

Timely N-Acetyl-Cysteine and Environmental Enrichment Rescue Oxidative Stress-Induced Parvalbumin Interneuron Impairments via MMP9/RAGE Pathway: A Translational Approach for Early Intervention in Psychosis

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Research in schizophrenia (SZ) emphasizes the need for new therapeutic approaches based on antioxidant/anti-inflammatory compounds and psycho-social therapy. A hallmark of SZ is a dysfunction of parvalbumin-expressing fast-spiking interneurons (PVI), which are essential for neuronal synchrony during sensory/cognitive processing. Oxidative stress and inflammation during early brain development, as observed in SZ, affect PVI maturation. We compared the efficacy of N-acetylcysteine (NAC) and/or environmental enrichment (EE) provided during juvenile and/or adolescent periods in rescuing PVI impairments induced by an additional oxidative insult during childhood in a transgenic mouse model with glutathione deficit (*Gclm* KO), relevant for SZ. We tested whether this rescue was promoted by the inhibition of MMP9/RAGE mechanism, both in the mouse model and in early psychosis (EP) patients, enrolled in a double-blind, randomized, placebo-controlled clinical trial of NAC supplementation for 6 months. We show that a sequential combination of NAC+EE applied after an early-life oxidative insult recovers integrity and function of PVI network in adult *Gclm* KO, via the inhibition of MMP9/RAGE. Six-month NAC treatment in EP patients reduces plasma sRAGE in association with increased prefrontal GABA, improvement of cognition and clinical symptoms, suggesting similar neuroprotective mechanisms. The sequential combination of NAC+EE reverses long-lasting effects of an early oxidative insult on PVI/perineuronal net (PNN) through the inhibition of MMP9/RAGE mechanism. In analogy, patients vulnerable to early-life insults could benefit from a combined pharmacological and psycho-social therapy.

Key words: antioxidant/physical exercise/mechanism/brain development/early psychosis/cognition

Introduction

The search for effective treatments in schizophrenia (SZ) is challenging due to the complexity of the disorder and the high heterogeneity of patients at genetic, pathophysiological, and clinical levels. Following a shift in clinical approach towards early intervention and preventive strategies for a better patient prognosis, there is also a need for preclinical studies to uncover pathophysiology and translational mechanism-based therapeutic approach linked to impaired brain function during adolescent development. SZ pathophysiology implicates both genetic and environmental factors interacting especially at early stage of brain development, triggering, among others, oxidative stress and neuroinflammation.¹⁻⁴ Environmental stressors such as obstetrical complication, maternal infection during pregnancy, but also adverse childhood experiences (ACE), that are well-known risk factors for SZ, induce oxidative stress and inflammation.^{1,5-15}

Based on these evidence, several clinical studies focusing on the use of antioxidants and anti-inflammatory compounds as add-on to antipsychotics show promising results. Specifically, the antioxidant and glutathione (GSH) precursor N-Acetyl Cysteine (NAC) has been shown to improve symptoms,¹⁶⁻¹⁹ white matter integrity in fornix,²⁰ and EEG mismatch negativity²¹ in SZ or early psychosis (EP) patients.

Although 2 recent meta-analyses^{22,23} concluded that, in general, NAC demonstrates some efficacy for the treatment of SZ symptoms, some studies found limited benefits²⁴ or improvement only in specific subgroups of patients.^{17,19}

In addition to pharmacological treatments, physical exercise, which modulates the immune system,²⁵⁻²⁷ showed

beneficial effects in SZ.^{28–34} In SZ rodent models, physical exercise and animal housing in an enriched environment (EE) reduces oxidative stress,^{35,36} neuroinflammation,^{37,38} and SZ-like behaviors.^{39–42} Thus, NAC and EE may have similar or overlapping effects on pathological mechanisms mediated by oxidative stress and neuroinflammation, such as impairments of parvalbumin interneurons (PVI). Anomaly of PVI and their enwrapping perineuronal nets (PNN) is a hallmark of SZ^{43–46} and contributes to abnormal high-frequency neuronal synchronization,^{47,48} impacting multiple information processing critical for sensory perception and cognition, and possibly promoting hyperdopaminergia related to positive symptoms.^{49–53} PVI are vulnerable to oxidative stress and inflammation, especially during their development.^{54–58} A deleterious feedforward interaction between oxidative stress and neuroinflammation through MMP9/RAGE pathway during specific developmental periods appears to be central to PVI impairments.⁵⁹

In order to intervene early on precise pathophysiological mechanisms mediated by oxidative stress/neuroinflammation, it is crucial to better define both the optimal timing and type of such interventions that would lead to long-term beneficial effects.

The aim of this study was to (1) compare the efficacy of NAC and/or EE provided during juvenile and/or adolescent periods for PVI/PNN impairments rescue in an SZ model, (2) understand the underlying mechanisms associated with the timely beneficial effects, and (3) explore in EP patients the effect of NAC on the described mechanisms.

Here, we studied *Gclm* KO mice which have a 70% decrease in brain GSH due to the lack of the GCL (glutamate-cysteine ligase) modulatory subunit (*Gclm*).^{60,61} These mice display increased oxidative stress in some brain regions, such as the anterior cingulate cortex (ACC) and show SZ-related phenotypes.^{54,60,62–67} Most importantly, an additional oxidative stress challenge applied during childhood, to mimic stressful events, induces perduring PVI impairments into adulthood, in line with the developmental aspect of SZ.⁵⁴

We evaluated the short and long-term benefits of NAC and/or EE provided at different timing on PVI, following the early-life oxidative challenge. We found that NAC administration during the juvenile/peripubertal period, when followed by EE during the adolescence, prevents the long-term impairment of PVIs through inhibition of the MMP9/RAGE cascade.⁵⁹ NAC add-on treatment in EP patients also mitigates the MMP9/RAGE mechanism in association with increased prefrontal GABA levels and improvements of clinical symptoms and cognitive function.

Materials and Methods

Animals

Gclm KO mice⁶⁸ were backcrossed with C57BL/6J mice as described previously.⁵⁴ To induce an additional oxidative

stress, GBR-12909 dihydrochloride (GBR, BioTrend, 5mg/kg), a dopamine reuptake inhibitor, was subcutaneously injected to *Gclm*-KO and WT mice, postnatal days (PND10)-PND20. NAC (900mg/L, PharmaNAC, BioAdvantexPharma) was delivered in drinking water, PND21-PND35. EE was provided either PND21-PND40 or PND36-PND57 ([supplementary information](#)).

Immunohistochemistry

Animals were sacrificed at PND40 or PND90 and coronal slices (40 μ m) were used for the IH quantification. The antibodies list is presented in the [supplementary information](#).

Confocal and Image Analysis

Immunohistological images were obtained with a Zeiss LSM780 Quasar confocal microscope. More details in the [supplementary information](#), and elsewhere.^{54,59}

Electrophysiology

Paracoronar slices (400- μ m, Bregma~1.4–0.6) containing the ACC were prepared as described in Steullet et al.⁶⁹ The specific details in the [supplementary information](#).

Subjects Recruitment

Data from EP patients included in the present study comes from the original sample of the NAC randomised controlled trial that was already published.^{17,70} More details in the [supplementary information](#).

Magnetic Resonance Spectroscopy Acquisition and Analysis

All MR measurements were carried out on a 3T-MR scanner (Magnetom TimTrio, Siemens Healthcare) with a transverse electromagnetic (TEM3000) head coil (MRIInstruments, Inc).⁷¹ More details elsewhere^{59,72,73} and in the [supplementary information](#).

Results

Short-Term Effect at Peripuberty: Juvenile/Peripubertal NAC Treatment but not EE Rescues PVI/PNN Through MMP9/RAGE-Dependent Mechanism

In *Gclm* KO mice, an early-life GBR (PND10-PND20) induces an additional oxidative stress which affects maturation of PVI in the ACC and subsequently their long-term impairments.^{54,59} These effects are mediated by a mechanism involving the shedding of RAGE by MMP9 during juvenile/peripubertal stage that maintains a perduring oxidative stress and neuroinflammation through a feedforward loop.⁵⁹

First, we examined the short-term effect (at PND40) of either a juvenile/peripubertal NAC (PND21-PND35) or EE (PND21-PND40), on the maturation of PVI in the ACC of GBR-treated KO mice (figure 1A). PVI maturation was evaluated by quantifying the density of parvalbumin-immunoreactive (PV-IR) cells and the number of PV-IR cells surrounded by PNN (stained with WFA).⁷⁴⁻⁷⁶ The decrease in PV-IR cell density and PNN in GBR-treated KO mice was completely abolished by NAC, but not EE (figure 1B).

Then, we investigated MMP9/RAGE pathway activation, by measuring MMP9 protein expression and RAGE shedding. The shedding of RAGE is assessed with 2 antibodies targeting its extra and intra-cellular domains, respectively. The extracellular-domain antibody binds to the uncleaved membrane-bound RAGE (extra-RAGE). The intracellular antibody recognizes the intracellular domain of RAGE (intra-RAGE) that translocates to the nucleus after shedding.⁵⁹ As previously shown,⁵⁹ MMP9 expression and RAGE shedding (ratio of intra- over extra-RAGE) were elevated in PND40 KO mice, and further increased by early-life GBR (figure 1B). Juvenile/peripubertal NAC, but not EE, decreased both MMP9 expression and RAGE shedding (figure 1B).

Finally, we observed that oxidative stress (8-oxoDG) and microglia activation (Iba1 and CD68) were high in PND40 KO mice and further increased by GBR (supplementary figure 1B). Juvenile/peripubertal NAC, but not EE, decreased these markers of oxidative stress and neuroinflammation to levels found in WT mice (supplementary figure 1B).

Collectively, these show that NAC treatment at PND21-35 blocks the oxidative stress/MMP9/RAGE pathway and rescues PVI alterations at peripuberty. This was linked to the antioxidant and/or anti-inflammatory property of NAC, as EE applied at the same time period had no effect on MMP9/RAGE and PVI recovery.

Long-Term Effect at Adulthood: Neither Juvenile/Peripubertal NAC Nor EE Rescue PVI/PNN

As juvenile/peripubertal NAC blocked the MMP9/RAGE pathway during the PVI maturation period allowing PVI recovery at PND40, we examined the long-term effect of such NAC treatment on PVI/PNN at adulthood (PND90). As previously shown,⁵⁴ early-life GBR decreased PV-IR cell density and PNN in ACC of adult KO but not WT mice (figure 2B). This was accompanied with increased MMP9 expression, microglial activation, and oxidative stress (figure 2B, supplementary figure 2B).

Although juvenile/peripubertal NAC restored PVI/PNN integrity at PND40 (figure 1B) and maintained low levels of oxidative stress, microglia activation, and MMP9 (figure 2B, supplementary figure 2B) until adulthood, it did not fully succeed in preserving long-term PVI/PNN integrity at PND90 (figure 2B).

As juvenile/peripubertal EE did not show any short-term protection of PVI/PNN in ACC of PND40 GBR-treated KO mice, we assessed the impact of EE when applied during the adolescent period (PND36-PND57). We found that adolescent EE also had no long-term effect on PVI/PNN (figure 2B). However, adolescent EE fully prevented oxidative stress and microglia activation at adulthood (supplementary figure 2B), while only partially decreasing MMP9 at the levels of PBS-treated KO, without reaching the levels observed in WT (figure 2B).

Long-Term Effect at Adulthood: Combined Juvenile/Peripubertal NAC and Adolescent EE Rescue PVI/PNN Through MMP9/RAGE-Dependent Mechanism

As both juvenile/peripubertal NAC and EE, individually failed to fully rescue PVI in adulthood, we tested the long-term effect of a combined treatment consisting of juvenile/peripubertal NAC (PND21-35) followed by EE during adolescence (PND36-57). We hypothesized that juvenile/peripubertal NAC would prevent oxidative stress-induced impairment of PVI/PNN by blocking MMP9/RAGE pathway, and the following EE during adolescence would further enhance their final maturation, function, and integrity, as well as keeping oxidative stress and neuroinflammation to low levels.^{36,56}

The combination of NAC+EE completely prevented the perduring GBR-induced PVI/PNN impairments in adult KO mice (figure 2B). NAC+EE also fully normalized MMP9 (figure 2B), microglia activation, and oxidative stress levels in adult KO mice (supplementary figure 2B). These results suggest that timely combination of NAC+EE following an early-life stress allows the full recovery of PVI/PNN integrity at adulthood.

Combined Juvenile/Peripubertal NAC and Adolescent EE Rescue High-Frequency Oscillations

We then examined whether an early-life GBR treatment also affected the fast-oscillatory neuronal activity in the ACC of adult KO mice, and if so, whether NAC combined with EE could restore it. High-frequency (β) oscillations were induced pharmacologically in ACC slices via the perfusion of carbachol, kainate, and quinpirole. In previous studies conducted in the ACC of adult mice (ie, 3 mo), we reported that these oscillations, generated within local networks of excitatory and inhibitory neurons,⁶⁷ were not significantly different between PBS-treated KO and WT mice,⁵⁴ and that WT mice were not affected by a GBR administration PND10-20.⁷⁷ Here, we observed that an early-life GBR treatment in KO mice significantly decreased β -power at PND90, concomitant with the impaired PVI/PNN network (figures 3A and 3B). More importantly, the sequential treatment of juvenile/peripubertal NAC followed by adolescent EE not only restored normal integrity of PVI/PNN (figure 2B)

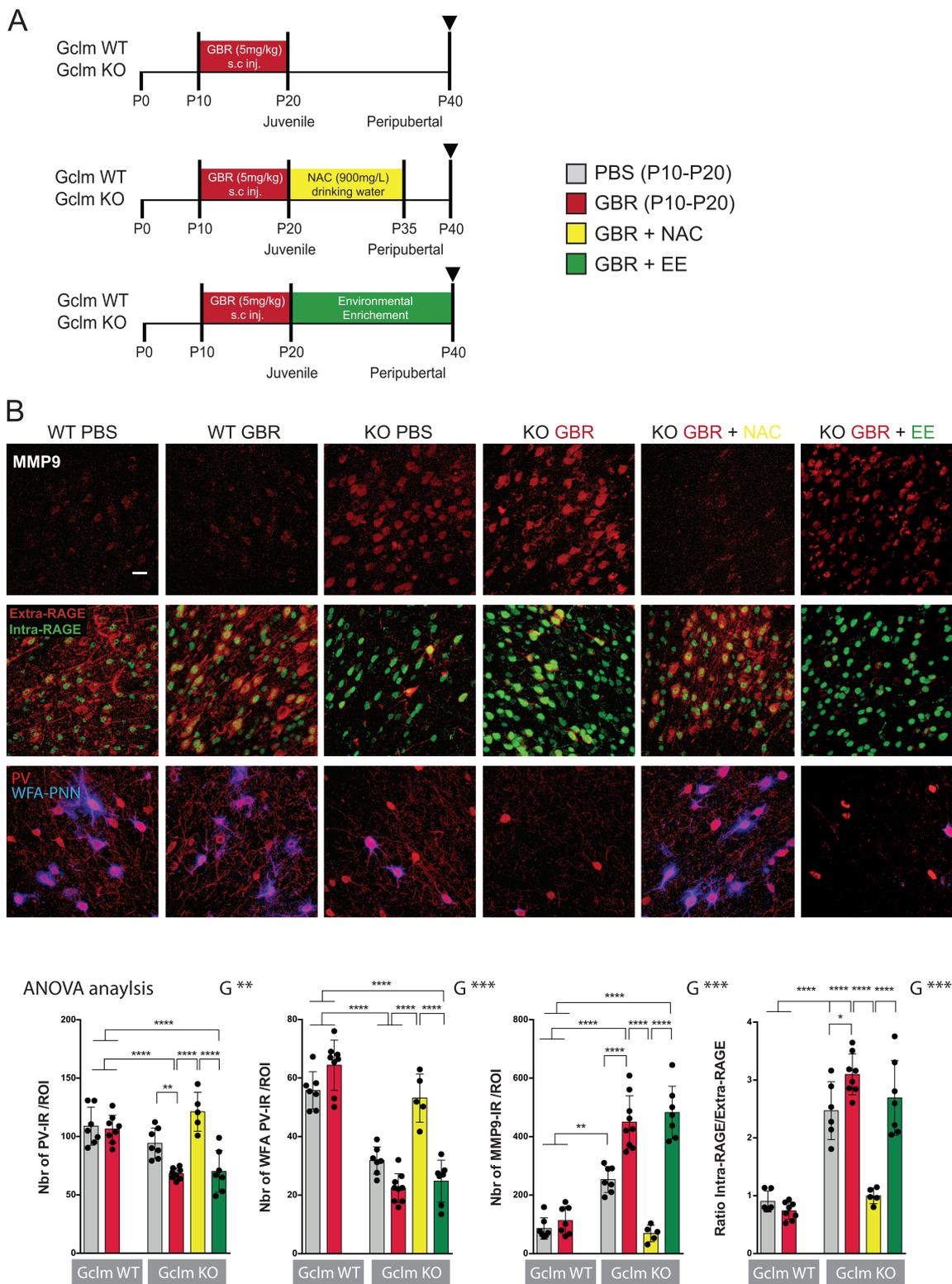


Fig. 1. NAC, but not EE, after an additional early-life oxidative challenge, prevents MMP9/RAGE activation and PV/PNN decrease in *Gclm* KO at PND40. (A) Protocol scheme. (B) MMP9, intraRAGE/extraRAGE ratio, PV and WFA in *Gclm* KO and WT mice: confocal images (scale bar:30 μ m) and quantification graphs. Data expressed as mean \pm s.e.d. (mice $N = 5-7$). ANOVA analysis: G = genotype, T = treatment, R = recovery, x = interaction; * $P < .05$; ** $P < .01$; *** $P < .001$, analyzed by Tukey post-hoc test.

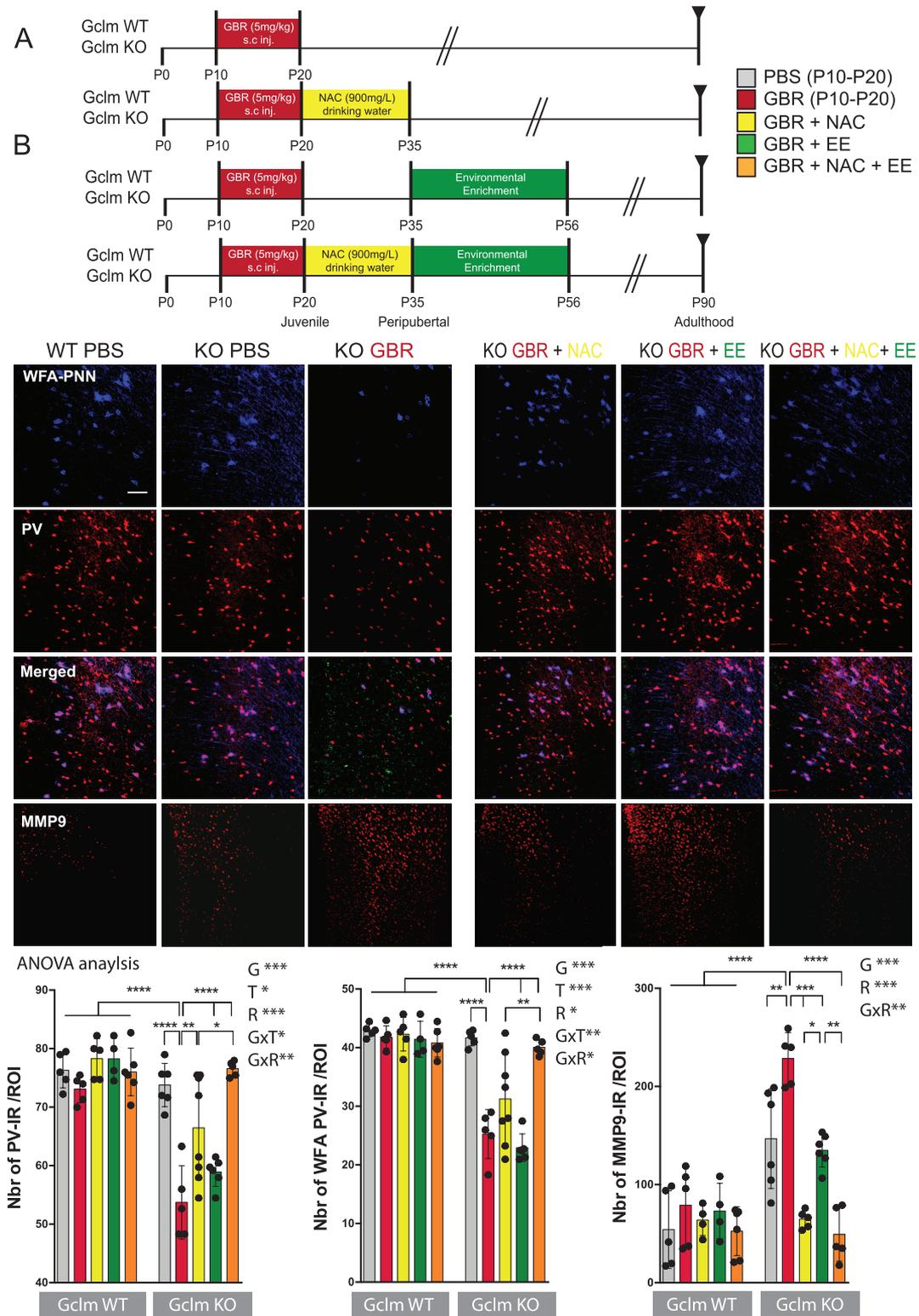


Fig. 2. NAC+EE after an additional early-life oxidative challenge recover PV/PNN and MMP9 in *Gclm* KO at adulthood. (A) Protocol scheme. (B) PV, WFA and MMP9 in *Gclm* KO and WT mice: confocal images (scale bar: 50 μ m) and quantification graphs. Data expressed as mean \pm s.e.d. (mice $N = 5-8$). ANOVA analysis: G = genotype, T = treatment, R = recovery, x = interaction; * $P < .05$; ** $P < .01$; *** $P < .001$, analyzed by Tukey post-hoc test.

but also fully restored the ability of the ACC to generate and sustain high-frequency oscillations at PND90.

*Reverse Translation to Early Psychosis Patients:
NAC Add-on Treatment Reduces MMP9/RAGE
Pathway Overactivation in Association With Increased
Prefrontal GABA Levels and Improvement in
Processing Speed and Positive Symptoms*

Previously, we reported that the soluble form of RAGE (sRAGE) was increased in the plasma of EP patients, reflecting an enhanced RAGE shedding via oxidative stress-induced MMP9 activation.⁵⁹ Moreover, higher sRAGE levels were associated with lower GABA levels in patients' prefrontal cortex as assessed by magnetic resonance spectroscopy (MRS),⁵⁹ suggesting a potential functional link between RAGE shedding and interneuron integrity. Here, we investigated whether NAC inhibits RAGE shedding in EP patients, as observed in brains of *Gclm* KO. We measured sRAGE in the plasma of EP patients enrolled in a double-blind, randomized, placebo-controlled trial of NAC supplementation for 6 months.¹⁷ Six-month NAC increased prefrontal GSH levels, improved processing speed, as well as positive symptoms in a sub-group of patients with high peripheral oxidative status.¹⁷ Here, we showed that NAC also decreased sRAGE levels, with no change in the placebo group (figure 4A). Moreover, in the NAC- but not placebo-treated group, we found a negative correlation between the changes in peripheral sRAGE and in prefrontal GABA (figure 4B), indicating that NAC leads to a normalization of sRAGE levels in association with an increase in prefrontal GABA levels. Interestingly, NAC-induced sRAGE decrease was significantly correlated with improvement in processing speed (figure 4C),

a domain that was already shown to be improved by NAC.¹⁷ A similar trend was found for working memory after NAC treatment (supplementary figure 3A). These suggest that decreased level of peripheral sRAGE following NAC is associated with amelioration of some cognitive functions in particular processing speed.^{17,78,79} Finally, in the NAC-treated group, we also found an association between decreased sRAGE and an improvement of clinical symptoms scores (PANSS positive and PANSS total) (figure 4D, supplementary figure 3B). Overall, our results indicate that the normalization of sRAGE levels by NAC treatment in EP patients correlates with an increase in prefrontal GABA levels and an improvement of cognition and psychotic symptoms.

Discussion

We show that the sequential combination of an antioxidant treatment and EE applied during the juvenile and adolescent periods respectively normalizes the integrity and function of PVI/PNN networks in ACC of adult *Gclm* KO after an early-life oxidative insult. This recovery is mediated by NAC, possibly via the inhibition of oxidative stress-induced MMP9/RAGE pathway. NAC interrupts this deleterious feedforward mechanism that maintains perduring high levels of oxidative stress and neuroinflammation, allowing PVI/PNN maturation. A subsequent EE during adolescence promotes the final maturation of PVI, providing a long-term neuroprotection to PVI/PNN networks. Our clinical study suggests that NAC engages similar neuroprotective mechanisms in EP patients. Indeed, a 6-month NAC add-on treatment reduces plasma sRAGE in association with an increase in prefrontal GABA level, and with improvements of working memory, processing speed, and positive symptoms.

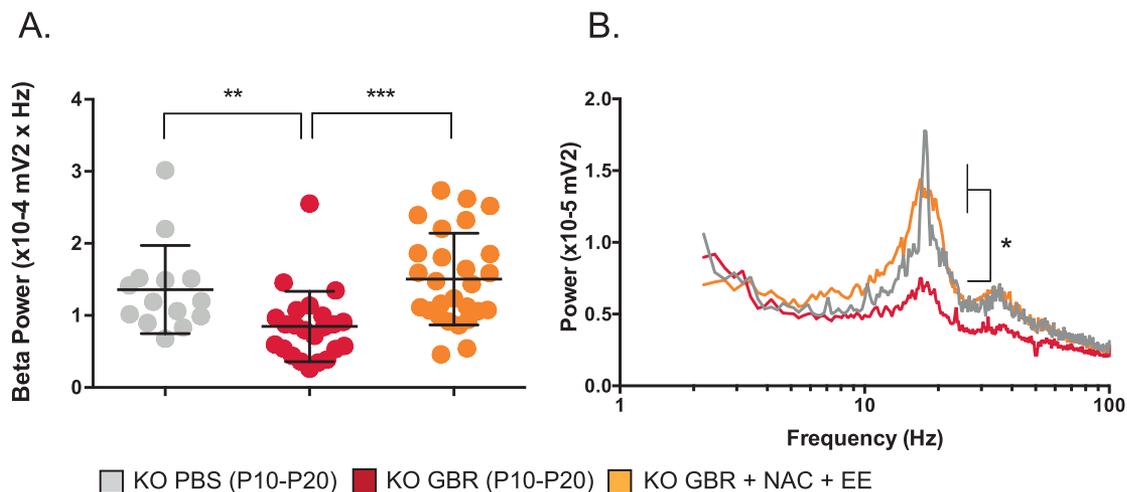


Fig. 3. Pharmacologically-induced high-frequency(β) oscillations in ACC slices are affected by an early-life oxidative challenge in *Gclm* KO mice, but are fully normalized by NAC+EE. (A–B) Effect of GBR and NAC+EE in KO mice. (D) Illustration of the average power spectrum for each experimental group. Box plots depict medians and quartiles; bars show values in the 1.5 box length. KO (mice $N = 5$, cells $N = 14$), KO+GBR(mice $N = 7$, cells $N = 23$), KO+GBR +NAC+EE (mice $N = 8$, cells $N = 27$). ** $P = .004$; *** $P < .001$.

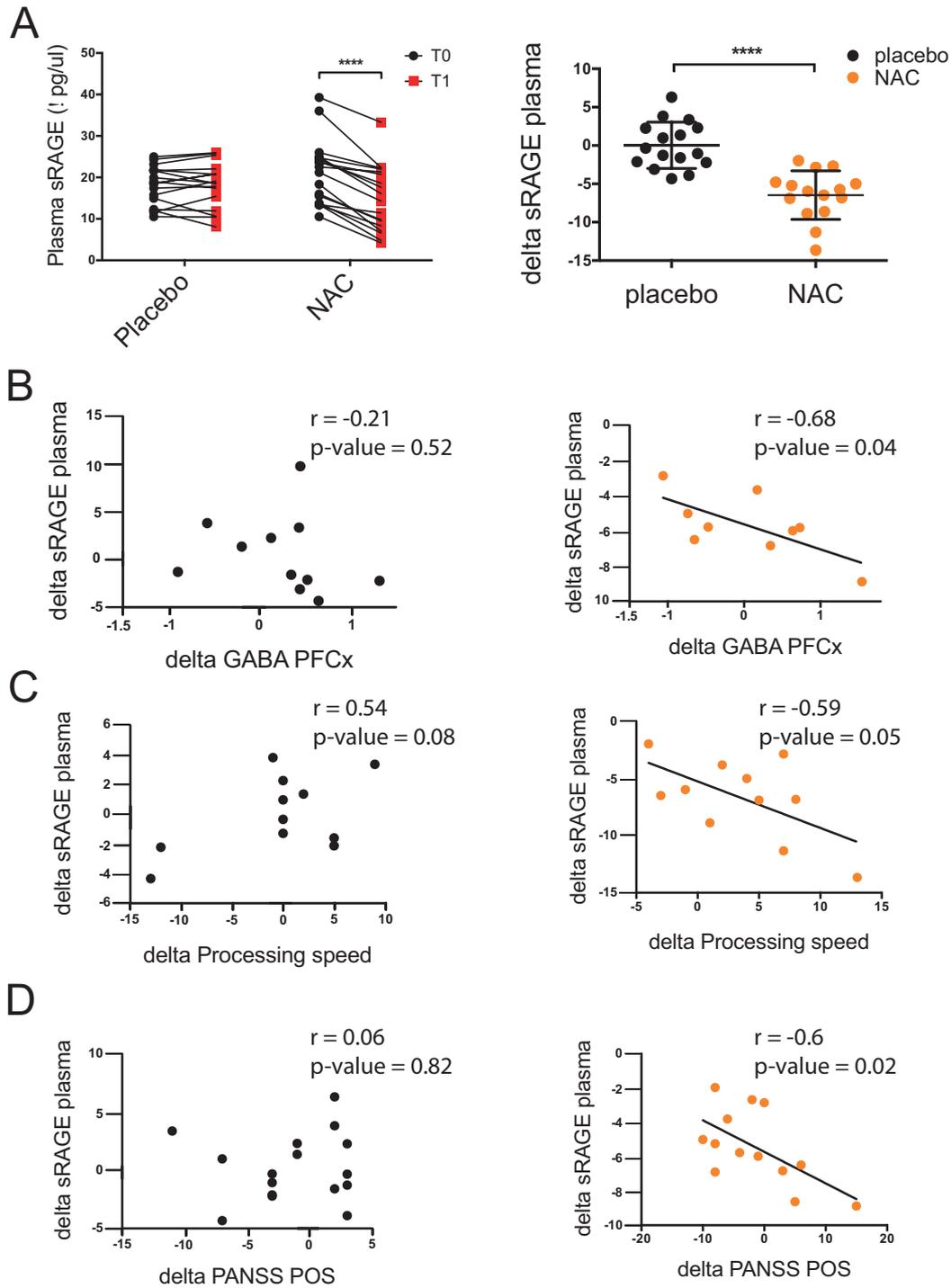


Fig. 4. sRAGE level is decreased after 6-mo NAC add-on treatment in EP patients and is associated with increased GABA level in the PFCx, as well as improvements in cognition and positive symptoms. (A) sRAGE decrease in the NAC-treated group ($N = 18$; $P < .0001$) compared to the placebo group ($N = 18$). (B) Negative correlation between delta GABA and delta sRAGE in NAC-treated group ($N = 9$; $r = -0.68$, $P = .04$). (C) Negative correlation between delta processing speed and delta sRAGE in NAC-treated group ($N = 11$; $r = -0.59$, $P = .05$). (D) Negative correlation between delta PANSS positive symptoms and delta sRAGE in NAC-treated group ($N = 13$; $r = -0.6$, $P = .02$).

Although converging evidence indicates that both NAC and EE can confer neuroprotection via overlapping mechanisms, our results show a different effect of NAC

and EE on neuroinflammation, oxidative stress, and PVI/PNN recovery in an animal model, with a genetic risk for impaired antioxidant defense (figure 5).

Effect of NAC Treatment

Juvenile/peripubertal NAC, but not EE, decreases microglia activation and oxidative stress, and fully recovers PVI/PNN maturation at PND40 (figure 5). Indeed, NAC during PVI maturation blocks the overactivation of the MMP9/RAGE pathway initiated by the early-life oxidative challenge that is responsible for the long-lasting PVI/PNN impairments in *Gclm* KO.⁵⁹ This may stop the perduring feedforward process between oxidative stress and microglia activation, allowing normal maturation of PNN, and subsequently PVIs at PND40. However, an early NAC treatment alone is not sufficient to fully maintain a long-term protection of PVI/PNN networks in adult *Gclm* KO (figure 2). This may be due to the genetically compromised antioxidant GSH system which may impinge the long-term maintenance of PVI/PNN integrity despite minimal oxidative stress and microglia activation. Previously, NAC has been shown to decrease oxidative stress, rescue PVI deficit and SZ-like behaviors in several animal models, including *Gclm* KO mice.^{54,77–81} However, in these studies, NAC was provided either before or during the early-life oxidative stress insult and maintained until the end of the experiments. The demonstration that a short NAC administration after the early-life oxidative challenge has also an effect is promising in view of clinical settings.

Effect of EE

EE alone during adolescence, but not during the juvenile/peripubertal period, reduces oxidative stress and microglial activation. The EE-effect during adolescence may be due to the combined reduction of ROS produced by microglia,³⁷ increase of neurotrophic factors such as BDNF, and activation of NMDAR,⁸² which enhances the antioxidant defenses.³ The lack of impact of EE during the juvenile/peripubertal period may be due to the impaired GSH system in *Gclm* KO and its lack of effect on MMP9.

Actually, in animals with intact antioxidant defenses, EE during juvenile/peripuberty boosts PVI-associated network maturation^{83–85} and prevents PVI impairment induced by either early postnatal hypoxia or anesthesia.^{36,56,86} This strongly suggests that the protective effect of EE on PVI/PNN during juvenile/peripubertal requires an intact GSH system. Juvenile/peripubertal EE favors PVI maturation by enhancing PV expression,⁸³ glutamatergic input onto PVI as well as inhibitory synapses on pyramidal neurons, in a NDMAR-dependent manner.^{84,87} This activity-dependent maturation of PVI induces more ROS production by the mitochondria, due to the fast-spiking interneurons high energy demand. Therefore, the beneficial effect of EE on PVI maturation may be limited in *Gclm* KO as they lack intact antioxidant system. Although EE at adolescence strongly diminishes oxidative stress and microglia activation, suggesting

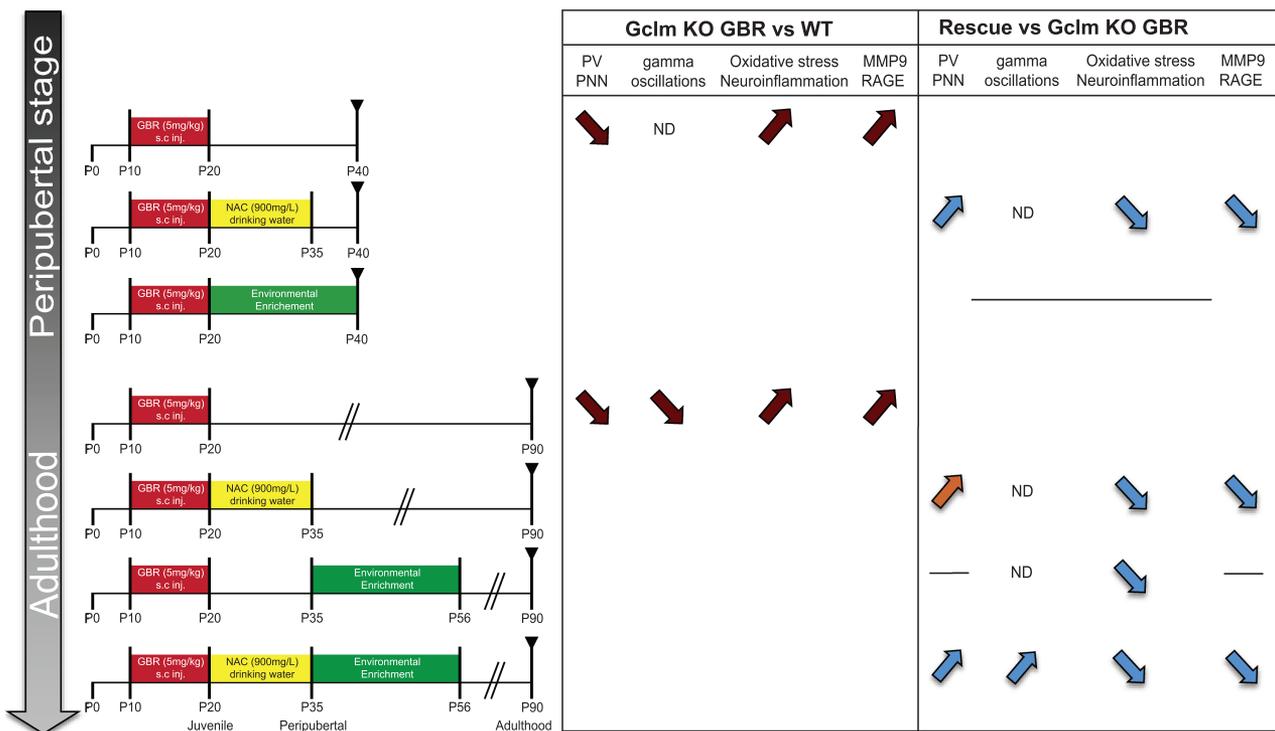


Fig. 5. Summary of NAC and/or EE effects at different developmental periods. Comparison of the *Gclm* KO+GBR to WT PBS/GBR, and to *Gclm* KO+GBR. Red-arrows = pathological changes; blue-arrows = rescue; orange-arrow = not significant rescue; line = no changes; ND = not determined (more details in the supplementary information).

a less critical role for the GSH system, this effect is not sufficient to recover PVI/PNN in adulthood, but still maintain PVI/PNN recovery provided by NAC treatment during juvenile/peripubertal (figure 5).

Effect of the Combination of NAC and EE

Collectively, juvenile/peripubertal NAC combined to EE during adolescence induces a long-term recovery of the PVI in the *Gclm* KO mice (figure 5). Our results suggest that NAC administration during the sensitive juvenile/peripubertal period of maturation, may not only decrease oxidative stress but may also block the activation of the MMP9/RAGE pathway. Addition of EE during adolescence may stimulate the final maturation of PVI in an activity-dependent manner, maintaining the integrity of these neurons. Interestingly, spontaneous excitatory postsynaptic currents (EPSC) measured on PVI in the PFCx increase drastically between the juvenile/peripubertal (PND25-35) and adolescent (PND45-55) periods,⁸⁸ suggesting that increased glutamatergic stimulation by EE (from PND36-57) could be beneficial to PVI maturation. This is in line with our findings regarding the functional recovery of the gamma oscillation power in the adult *Gclm* KO after NAC and EE (figure 3). Altogether, our results reveal a pathological mechanism involving MMP9/RAGE, that can be blocked by NAC at a precise timing, followed by a beneficial effect of EE during adolescence, contributing to the final recovery of the inhibitory network.

Translation to Patients

We investigated a novel combination of 2 therapeutic approaches in a mouse model carrying a genetic vulnerability to redox dysregulation and exposed to an environmental insult during brain development. Indeed, the GBR injections during early postnatal days mimic the dopamine increase in the PFCx, possibly induced by a psychosocial stress⁸⁹ and leading to ROS increase via the catabolism of dopamine.^{90,91} This study paves the way for a novel therapeutic approach that could be proposed to patients *after* an early psychotraumatic event. This consists of: (1) reestablishing the redox/antioxidant balance and neutralizing the deleterious feedforward interaction between oxidative stress and neuroinflammation with NAC during the juvenile/peripubertal period, and (2) stimulating the activity-dependent PVI/PNN microcircuitry with EE during adolescence, eventually leading to the timely maturation of functional local network, critical for gamma oscillations and cognition. Noteworthy, NAC decreases plasmatic sRAGE in EP patients in association with increased prefrontal GABA levels, suggesting that NAC in EP may act on similar oxidative stress-mediated pathological mechanisms than those underlying PVI/

PNN impairment in the animal model. This is further supported by the observation that elevated plasmatic sRAGE levels correlate with low prefrontal GABA in EP who carry a genetic susceptibility to redox dysregulation.⁵⁹ Together, these results highlight that peripheral sRAGE may be a valid proxy marker of central I/E imbalance in EP. Moreover, the NAC-induced decrease in sRAGE is associated with improvement of positive symptoms and cognitive functions, including working memory and processing speed. NAC has been previously reported to reduce symptoms and improve cognition in EP,^{22,23} including processing speed in EP.¹⁷ Interestingly, the beneficial effect of NAC on symptoms was observed mostly in EP displaying high peripheral oxidative status.¹⁷ This suggests the need of mechanism based biomarker allowing patients stratification and targeting precise underlying mechanism.

In a translational train of thought, it is tempting to hypothesize that individuals who carry a genetic vulnerability have a risk to develop psychosis if they are exposed to environmental insults during brain development. Those insults, which can be infectious, traumatic or psychosocial, generate additional oxidative stress. Indeed, ACE during childhood are more prevalent in at-risk-mental-state (ARMS) for psychosis as compared to control individuals,^{15,92} increase the risk for developing psychosis^{93,94} and are associated with a redox dysregulation along with reduced hippocampal volume and more severe symptoms and cognitive deficits in EP.¹¹ Moreover, in various animal models,^{46,80,95-97} known genetic and environmental risks converge to a pathophysiological hub of oxidative stress and consequently long-term decreased PVI, affecting cognitive functions.⁹⁸

Altogether, the present findings highlight the need to (1) identify individuals who are most likely to profit from antioxidant treatment based on mechanism-based biomarker and (2) timely involve the equivalent of “EE” in the treatment, which could include physical training, nutrition, social activities and psychotherapy. Physical exercise and cognitive remediation were shown to improve cognitive performance, psychosocial functioning, and overall symptoms in SZ.^{33,99-104} Besides, a decrease in schizotypal personality was observed in adolescents enrolled when they were children in an enrichment program, consisting of nutrition, education, and physical exercise, compared with those that were not,¹⁰⁵ stressing the importance of childhood environment. These data provide a framework for the implementation of early therapeutic strategies based on antioxidants and psychosocial/cognitive therapy/physical exercise/nutrition, oriented mostly towards individuals exposed to ACE presenting high oxidative status. A limitation of this paradigm is to directly translate these findings into real-world clinic, in particular regarding the optimal timing and duration of NAC treatment followed by physical/psychosocial intervention. Moreover, the characterization of precise cognitive/

dimension-related brain circuitry to be stimulated still need to be clarified.

To conclude, our results highlight an innovative mechanism-based therapeutic strategy, aiming to block MMP9/RAGE pathway inducing oxidative stress and neuroinflammation during childhood, to improve long-lasting integrity and function of PVI/PNN networks. This may pave the way to novel interventions that may improve symptoms and cognition EP patients.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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