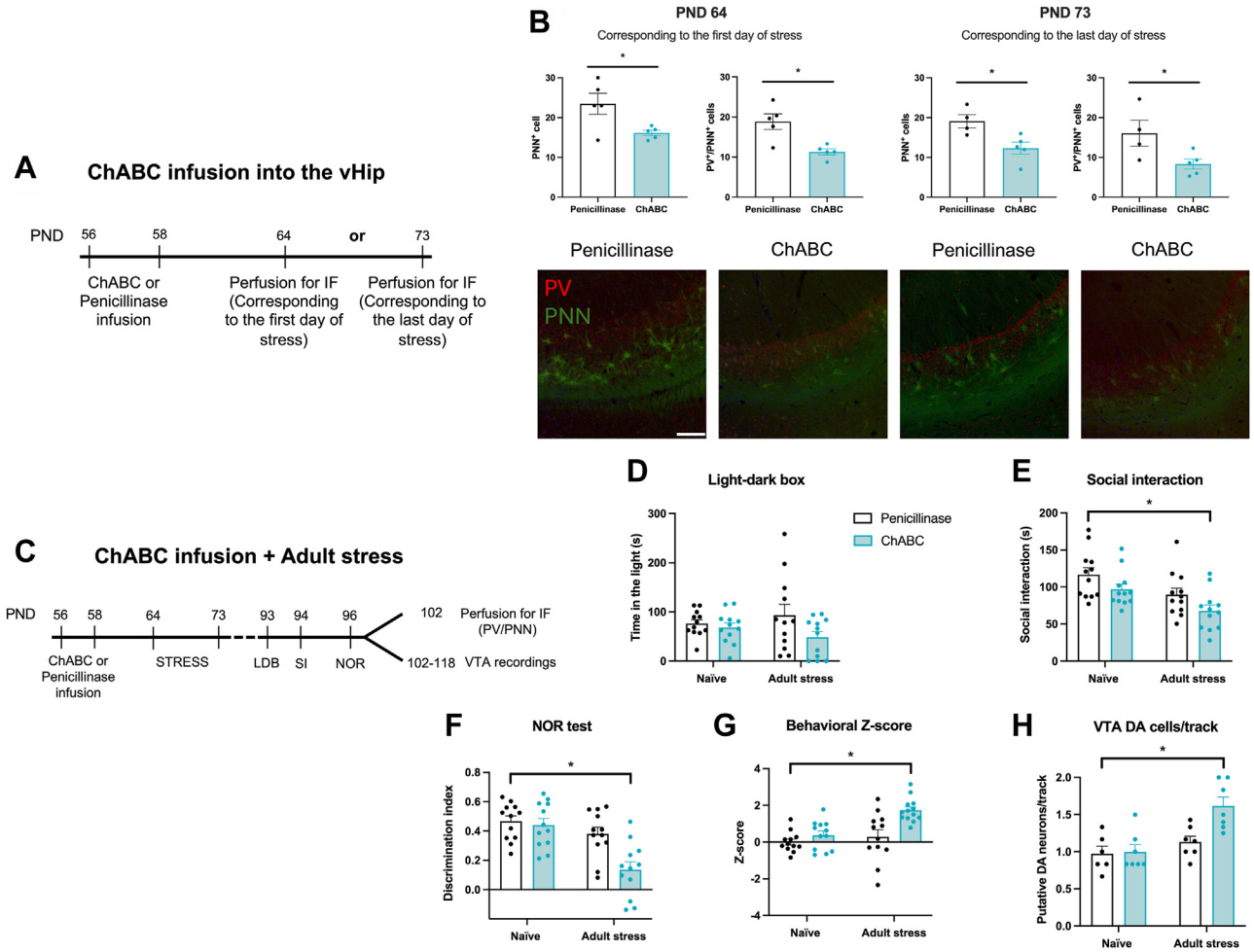

Figure 3. ChABC intra-vHip combined with adult stress causes the rats to show behavioral deficits and changes in VTA dopamine system activity similar to those found after adolescent stress. **(A)** ChABC or penicillinase (control) was infused into the vHip of an independent group of animals (not exposed to stress) that were perfused on what would be the first (PND 64) and last (PND 73) day of adult stress to evaluate local PNN degradation caused by ChABC (n = 4–5/group). **(B)** ChABC intra-vHip decreased the number of PNN^+ and PV^+/PNN^+ cells on PND 64 and PND 73, as evidenced by their respective representative images of the vHip (green: PNNs; red: PV; blue: cell nucleus). Image acquired on the Leica TCS SP5 confocal microscope at 20× magnitude. Scale bar = 100 μm. Data represent mean ± SEM. *$p < .05$, unpaired Student's t test. **(C)** After ChABC or penicillinase infusion into the vHip, animals were subjected to adult stress following behavioral (n = 12/group) and electrophysiological (n = 6–7/group) assessment. Animals that did not undergo VTA recordings were perfused to evaluate PV and PNN expression in the vHip (Figure 4). **(D)** No change in the amount of time spent in the light compartment of the LDB was observed. However, the combination of ChABC intra-vHip and adult stress caused the animals to show **(E)** decreased social interaction and **(F)** cognitive impairment on the NOR test and **(G)** led to a higher behavioral z score than the other groups, indicating a higher impact of stress on behavior that is similar to that found after adolescent stress. In addition, similar to adolescent stress, **(H)** the combination of ChABC intra-vHip and adult stress caused the animals to show increased VTA dopamine neuron population activity. Data represent mean ± SEM. For social interaction time, behavioral z score, and the number of spontaneously active VTA dopamine neurons, *$p < .05$ after the Kruskal-Wallis test followed by Dunn's posttest. For the other parameters, *$p < .05$ after 2-way ANOVA followed by Tukey's posttest. ChABC, chondroitinase ABC; DA, dopamine; IF, immunofluorescence; LDB, light-dark box; NOR, novel object recognition; PND, postnatal day; PNN, perineuronal net; PV, parvalbumin; SI, social interaction; vHip, ventral hippocampus; VTA, ventral tegmental area.

64) and last (PND 73) day of the adult stress protocol to verify PNN degradation (Figure 3B). At both time points, Student's t tests indicated that ChABC caused the animals to present significantly lower number of PNN^+ cells (PND 64: 31% reduction, $t_8 = 2.67$, $p < .05$; PND 73: 25% reduction, $t_7 = 3.00$, $p < .05$), including PV^+ cells surrounded by PNNs (PND 64: 39% reduction, $t_8 = 3.60$, $p < .05$; PND 73: 48% reduction, $t_7 = 2.42$, $p < .05$) (Figure 3B). There was no difference in the number of PV^+ cells (Figure S2). These findings suggest that intra-vHip ChABC degraded PNNs during this period, potentially rendering PVIs more vulnerable to the adverse effects of stress.

After confirming PNN degradation, animals (n = 12/group) were subjected to stereotaxic surgery for ChABC or penicillinase infusion into the vHip and were subsequently exposed to adult stress (PND 63–74), being tested 3–4 weeks later (Figure 3C).

In the LDB test, no change in the time spent in the light compartment was observed (Figure 3D). In the social

interaction, the Kruskal-Wallis test indicated a significant effect (H = 14.78, $p < .05$). Dunn's posttest revealed that ChABC-treated animals submitted to adult stress had a lower social interaction time ($p < .05$ vs. naïve+penicillinase), similar to what was found after the adolescent stress. Regarding discrimination memory in the NOR test, a 2-way ANOVA revealed an effect of treatment ($F_{1,44}$ = 8.84, $p < .05$), stress ($F_{1,44}$ = 18.55, $p < .05$), and the interaction between treatment and stress ($F_{1,44}$ = 5.82, $p < .05$) (Figure 3F). Tukey's posttest indicated that ChABC-treated animals submitted to adult stress had a significantly lower discrimination index ($p < .05$ vs. naïve+penicillinase), suggesting cognitive impairment similar to that observed after the adolescent stress.

Like adolescent stress, the behavioral z score indicated that the combination of intra-vHip ChABC infusion and adult stress caused marked, long-lasting behavioral deficits (Figure 3G). The Kruskal-Wallis test showed a significant effect (H = 19.40, $p < .05$). Dunn's posttest indicated that animals that received intra-vHip ChABC infusion and were stressed had a higher z score, indicating worse behavioral performance ($p < .05$ vs. all groups).

Regarding VTA dopamine neuron activity, stressed ChABC-treated animals showed enhanced VTA dopamine neuron population activity (n = 6–7/group) (Figure 3H). The Kruskal-Wallis test indicated a significant effect (H = 12.67, $p < .05$). Dunn's posttest revealed a significant increase in the number of spontaneously active VTA dopamine neurons of animals that received ChABC intra-vHip and were subjected to stress during adulthood ($p < .05$ vs. naïve+penicillinase). No change in the firing rate or burst activity was found in the groups (Figure S3).

Overall, these findings support our hypothesis that removing PNNs in the vHip recreates an adolescent-like phenotype of stress susceptibility.

Degradation of PNNs in the vHip Combined With Adult Stress Causes a Long-Term Decrease in Local PV^+ and PNN^+ Cells

Animals that were not subjected to electrophysiology were perfused for immunofluorescence (n = 5–6/group) (Figure 4A, B). A 2-way ANOVA indicated an effect of treatment ($F_{1,17}$ = 9.04, $p < .05$), stress ($F_{1,17}$ = 23.42, $p < .05$), and the interaction between treatment and stress ($F_{1,17}$ = 31.09, $p < .05$) on the number of PV^+ cells. This was also observed for the number of PNN^+ cells (treatment, $F_{1,17}$ = 5.53, $p < .05$) (stress, $F_{1,17}$ = 12.32, $p < .05$) (treatment × stress interaction, $F_{1,17}$ = 8.79, $p < .05$). Conversely, for the number of PV^+/PNN^+ cells, there was no significant effect of treatment but a significant effect of stress ($F_{1,17}$ = 12.31, $p < .05$) and interaction between treatment and stress ($F_{1,17}$ = 6.21, $p < .05$). Tukey's posttest revealed a lower number of PV^+, PNN^+, and PV^+/PNN^+ cells in animals that received ChABC intra-vHip and were stressed ($p < .05$ vs. naïve+penicillinase), indicating that, similar to adolescent stress, after local PNNs' degradation combined with adult stress, there was a long-lasting decrease in the number of PV^+ (36% reduction), PNN^+ (36% reduction), and PV^+/PNN^+ cells (31% reduction) in the vHip.

DISCUSSION

Both clinical and preclinical evidence suggest that stress may increase susceptibility to various mental disorders, including anxiety, depression, and schizophrenia (4,18,30,31). It has been hypothesized that repeated stress exposure accumulates after some time, increasing stress reactivity and risk for positive symptoms (31). However, the timing of stress exposure could be crucial to determining the outcomes observed. Adolescence is a period of neurodevelopment characterized

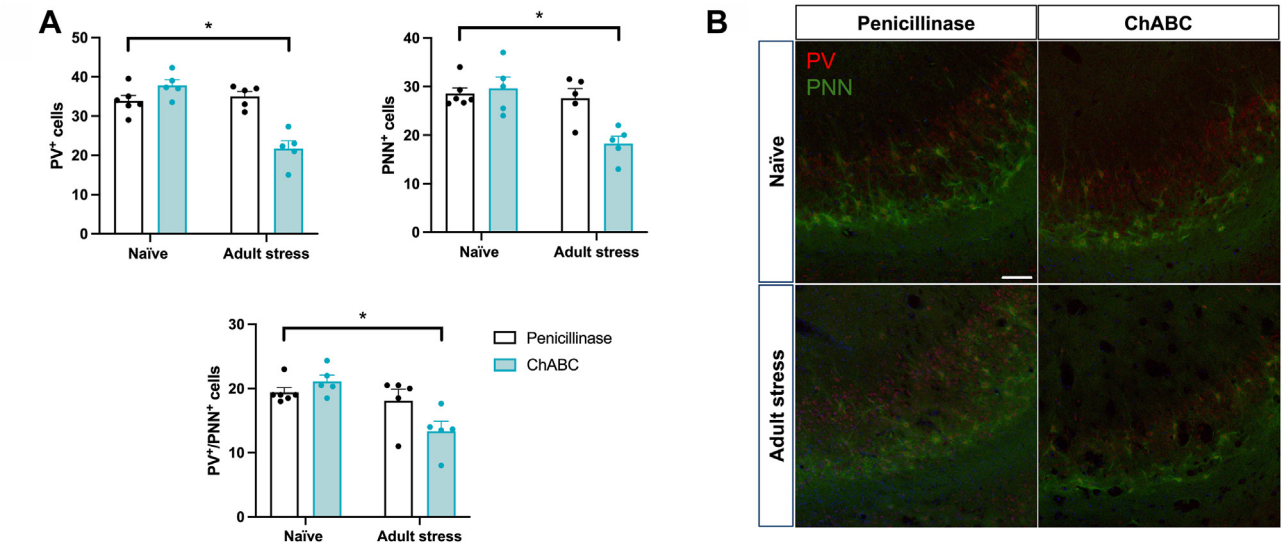

Figure 4. Effects of ChABC intra-vHip combined with adult stress on PV^+, PNN^+, and PV^+/PNN^+ cells in the vHip. **(A)** Animals that received ChABC intra-vHip and were stressed in adulthood had fewer PV^+, PNN^+, and PV^+/PNN^+ cells (n = 5–6/group). **(B)** Representative images of the vHip of a naïve+penicillinase, naïve+ChABC, adult stress+penicillinase, and adult stress+ChABC animals indicate these changes (green: PNNs; red: PV; blue: cell nucleus). Image acquired on the Leica TCS SP5 confocal microscope at 20× magnitude. Scale bar = 100 μm. Data represent mean ± SEM. *$p < .05$ after 2-way ANOVA followed by Tukey's posttest. ChABC, chondroitinase ABC; PNN, perineuronal net; PV, parvalbumin; vHip, ventral hippocampus.

by heightened vulnerability to stressors, which may cause long-term complications (2,32,33).

In this study, we first evaluated the impact of stress during adolescence and adulthood on behavior and VTA dopamine neuron activity later in life. In agreement with previous studies from our group (18,21,22), we observed that adolescent stress caused anxiety-like behavior, decreased sociability, and induced cognitive impairments in adulthood. We also found that adolescent stress increased the number of spontaneously active VTA dopamine neurons, which was not observed in animals exposed to stress during adulthood. Decreased PV^+, PNN^+, and PV^+/PNN^+ cells in the vHip were also observed after adolescent but not adult stress.

In rats, the vHip corresponds to the anterior hippocampus in humans, a structure involved in emotional and affective processing and stress response (34). Recent research suggests that the hippocampus is a central structure in several dysfunctions observed in stress-related psychopathologies (2,35). For example, VTA hyperactivity may be explained by PV loss or dysfunction in the vHip, which leads to heightened activity of pyramidal neurons and subsequent hyperactivation of dopaminergic neurons that project to striatal regions (2,36). This phenomenon has been associated with the emergence of psychotic symptoms (37,38). Also, through its projections to the PFC and amygdala, abnormal vHip activity impacts cognition and affective behaviors (37). PVI desynchronization has been linked to the various symptoms observed in schizophrenia (39). Reduced hippocampal PV expression has been observed in postmortem brain samples from individuals with schizophrenia (40,41), as well as in animal models of the disorder (42,43).

Our results suggest that the well-known heightened susceptibility to stress that occurs during adolescence is due to the vulnerability of PVIs in the vHip during that sensitive period, when PNNs are not yet fully established. PVIs are more vulnerable to oxidative and metabolic stress during this sensitive period of development, the moment of their maturation, and the refinement of GABAergic neurotransmission (2,44). The window of vulnerability for PVIs persists until the emergence of PNNs, a glycosaminoglycan extracellular matrix (16). PNNs typically consist of a hyaluronate backbone that connects CSPGs to the cell surface along with glycoproteins, such as tenascin-R and link proteins (45). These structures are crucial in protecting PVIs from potential harm and facilitating their activity states (17,46).

Given that PVIs have high activity and receive numerous glutamatergic projections from afferent structures, there is a risk of excessive activity-dependent Ca^{2+} influx into these cells, particularly when PV levels are low. This imbalance can generate reactive oxygen species, mitochondrial membrane rupture, and subsequent activation of apoptotic pathways (13,47). Therefore, PNNs act as a physical barrier, offering stability to glutamatergic inputs and shielding PVIs from oxidative and metabolic damage (19,20). Additionally, PNNs play a role in synaptic homeostasis by limiting axonal growth, synaptic rearrangement, and plasticity (16,48). Several studies have revealed the involvement of PNNs in the pathogenesis of different psychiatric disorders. For example, animal models of schizophrenia show a reduction in PNNs in the PFC (49,50), amygdala (50), and hippocampus (51,52). Corroborating this evidence, postmortem samples from individuals with schizophrenia also indicate a decrease in PNNs in the PFC (53), amygdala (54), and temporal lobe (55).

Recent studies estimate that in a mature brain, approximately 60% of PNNs are situated around PVIs and that, specifically in the hippocampal formation, PNNs surround 40% to 50% of PVIs (56). The absence of PNNs decreases the relative expression of PV in the vHip, which suggests that its protective effect may underlie the vulnerability of PVIs (57). PNNs undergo a prolonged maturation process, reaching complete formation in mid-to-late adolescence (53). Therefore, during adolescence, when PNNs are not fully formed, PVIs would be more susceptible to stress and potentially influence the development of psychiatric disorders (2). Consistent with our results, fewer PNNs during adolescence could underlie the greater vulnerability of PVIs to stress, thereby causing the long-lasting behavioral and electrophysiological changes induced by adolescent stress.

Modulating plasticity in adult life through pharmacological strategies that interfere with molecular brakes, including PNNs, offers insights into how stress may impact the organism during periods of greater plasticity. Previous studies by our group indicate that adult stress, along with sensitive period reopening induced by repeated administration of valproic acid (an inhibitor of histone deacetylase, an enzyme essential for limiting neuroplasticity in the adult brain), promotes changes in the number of PV^+/PNN^+ cells and behavioral and electrophysiological impairments similar to those observed after adolescent stress (18). Furthermore, knockout animal models of PNN components exhibit structural disorganization of these networks, a reduced number of PV^+ and PV^+/PNN^+ cells, and an excitatory-inhibitory imbalance (58,59). Local infusion of ChABC in adult animals is another approach that increases brain plasticity, similar to the sensitive period. ChABC is a bacterial enzyme that degrades the glycosaminoglycan chains in CSPGs, thereby removing the protection mediated by PNNs (23). Enzymatic ablation or rendering PNNs to a looser state can induce a juvenile-like phenotype characterized by heightened plasticity (60,61). Studies indicate that ChABC can reopen plasticity in the visual cortex (48), and this plasticity depends on intracellular signaling in PVIs (62).

The relationship between stress, PVIs, and PNNs has also been observed in animals that lack the PGC-1α coactivator, which acts as an antioxidant in GABAergic neurons. These animals have higher levels of oxidative stress and a reduction in PV^+ and PNN^+ cells. ChABC infusion into the retrosplenial cortex of these animals increases the impact of oxidative stress on PV^+ cell reduction (63). Furthermore, it has been observed that ChABC infusion into the prelimbic PFC of adult animals induces depression-like behaviors after an unpredictable chronic stress protocol, similar to that observed after adolescent stress (64). Animals presented the same behavioral impairments after a virus injection that degrades one of the PNNs components, neurocan. These studies highlight the importance of PNNs in protecting and preserving PVIs and support the hypothesis that degradation of PNNs may increase PVI vulnerability to stress.

To corroborate these findings and reinforce the idea that PVIs are more susceptible during periods of greater plasticity, we tested a pharmacological intervention that alters PNN

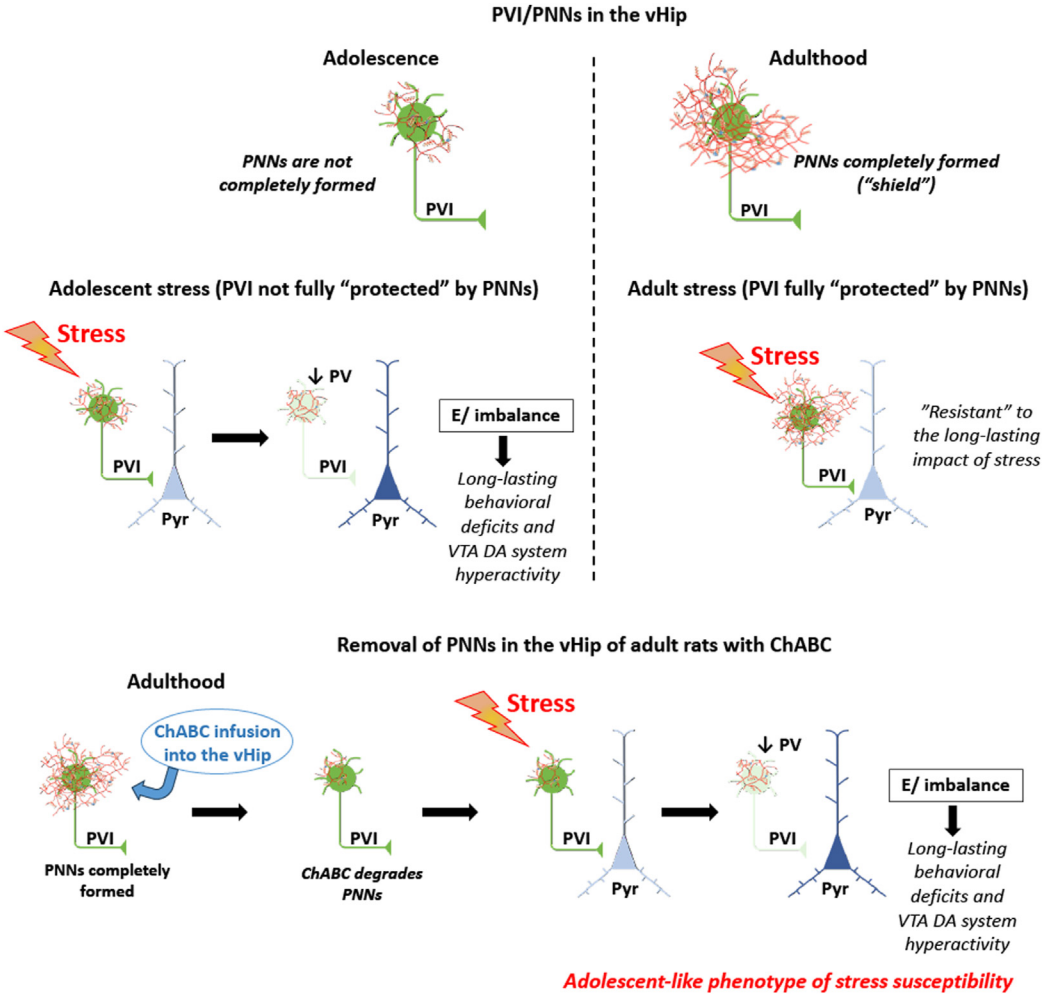

Figure 5. Schematic diagram outlining stress susceptibility and PNN dynamics. During adolescence, the incomplete formation of PNNs renders PVIs more vulnerable to the effects of stress, leading to PVI damage/loss. This, in turn, is proposed to lead to increases in the activity of glutamatergic pyramidal neurons and deficits in excitatory/inhibitory balance, causing long-lasting behavioral deficits and increased VTA DA system activity. In contrast, in adulthood, fully formed PNNs confer protection to PVIs, making them less susceptible to stress-induced damage. The local infusion of ChABC into the vHip of adult rats degrades PNNs. Consequently, PVIs become more vulnerable to stress, which results in similar behavioral and electrophysiological deficits observed after adolescent stress, recreating an adolescent-like phenotype of stress susceptibility. ChABC, chondroitinase ABC; DA, dopamine; PNN, perineuronal net; PV, parvalbumin; PVI, parvalbumin interneuron; Pyr, pyramidal neuron; vHip, ventral hippocampus; VTA, ventral tegmental area.

structure. Therefore, to degrade PNNs and recreate an adolescent-like phenotype of stress susceptibility, we infused ChABC bilaterally into the vHip of adult animals. After a few days of recovery, the animals were subjected to the same stress protocol. We observed that ChABC could degrade PNNs on what would be the first and last day of stress without affecting the number of PV^+ cells, thereby potentially rendering PVIs more vulnerable during the stress protocol.

We found that, similar to adolescent stress, removing PNNs from the vHip before adult stress caused cognitive and social interaction impairments and a hyperdopaminergic state in the VTA. Through immunofluorescence analysis, we found fewer PV^+, PNN^+, and PV^+/PNN^+ cells in animals that received ChABC intra-vHip before being subjected to adult stress. This suggests that stress may exacerbate degradation or delay PNN regeneration induced by ChABC because naïve animals that received this treatment did not show this long-lasting reduction. The absence of PNN protection around PVIs may explain the greater susceptibility of these animals to stress, further manifested by a decrease in PV^+ cells, indicative of potential PVI dysfunction. It is noteworthy that, despite the similarities for most of the deficits found in animals exposed to adolescent stress and animals that received ChABC intra-vHip and were exposed to adult stress intra-ChABC, some differences were found. For example, anxiety-related changes in the LDB were observed only after adolescent stress. Although more studies are needed to assess these differences, one putative mechanism could involve compensatory mechanisms in anxiety-related pathways.

Interestingly, we did not observe behavioral and electrophysiological changes in naïve animals that received ChABC intra-vHip. It has been observed previously that bilateral injection of ChABC into the vHip increased the activity of vHip pyramidal neurons and VTA dopamine neurons 1 week after drug infusion (65). ChABC rapidly degrades PNNs by about 90%, and PNNs gradually regenerate over time (66). In the current study, behavioral tests and electrophysiological recordings were performed between 5 and 8 weeks after surgery, when PNNs were reconstituted. This suggests that removing PNNs alone would not be sufficient to cause long-lasting behavior and dopaminergic changes but, in the face of stress, it leads to persistent downregulation of PVI numbers.

While the current study yielded consistent results that support our hypothesis, it is important to acknowledge some limitations. The *Wisteria floribunda* agglutinin marker, although widely used to detect PNNs in immunofluorescence, does not equally bind to all CSPGs. Therefore, it would be relevant to explore other markers specific to different proteoglycans (16). Additionally, we have discussed our findings focusing on the impact of stress on PVIs in their associated PNNs in the ventral subiculum of the vHip. Although PNNs primarily surround PVIs, PNNs also ensheath pyramidal neurons in the dorsal CA2 subregion of the hippocampus (67). While it is conceivable that pyramidal neurons present in the ventral subiculum may also have PNNs and could be affected by stress and/or ChABC infusion, this remains speculative and lacks substantial evidence. Finally, our research focused exclusively on male animals. Previous studies by our group have already evaluated the impact of the same stress protocol on adolescent females, but no behavioral or electrophysiological changes were observed (25). This is proposed to occur due to estrogen interference in PV maturation, which leads to their earlier maturation in females than males. However, replicating the study aiming for PNN degradation in females would be valuable.

This study focused on how increasing plasticity in adult life may negatively impact development. Nevertheless, it is essential to recognize the potential positive outcomes associated with this approach, such as amblyopia recovery in the visual system (48), functional improvement after lesions in the nigrostriatal tract (68) and the spinal cord (69), and extinction of conditioned fear memories (70). While these studies suggest that compromising PNN integrity may enhance cognition and plasticity, such manipulations can also weaken the neuroprotective barrier that this matrix provides, thereby increasing PVI's vulnerability to damage.

Conclusions

Overall, our findings indicate that the long-lasting impairments caused by adolescent stress may be attributed to the impact of stress on PVIs in the vHip, which are not yet protected by PNNs during this period. Furthermore, we suggest that degradation of PNNs in the vHip through ChABC infusion recapitulates an adolescent-like phenotype of stress susceptibility, highlighting the protective role of PNNs against stress-induced damage in PVIs (Figure 5). Our study provides insights into potential therapeutic strategies targeting these pathways to prevent and treat stress-related psychiatric disorders.

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REFERENCES

1. Brietzke E, Kauer Sant'anna M, Jackowski A, Grassi-Oliveira R, Bucker J, Zugman A, *et al.* (2012): Impact of childhood stress on psychopathology. Braz J Psychiatry 34:480–488.
2. Gomes FV, Zhu X, Grace AA (2019): Stress during critical periods of development and risk for schizophrenia. Schizophr Res 213:107–113.
3. Hensch TK (2005): Critical period plasticity in local cortical circuits. Nat Rev Neurosci 6:877–888.
4. Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, *et al.* (2010): Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. Br J Psychiatry 197:378–385.
5. Levitan RD, Parikh SV, Lesage AD, Hegadoren KM, Adams M, Kennedy SH, Goering PN (1998): Major depression in individuals with a history of childhood physical or sexual abuse: Relationship to neurovegetative features, mania, and gender. Am J Psychiatry 155:1746–1752.
6. Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC (2010): Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. Arch Gen Psychiatry 67:113–123.
7. Álvarez MJ, Roura P, Osés A, Foguet Q, Solà J, Arrufat FX (2011): Prevalence and clinical impact of childhood trauma in patients with severe mental disorders. J Nerv Ment Dis 199:156–161.
8. Kelleher I, Keeley H, Corcoran P, Ramsay H, Wasserman C, Carli V, *et al.* (2013): Childhood trauma and psychosis in a prospective cohort study: Cause, effect, and directionality. Am J Psychiatry 170:734–741.
9. Belujon P, Grace AA (2015): Regulation of dopamine system responsivity and its adaptive and pathological response to stress. Proc Biol Sci 282:20142516.
10. McEwen BS, Nasca C, Gray JD (2016): Stress Effects on Neuronal Structure: Hippocampus, amygdala, and Prefrontal Cortex. Neuropsychopharmacology 41:3–23.
11. Santos-Silva T, Hazar Ülgen D, Lopes CFB, Guimarães FS, Alberici LC, Sandi C, Gomes FV (2023): Transcriptomic analysis reveals mitochondrial pathways associated with distinct adolescent behavioral phenotypes and stress response. Transl Psychiatry 13:351.

12. Santos-Silva T, Lopes CFB, Hazar Ülgen D, Guimarães DA, Guimarães FS, Alberici LC, *et al.* (2024): Adolescent stress-induced ventral hippocampus redox dysregulation underlies behavioral deficits and excitatory/inhibitory imbalance related to schizophrenia. Schizophr Bull:sbae033.
13. Hu H, Gan J, Jonas P (2014): Interneurons. Fast-spiking, parvalbumin^{+} GABAergic interneurons: From cellular design to microcircuit function. Science 345:1255263.
14. Kann O, Papageorgiou IE, Draguhn A (2014): Highly energized inhibitory interneurons are a central element for information processing in cortical networks. J Cereb Blood Flow Metab 34:1270–1282.
15. Marín O (2016): Developmental timing and critical windows for the treatment of psychiatric disorders. Nat Med 22:1229–1238.
16. Fawcett JW, Oohashi T, Pizzorusso T (2019): The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. Nat Rev Neurosci 20:451–465.
17. Santos-Silva T, Colodete DAE, Lisboa JRF, Silva Freitas Í., Lopes CFB, Hadera V, *et al.* (2024): Perineuronal nets as regulators of parvalbumin interneuron function: Factors implicated in their formation and degradation. Basic Clin Pharmacol Toxicol 134:614–628.
18. Gomes FV, Zhu X, Grace AA (2020): The pathophysiological impact of stress on the dopamine system is dependent on the state of the critical period of vulnerability. Mol Psychiatry 25:3278–3291.
19. Cabungcal JH, Steullet P, Kraftsik R, Cuenod M, Do KQ (2013): Early-life insults impair parvalbumin interneurons via oxidative stress: Reversal by N-acetylcysteine. Biol Psychiatry 73:574–582.
20. Testa D, Prochiantz A, Di Nardo AA (2019): Perineuronal nets in brain physiology and disease. Semin Cell Dev Biol 89:125–135.
21. Gomes FV, Grace AA (2017): Prefrontal cortex dysfunction increases susceptibility to schizophrenia-like changes induced by adolescent stress exposure. Schizophr Bull 43:592–600.
22. Cavichioli AM, Santos-Silva T, Grace AA, Guimarães FS, Gomes FV (2023): Levetiracetam attenuates adolescent stress-induced behavioral and electrophysiological changes associated with schizophrenia in adult rats. Schizophr Bull 49:68–77.
23. Bucher EA, Collins JM, King AE, Vickers JC, Kirkcaldie MTK (2021): Coherence and cognition in the cortex: The fundamental role of parvalbumin, myelin, and the perineuronal net. Brain Struct Funct 226:2041–2055.
24. Uliana DL, Gomes FV, Grace AA (2021): Stress impacts cortico-amygdalar connectivity in an age-dependent manner. Neuropsychopharmacology 46:731–740.
25. Klinger K, Gomes FV, Rincón-Cortés M, Grace AA (2019): Female rats are resistant to the long-lasting neurobehavioral changes induced by adolescent stress exposure. Eur Neuropsychopharmacol 29:1127–1137.
26. Uliana DL, Gomes FV, Grace AA (2022): Nucleus reuniens inactivation reverses stress-induced hypodopaminergic state and altered hippocampal-accumbens synaptic plasticity. Neuropsychopharmacology 47:1513–1522.
27. Ungless MA, Grace AA (2012): Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. Trends Neurosci 35:422–430.
28. Härtig W, Meinicke A, Michalski D, Schob S, Jäger C (2022): Update on perineuronal net staining with Wisteria floribunda Agglutinin (WFA). Front Integr Neurosci 16:851988.
29. Guilloux JP, Seney M, Edgar N, Sibille E (2011): Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: Relevance to emotionality and sex. J Neurosci Methods 197:21–31.
30. Wang R, Wang W, Xu J, Liu D, Jiang H, Pan F (2018): Dynamic effects of early adolescent stress on depressive-like behaviors and expression of cytokines and JMJD3 in the prefrontal cortex and hippocampus of rats. Front Psychiatry 9:471.
31. Myin-Germeys I, van Os J (2007): Stress-reactivity in psychosis: Evidence for an affective pathway to psychosis. Clin Psychol Rev 27:409–424.
32. Dean J, Keshavan M (2017): The neurobiology of depression: An integrated view. Asian J Psychiatr 27:101–111.
33. Leonardo ED, Hen R (2008): Anxiety as a developmental disorder. Neuropsychopharmacology 33:134–140.
34. Fanselow MS, Dong H-W (2010): Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7–19.
35. Albrecht A, Redavide E, Regev-Tsur S, Stork O, Richter-Levin G (2021): Hippocampal GABAergic interneurons and their co-localized neuropeptides in stress vulnerability and resilience. Neurosci Biobehav Rev 122:229–244.
36. Lodge DJ, Grace AA (2007): Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. J Neurosci 27:11424–11430.
37. Grace AA, Gomes FV (2019): The circuitry of dopamine system regulation and its disruption in schizophrenia: Insights into treatment and prevention. Schizophr Bull 45:148–157.
38. McCutcheon RA, Reis Marques T, Howes OD (2020): Schizophrenia-an overview. JAMA Psychiatry 77:201–210.
39. Lodge DJ, Behrens MM, Grace AA (2009): A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia. J Neurosci 29:2344–2354.
40. Konradi C, Yang CK, Zimmerman EI, Lohmann KM, Gresch P, Pantazopoulos H, *et al.* (2011): Hippocampal interneurons are abnormal in schizophrenia. Schizophr Res 131:165–173.
41. Zhang ZJ, Reynolds GP (2002): A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res 55:1–10.
42. Santos-Silva T, Dos Santos Fabris D, de Oliveira CL, Guimarães FS, Gomes FV (2024): Prefrontal and hippocampal parvalbumin interneurons in animal models for schizophrenia: A systematic review and meta-analysis. Schizophr Bull 50:210–223.
43. Steullet P, Cabungcal JH, Coyle J, Didriksen M, Gill K, Grace AA, *et al.* (2017): Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. Mol Psychiatry 22:936–943.
44. Caballero A, Tseng KY (2016): GABAergic function as a limiting factor for prefrontal maturation during adolescence. Trends Neurosci 39:441–448.
45. Carceller H, Gramuntell Y, Klimczak P, Nacher J (2023): Perineuronal nets: Subtle structures with large implications. Neuroscientist 29:569–590.
46. Soleman S, Filippov MA, Dityatev A, Fawcett JW (2013): Targeting the neural extracellular matrix in neurological disorders. Neuroscience 253:194–213.
47. Ruden JB, Dugan LL, Konradi C (2021): Parvalbumin interneuron vulnerability and brain disorders. Neuropsychopharmacology 46:279–287.
48. Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002): Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248–1251.
49. Matuszko G, Curreli S, Kaushik R, Becker A, Dityatev A (2017): Extracellular matrix alterations in the ketamine model of schizophrenia. Neuroscience 350:13–22.
50. Paylor JW, Lins BR, Greba Q, Moen N, de Moraes RS, Howland JG, Winship IR (2016): Developmental disruption of perineuronal nets in the medial prefrontal cortex after maternal immune activation. Sci Rep 6:37580.
51. Fujikawa R, Yamada J, Jinno S (2021): Subclass imbalance of parvalbumin-expressing GABAergic neurons in the hippocampus of a mouse ketamine model for schizophrenia, with reference to perineuronal nets. Schizophr Res 229:80–93.
52. Klimczak P, Rizzo A, Castillo-Gómez E, Perez-Rando M, Gramuntell Y, Beltran M, Nacher J (2021): Parvalbumin interneurons and perineuronal nets in the hippocampus and retrosplenial cortex of adult male mice after early social isolation stress and perinatal NMDA receptor antagonist treatment. Front Synaptic Neurosci 13:733989.
53. Mauney SA, Athanas KM, Pantazopoulos H, Shaskan N, Passeri E, Berretta S, Woo T-UW (2013): Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. Biol Psychiatry 74:427–435.

54. Pantazopoulos H, Markota M, Jaquet F, Ghosh D, Wallin A, Santos A, *et al.* (2015): Aggrecan and chondroitin-6-sulfate abnormalities in schizophrenia and bipolar disorder: A postmortem study on the amygdala. Transl Psychiatry 5:e496.
55. Pantazopoulos H, Woo T-UW, Lim MP, Lange N, Berretta S (2010): Extracellular matrix-glial abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia. Arch Gen Psychiatry 67:155–166.
56. Lupori L, Totaro V, Cornuti S, Ciampi L, Carrara F, Grilli E, *et al.* (2023): A comprehensive atlas of perineuronal net distribution and colocalization with parvalbumin in the adult mouse brain. Cell Rep 42:112788.
57. Yamada J, Ohgomori T, Jinno S (2015): Perineuronal nets affect parvalbumin expression in GABAergic neurons of the mouse hippocampus. Eur J Neurosci 41:368–378.
58. Gottschling C, Wegrzyn D, Denecke B, Faissner A (2019): Elimination of the four extracellular matrix molecules tenascin-C, tenascin-R, brevican and neurocan alters the ratio of excitatory and inhibitory synapses. Sci Rep 9:13939.
59. Mueller-Buehl C, Reinhard J, Roll L, Bader V, Winklhofer KF, Faissner A (2022): Brevican, neurocan, tenascin-C, and tenascin-R act as important regulators of the interplay between perineuronal nets, synaptic integrity, inhibitory interneurons, and Otx2. Front Cell Dev Biol 10:886527.
60. Willis A, Pratt JA, Morris BJ (2022): Enzymatic degradation of cortical perineuronal nets reverses GABAergic interneuron maturation. Mol Neurobiol 59:2874–2893.
61. Reichelt AC, Hare DJ, Bussey TJ, Saksida LM (2019): Perineuronal nets: Plasticity, protection, and therapeutic potential. Trends Neurosci 42:458–470.
62. Lesnikova A, Casarotto PC, Fred SM, Voipio M, Winkel F, Steinzeig A, *et al.* (2021): Chondroitinase and antidepressants promote plasticity by releasing TRKB from dephosphorylating control of PTPσ in parvalbumin neurons. J Neurosci 41:972–980.
63. Wang J, Song H-R, Guo M-N, Ma S-F, Yun Q, Liu W-J, *et al.* (2020): PGC-1α regulate critical period plasticity via gene × environment interaction in the developmental trajectory to schizophrenia. Biochem Biophys Res Commun 525:989–996.
64. Yu Z, Han Y, Hu D, Chen N, Zhang Z, Chen W, *et al.* (2022): Neurocan regulates vulnerability to stress and the anti-depressant effect of ketamine in adolescent rats. Mol Psychiatry 27:2522–2532.
65. Shah A, Lodge DJ (2013): A loss of hippocampal perineuronal nets produces deficits in dopamine system function: Relevance to the positive symptoms of schizophrenia. Transl Psychiatry 3:e215.
66. Smail MA, Smith BL, Shukla R, Alganem K, Eby HM, Bollinger JL, *et al.* (2023): Molecular neurobiology of loss: A role for basolateral amygdala extracellular matrix. Mol Psychiatry 28:4729–4741.
67. Carstens KE, Phillips ML, Pozzo-Miller L, Weinberg RJ, Dudek SM (2016): Perineuronal nets suppress plasticity of excitatory synapses on CA2 pyramidal neurons. J Neurosci 36:6312–6320.
68. Moon LDF, Asher RA, Rhodes KE, Fawcett JW (2001): Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci 4:465–466.
69. Bradbury EJ, Moon LDF, Popat RJ, King VR, Bennett GS, Patel PN, *et al.* (2002): Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636–640.
70. Gogolla N, Caroni P, Lüthi A, Herry C (2009): Perineuronal nets protect fear memories from erasure. Science 325:1258–1261.