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Thalamic reticular nucleus impairments and abnormal prefrontal control of dopamine system in a developmental model of schizophrenia: prevention by N-acetylcysteine

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Abstract

Recent evidence showed thalamic abnormalities in schizophrenia involving disruptions to the parvalbumin neurons in the thalamic reticular nucleus (TRN). However, their functional consequences, as well as a potential linkage to oxidative stress, are unclear. The TRN is posited to gate prefrontal control of dopamine neuron activity in the ventral tegmental area (VTA). Thus, we hypothesized that schizophrenia-related TRN abnormalities might contribute to dopamine dysregulation, a well-known feature of the disorder. To test this, in adult rats exposed prenatally to methylazoxymethanol acetate (MAM rats), oxidative impairments to the parvalbumin neurons in the anterior TRN were assessed by immunohistochemistry. Using *in vivo* electrophysiology, we investigated whether inactivation of the prefrontal cortex would produce differential effects on VTA dopamine neurons in MAM rats. We show that MAM rats displayed reduced markers of parvalbumin and *wisteria floribunda agglutinin-labeled* perineuronal nets, correlating with increased markers of oxidative stress (8-oxo-7, 8-dihydro-20-deoxyguanosine and 3-Nitrotyrosine). Moreover, MAM rats displayed heightened baseline and abnormal prefrontal control of VTA dopamine neuron activity, as tetrodotoxin-induced inactivation of the infralimbic prefrontal cortex decreased the dopamine population activity, contrary to the normal increase in controls. Such dopamine neuron dysregulation was recapitulated by enzymatic perineuronal net digestion in the TRN of normal rats. Furthermore, juvenile (postnatal day 11-25) antioxidant treatment (N-acetyl-cysteine; 900mg/L; drinking water) prevented all these impairments in MAM

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rats. Our findings suggest that early accumulation of oxidative stress in the TRN may shape the later onset of schizophrenia pathophysiology, highlighting redox regulation as a potential target for early intervention.

Keywords

Schizophrenia; Thalamic Reticular Nucleus; Oxidative Stress; Dopamine; Parvalbumin; Prefrontal Cortex

Introduction

Developmental interneuron deficits have been linked to the pathophysiology of schizophrenia (1). Specifically, the fast-spiking, parvalbumin (PV) interneurons and aberrant PV neural circuits are posited to underlie circuit dysfunction in schizophrenia (2). Because of their widespread distribution and functional importance in high-frequency neural synchronization, disturbances in PV networks can induce dramatic functional impairments (3), manifesting as diverse attributes of schizophrenia symptomatology (4). Therefore, understanding the mechanisms driving abnormalities in the PV network is critical to the development of novel therapeutics.

Oxidative stress is a candidate mechanism underlying PV disruption in schizophrenia (5). PV neurons are fast-spiking with heightened metabolic rates and elevated mitochondrial functions (6), resulting in increased vulnerability to oxidative stress (7). Clinical evidence suggests a strong association between redox dysregulation and schizophrenia. For example, antioxidant deficits and accumulation of oxidative stress have been observed in schizophrenia patients (8, 9), including the most abundant endogenous non-protein antioxidant, glutathione (GSH) (10). Such deficits may arise from a genetic origin, as gene polymorphisms relevant to GSH synthesis are reported in schizophrenia patients (11). Moreover, oxidative stress-induced impairments are found in animal models carrying genetic and environmental risks for schizophrenia (2), and permanent or transitory disruptions to endogenous antioxidant defense (e.g., GSH) can recapitulate selective endophenotypes relevant to schizophrenia (12, 13). Lastly, the GSH precursor, N-acetylcysteine (NAC), shows efficacy in individuals with early-phase schizophrenia (14, 15). Given the heterogeneity in schizophrenia etiology (16), these findings suggest that diverse risk factors of schizophrenia may converge onto deficits in redox pathways to disrupt PV circuits, ultimately leading to the development of psychosis (2).

Many studies of oxidative PV impairments focused on cortical structures (5), whereas the vulnerability of subcortical PV neurons is less clear. Specifically, the vulnerability of thalamic PV neurons to oxidative stress is underexplored, although thalamus-related abnormalities are reported in schizophrenia (17). Among thalamic nuclei, the fast-spiking PV neurons are almost exclusively located in the thalamic reticular nucleus (TRN) (18, 19), and are vulnerable to oxidative stress (20, 21). Anatomically, the TRN receives collaterals from both thalamocortical and corticothalamic projections, enabling this region to exert strong feedforward inhibition of thalamic relay neurons and hence gating corticothalamic information flow (22). This organizational scheme was first characterized

in sensory processing but applies to other functional modalities (22). Given its complex interconnections, TRN deficits can cause network-level dysfunctions and behavioral abnormalities relevant to neurodevelopmental disorders (23), including schizophrenia (24–26). Indeed, 19 risk genes are robustly expressed in the TRN of schizophrenia patients (27). In animal models, NMDA receptor antagonists can induce metabolic malfunction and lesions to the TRN (28–30), which are posited to precipitate psychosis-like phenotypes (25). A recent study (21) provided the first direct evidence of morphological abnormalities in the TRN of schizophrenia patients; i.e., decreased PV expression and associated extracellular matrix (i.e., perineuronal nets; PNNs) deficits. Such TRN alterations were proposed to be induced by oxidative stress, as transgenic mice with compromised antioxidant defense (i.e., *Gclm* knockout mice (31)) exhibited similar TRN pathology and abnormal TRN activity (21). Consistently, *Gclm* knockout mice exhibit increased susceptibility to oxidative stress, and oxidative damage to TRN PV neurons preceded that in other regions (20). Whether oxidative stress-induced TRN pathology functionally contributes to core schizophrenia pathophysiology, especially dopamine dysregulation, is unclear.

The midbrain dopamine system is regulated by complex afferent inputs (32). In schizophrenia, the primary deficits are thought to be in the afferent regulation of dopamine neurons, rather than in dopamine neurons themselves (33). The hippocampus (34) and the prefrontal cortex (PFC) (35), both of which are capable of regulating dopamine neuron activity in the ventral tegmental area (VTA), have emerged as potential sites of dopamine dysregulation in schizophrenia. We have demonstrated that pharmacological manipulation of the infralimbic PFC (ilPFC) can bidirectionally regulate VTA dopamine neuron population activity (i.e., number of active DA neurons) (36). Whereas activation of the ilPFC decreased dopamine neuron activity dependent on the amygdala, inactivation of the ilPFC increased dopamine population activity via the vHipp (36). However, unlike the PFC-BLA pathways, there are no direct PFC-vHipp projections, and thus the ilPFC input to vHipp must go through a thalamic relay (37), which we determined to be the reuniens of the midline thalamus (RE). Specifically, we demonstrated that the anterior TRN (aTRN) is under afferent control by the ilPFC to inhibit RE activity (38). Moreover, changes in RE output are sufficient to bidirectionally regulate ventral hippocampus (vHipp) activity, impacting VTA dopamine neuron firing (39) via a well-established pathway comprising the vHipp, the nucleus accumbens (NAc), and the ventral pallidum (VP) (32). These findings implicate the TRN and RE as novel regions involved in the control of the dopamine system. Therefore, the newly discovered TRN deficits in schizophrenia (21) may directly contribute to dopamine abnormalities purported to underlie psychosis (40). This was tested using a neurodevelopmental model of schizophrenia risk (i.e., methylazoxymethanol acetate (MAM) E17 model (40) to examine whether oxidative stress and the associated PV/PNN deficits are present in the TRN, and whether this leads to abnormal ilPFC control of dopamine neurons. Moreover, since redox dysregulation is a putative prophylactic target of schizophrenia (41), we tested whether treating early oxidative damage can avert schizophrenia-relevant histological and neurophysiological phenotypes of the MAM model. Furthermore, to assess the direct involvement of oxidative stress-associated PV/PNN damage in dopamine dysfunction, we enzymatically digested PNNs in the aTRN of normal rats and investigated

whether this targeted manipulation would cause similar neurophysiological alterations as observed in MAM rats.

Methods and Materials

Animals

Experiments were performed on adult male offspring (aged 75-125 days) of rats injected gestationally with 0.9% saline (SAL) or methylazoxymethanol acetate (20mg/kg, i.p.; MRI Global), based on prior studies showing significant susceptibility differences between male and female MAM offspring (42, 43). To avoid litter effects, individual experimental groups were comprised of animals from at least 4 independent litters (range: 4-6) from 2-3 independent cohorts. No more than two pups from a single litter were used in each experimental group, and all the experimental groups within an experiment were counterbalanced. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. See Supplementary Information for details on animal preparation and histological confirmation of recording and infusion sites.

NAC treatment

Daily freshly made solution (900 mg/L) of NAC (A7250, Sigma-Aldrich, USA), an antioxidant and a precursor of GSH, was provided as drinking water to lactating mothers and their pups from postnatal day (P) 11-25. Previous rodent research has indicated that NAC orally consumed at similar doses is transmitted to pups via breast milk to achieve antioxidative and protective effects on PV neurons (44, 45). Animals were weaned at P25 and thereafter received normal drinking water until euthanasia.

Immunohistology

Perfusion, tissue preparation, immunohistochemistry, confocal microscopy, and analyses were similar to previous reports with minor modifications (21, 46). See Supplementary Information for details.

Electrophysiology

Single-unit extracellular recordings of VTA dopamine neurons were performed on rats anesthetized with chloral hydrate (Sigma-Aldrich; 400mg/kg, i.p.), as previously described (39). Briefly, glass microelectrodes were filled with 2M NaCl containing 2% Chicago Sky Blue (impedance 7 to 15 MΩ *in situ*) and lowered through the VTA (beginning at A/P: −5.4 mm, M/L: +0.6 mm, D/V: −6.5 to −9 mm) via 4-6 vertical tracks (each separated by 0.2 mm) in a predetermined pattern. Dopamine neurons were identified by well-established criteria (47) and recorded for at least 90 seconds. Population activity (i.e., average number of spontaneously active neurons recorded each track), average firing rate, and the average percentage of spikes in burst were analyzed. See Supplementary Information for details.

Acute chondroitinase treatment and confirmation of PNN degradation

Following chloral hydrate-induced anesthesia, and at least one hour before the extracellular recordings, a subset of rats was infused bilaterally with 0.5μl chondroitinase ABC from

Proteus Vulgaris (ChABC, 0.05U/μl), or Dulbecco's PBS (dPBS), into the anterior TRN (A/P: −1.5, M/L: ±2.5, D/V: −5.5 mm). This treatment was designed to acutely digest PNNs and was based on ChABC's rapid action *in vitro* at a significantly lower concentration (48, 49). The time course of PNN degradation within the time frame of a recording experiment (i.e. 4-5 hours) was also characterized in this study. See Supplementary Information for details.

Intra-ilPFC and -TRN microinjection

Immediately before VTA recordings, tetrodotoxin (TTX; 1 M, 0.5 μl) or dPBS vehicle (Veh) was infused into the ilPFC at the following coordinates (mm): A/P: +2.7; M/L: +0.6; D/V: −3.9. This microinfusion protocol has been validated previously, and the spread was limited to the ilPFC (50). See Supplementary Information for details of cannula-based microinfusion.

Statistical analysis

Sample sizes were determined based on similar previous studies (38, 44). Group data were tested for normal distribution by the Shapiro-Wilk test. If passed, group data were analyzed by two-way ANOVA, with treatment (SAL or MAM) and TTX infusion (Veh or TTX) as main factors, followed by Tukey's *post hoc* test when a significant effect was detected. In the electrophysiological experiments in a within-subject design, group data were analyzed with two-way repeated measure ANOVA with developmental (SAL:H_2O, SAL:NAC, MAM:H_2O, or MAM:NAC) or TRN treatment (PBS vs. ChABC) condition as a main factor and TTX infusion (pre-TTX vs. post-TTX) as a repeated measure, followed by Bonferroni *post hoc* test. If data failed to pass the normality test, a Kruskal–Wallis one-way ANOVA on Ranks, followed by Dunn's *post hoc* analysis, was applied. Statistics were calculated using Prism8 (GraphPad Software). Post-hoc Dunnett's t-tests were performed to compare the mean number of PV cells, Wisteria floribunda agglutinin (WFA; a PNN marker)/PV cells, and the overall oxidative stress immunolabeling between the groups. These statistics were performed using JMP 12 (SAS Institute Inc., USA). All data are represented as the mean ±SD or SEM. Differences were considered significant at $p<0.05$.

Results

Increased oxidative stress and reduced PV and WFA/PNNs in the TRN of adult MAM rats was prevented by early antioxidant treatment

To assess TRN oxidative stress in MAM rats, mitochondrial DNA oxidation was labeled using 8-oxo-7, 8-dihydro-20-deoxyguanosine (8-oxo-dG) and quantified (44). Additionally, subcellular nitration of proteins and free tyrosine residues, as another evidence of oxidative stress, was confirmed using 3-Nitrotyrosine (3-NT) immunolabeling. Dunnett's *post hoc* analysis revealed an increased level of oxidative stress labeling by ~350% with 8-oxo-DG and ~320% with 3-NT in the anterior segment of the TRN in adult MAM:H_2O rats compared to SAL:H_2O rats (8-oxo-dG: Figure 1A, B; $D=0.87$, $p<0.0001$, and 3-NT: Figure 1E,F; $D=1.96$, $p<0.0001$). Since PV neurons are vulnerable to oxidative stress (51), the expression of PV in the TRN was assessed. The number of PV neurons and PV intensity significantly decreased by 31% and 460%, respectively, in MAM:H_2O group compared to

SAL:H_2O group (Figure 1A, C; D=241.6, p<0.0001 and Figure 1E,G; D=5.86, p<0.0001), revealing prominent impairments to the PV circuitry in MAM rats. PNNs, labeled by WFA, are maturational markers of PV neurons and are protective against oxidative stress (51). Thus, to further determine if the observed oxidative stress affects the maturation and resilience of PV circuitry, the extent of PV neurons associated with PNNs and PNN (WFA) labeling intensity were assessed. Compared to SAL:H_2O rats, a significantly lower number of PV/PNN neuronal colocalizations (Figure 1A, D; D=185, p<0.0001) and lower PNN intensity (Figure 1E and H; D=2.2, p<0.001) were found, suggesting a relatively immature state of PV circuitry in MAM rats with increased vulnerability to oxidative stress.

Accumulation of oxidative damage occurs over an extended period (20), which is potentially preventable by early interventions that restore redox balance, such as NAC supplementation (44). To test whether limiting developmental oxidative stress can prevent TRN abnormalities of MAM rats, we administered NAC during P11-25 and assessed the integrity of TRN PV circuitry in adult MAM rats. MAM:NAC rats exhibited decreased oxidative stress labeling compared to MAM:H_2O rats (8-oxo-dG: Figure 1A, B; D=1.08, p<0.0001 and 3-NT: Figure 1E, F; D=1.95, p<0.0001). Furthermore, NAC treatment prevented the decreased PV neuron number (Figure 1A, C; D=231.9, p<0.0001), PV intensity (Figure 1E, G; D=1.95, p<0.0001), decreased number of PV/PNN co-labeled cells (Figure 1A, D; D=197.8, p<0.0001) and PNN intensity (Figure 1E, H; D=3.07, p<0.0001), which was not found in SAL:NAC rats compared to SAL:H_2O rats. Collectively, these data indicate that early NAC treatment was able to prevent circuit impairments to both PV and PV/PNN neurons in the TRN of MAM rats.

Inverse effects of ilPFC inactivation on VTA dopamine neurons in MAM rats

The medial PFC is a key regulator of dopamine activity (36). Specifically, the ilPFC controls VTA dopamine activity via a thalamic relay under the feedforward inhibitory control from the TRN (39). Considering the extensive oxidative damage to TRN PV neurons, we determined whether such morphological deficits could functionally affect the ability of the ilPFC to modulate dopamine system activity. ilPFC inactivation specifically upregulates dopamine neuron firing in a manner dependent on the vHipp and the thalamic relay involving the TRN (36, 38, 39), and is therefore the focus of this study. Following pharmacological inactivation of the ilPFC by TTX, *in vivo* extracellular recordings were performed on identified VTA dopamine neurons, displaying well-established physiological characteristics such as irregular firing and long spike duration (Figure 2A) (47). For population activity (i.e., number of neurons firing spontaneously; Figure 2B), a two-way ANOVA revealed a significant interaction [F(1, 34)=21.08, p<0.001]. SAL:Veh group (n=12 rats/78 cells) displayed 0.79±0.08 spontaneously active dopamine neurons per track. In contrast, SAL:TTX group (n=7 rats/60 neurons) had significantly increased VTA dopamine neuron population activity (1.30±0.17 cells/track) compared to SAL:Veh group (Tukey's *post hoc* test, p<0.05), confirming previous results that ilPFC inactivation upregulates population activity (36, 39, 50). The MAM:Veh group (n=10 rats/98 neurons) displayed significantly higher dopamine population activity (1.36±0.13 cells/track) compared to SAL:Veh group (Figure 2B, p<0.01) as reported previously (52, 53). MAM:TTX rats (n=10 rats/69 cells) exhibited significantly decreased population activity (0.82±0.11 cells/track)

compared to MAM:Veh rats ($p<0.01$). Altogether, these results revealed opposite effects of ilPFC inactivation on dopamine system activity between SAL and MAM rats, suggesting abnormal prefrontal control of dopamine neurons in the MAM offspring.

No significant effect was detected in either firing rate or burst firing (Figure 2C and D). These results are consistent with previous reports that ilPFC inactivation primarily affects dopamine neuron tonic firing, with only limited effects on phasic firing (36, 39, 50).

Early antioxidant treatment prevented the increased baseline activity and the abnormal prefrontal modulation of dopamine neurons in MAM rats

Since TRN PV deficits in MAM rats can be prevented by early NAC treatment (Figure 1B–D, F–G), we tested whether the dopamine dysfunction in MAM rats can also be prevented. Using a within-subject design, we performed *in vivo* extracellular recordings from dopamine neurons before and after TTX-induced ilPFC inactivation. For population activity (Figure 3A), a two-way repeated measure ANOVA revealed a significant main effect of TTX infusion [$F(1, 24)=14.34$, $p<0.001$] and a significant interaction [$F(3, 24)=10.46$, $p<0.001$]. For baseline dopamine population activity (i.e., “pre-TTX” groups), Bonferroni *post hoc* analysis showed that MAM:H_2O group (n=6 rats/55 neurons) exhibited increased dopamine population activity (1.70±0.20 cells/track) compared to SAL:H_2O controls (n=6 rats/38 neurons; 0.86±0.17; $p<0.05$). Juvenile NAC treatment significantly lowered baseline dopamine population activity in MAM rats, and MAM:NAC group (n=10 rats/48 neurons; 0.84±0.15 cells/track) exhibited lower dopamine population activity compared to MAM:H_2O group ($p<0.01$). No significant difference in baseline population activity was detected between SAL:H_2O and SAL:NAC (6 rats/25 cells; 0.87±0.10 cells/track; $p>0.05$) groups. For the effect of ilPFC infusion (i.e., “post-TTX” groups), in SAL:H_2O group (post-TTX: 6 rats/50 neuron) we observed increased population activity (1.53±0.29 cells/track; $p<0.01$), which is comparable to previous results (Figure 2B). In contrast, in MAM:H_2O group (post-TTX: 6 rats/33 cells), ilPFC inactivation decreased dopamine population activity (1.17±0.17 cells/track; $p<0.01$), confirming the abnormal prefrontal control observed in Figure 2B. Notably, this effect was prevented in MAM:NAC group (post-TTX: n=10 rats/66 cells), in which MAM:NAC rats displayed increased dopamine population activity in response to TTX infusion (post-TTX: 1.24±0.19 cells/track; $p<0.05$). SAL:NAC group (post-TTX: 6 rats/48 cells) similar to that observed in the SAL:H_2O group, in which TTX infusion increased dopamine population activity (1.60±0.12 cells/track; $p<0.001$).

Firing rate and burst firing of dopamine neurons were also analyzed (Figure 3B and C). For firing rate (Figure 3B), no significant effect among groups was detected, which was consistent with previous results showing inactivation of ilPFC alone selectively affected tonic firing without affecting the average firing rate of dopamine neurons (36). There was a significant difference in burst firing among groups [Figure 3C; Kruskal-Wallis H test, $H=16.90$, $p<0.05$]. Dunn’s *post hoc* analysis revealed significantly reduced post-TTX burst firing of dopamine neurons in MAM:H_2O ($p<0.01$) and MAM:NAC ($p<0.05$) groups.

ChABC-induced PNN digestion in the TRN of normal rats recapitulated the MAM-like electrophysiological phenotypes.

To further elucidate the functional implications of TRN abnormalities in MAM rats, we acutely degraded PNNs using ChABC in the TRN of normal rats and recorded the response of VTA dopamine neurons. Specifically, in rats injected bilaterally with ChABC or PBS control for one hour, we recorded dopamine neurons before and after TTX-induced ilPFC inactivation (Figure 4A–C). A two-way repeated measure ANOVA revealed a significant interaction in dopamine neuron population activity [$F(1, 11) = 127.6$, $p<0.0001$]. Comparable to previous findings and similar to SAL controls in this study (Figure 2A and 3A), PBS controls show baseline population activity of 1.01±0.17 cell/track (n=6 rats), which is significantly increased after TTX-induced ilPFC inactivation (1.88±0.15 cells/track; $p<0.0001$). In contrast, in ChABC-treated rats, the baseline population activity is significantly increased compared to PBS controls (1.59±0.10 cells/track, n=7 rats; $p<0.01$), resembling MAM rats. After ilPFC inactivation, ChABC-treated rats showed decreased population activity of 0.80±0.09 cells/track ($p<0.0001$, vs. ChABC:Pre), which is significantly lower compared to the post-TTX recording in PBS controls (Figure 4A).

To verify the extent of PNN degradation, immediately after the recording TRN brain sections were collected and processed for immunohistochemistry (Figure 4D). We found decreased mean TRN WFA intensity in ChABC-treated (80.44±11.69 a.u., n=7) versus control animals (137.7±12.17 a.u., n=6; $t_{(11)}=3.369$; $p<0.01$), whereas the TRN PV intensity was not different between these groups (76.47±5.88 vs. 82.18±4.79 a.u.; $p>0.05$). This result confirmed the TRN PNN degradation in recorded animals, indicating that ChABC is capable of effectively degrading PNNs within the typical time frame of VTA recording experiments (~5 hours). To further elucidate the temporal dynamics of PNN degradation, we performed unilateral intra-TRN ChABC injection and compared that with the PBS-treated side on the same coronal sections (n=2-3 rats; N=2-4 sections/rat) throughout post-infusion time points (Figure 4E). Consistent with in vitro studies(48), PNN degradation was observable one hour after the ChABC treatment (Figure 4F), and the WFA signal was reduced to 51.6±6.5%, which is significantly lower compared to the control PBS injection sides (paired t-test, $p<0.0001$) (Figure 4G). Moreover, the reduction in WFA intensity was stable throughout the recording period, as significant PNN degradation was still observed at 2- and 4-hour post-infusion. Collectively, these results demonstrate that direct adult perturbation to TRN PNNs is sufficient to elevate baseline activity and disrupt infralimbic prefrontal control of VTA dopamine neurons, reminiscent of the dopamine dysregulation of MAM rats.

Discussion

We found increased oxidative damage in the TRN, along with decreased PV immunoreactive cell count and fewer PV cells associated with PNNs in the MAM developmental disruption model of schizophrenia. Such TRN deficits were accompanied by elevated dopamine population activity and abnormal prefrontal control of dopamine neurons. Targeted digestion of TRN PNNs in normal rats recapitulated these neurophysiological abnormalities. All of these TRN morphological deficits and dopamine neurophysiological dysfunctions in MAM rats were prevented by early (P11-25) antioxidant treatment.

Several studies on redox dysregulation in schizophrenia focused on the PFC and the hippocampus, providing consistent findings of increased oxidative damage and decreased capacity of the redox system in this disorder (5). Emerging evidence, however, suggested that oxidative stress in schizophrenia can affect subcortical regions, particularly the TRN (17). Noteworthy, in young mice with genetically induced redox dysregulation, oxidative impairments to PV circuits tend to occur earlier in subcortical regions (e.g., at P20 in the TRN and P40 in the amygdala) compared to the cortex and the hippocampus, raising the possibility that early perturbation to TRN integrity might affect the development of other regions, eventually leading to brain-wide deficits (20). These recent breakthroughs inspired this study.

The MAM model is based on *in utero* disruption of neural development by the mitotoxin (54), and adult MAM-exposed offspring recapitulate histological, neurophysiological, and behavioral deficits analogous to several clinical features observed in schizophrenia, particularly those consistent with circuit disruption (40, 43). Previous research of redox dysregulation in the MAM model is limited. To date, only two studies examined the oxidative impairments in MAM rats, both reporting increased oxidative-stress markers and substantial PV damage in the cortex (2, 55). Complementing these studies, we now demonstrate oxidative impairments in the TRN of adult MAM rats. Together, current evidence indicates putative widespread oxidative pathology in PV circuits in MAM rats, encompassing cortical and subcortical regions.

The increased oxidative stress in MAM rats may relate to GSH disruption. In schizophrenia patients, several magnetic resonance spectroscopy (MRS) studies indicated brain-wide GSH reduction, including the PFC, the anterior cingulate cortex, and the striatum (56–60). A recent MRS study further revealed a thalamic-selective GSH reduction in first-episode psychosis patients (61), linking regional GSH dysregulation to schizophrenia pathogenesis. In MAM rats, gestational exposure to MAM is known to reduce brain GSH level, leading to progressive neuronal death in GABAergic interneurons (62). Thus, an early GSH dysregulation in the TRN of MAM rats may contribute to the observed oxidative damage to PV neurons. Noteworthy, vulnerability to early stress substantially mediates MAM-induced pathology (63). Since exposure to environmental stressors impairs redox processes at cellular and molecular levels (64, 65), the oxidative damage in MAM rats might exacerbate their heightened susceptibility to a negative environment (66). Thus, future studies should elucidate possible interactions between deficits in the GSH system and excessive environmentally induced oxidative stress in MAM rats.

To test whether early antioxidant prevents later development of oxidative deficits, MAM rats were treated with a GSH precursor, NAC, during the juvenile period (P11 – 25). This NAC treatment regimen was based on numerous studies on early insults to the redox system, all of which demonstrated that oral NAC treatment was especially effective against oxidative pathology when given during a preweaning period (44–46, 67, 68). Such efficacy of early treatment might be related to maturational changes of GSH inhibitory feedback control (69). The present treatment was also designed to parallel TRN PV development and account for their early onset of susceptibility to oxidative stress. Specifically, TRN PV expression in rodents is detectable at P0 and continues to increase until approximately P30 (70, 71).

Moreover, PV neurons manifest vulnerability to oxidative stress as early as P20 (20). Thus, a preweaning period (i.e., P11-25) was selected to account for these developmental hallmarks. Notably, both the increased oxidative stress markers and the morphological deficits in TRN PV circuits in MAM rats were prevented by juvenile NAC treatment (Figure 1). These findings highlight early oxidative stress as a promising prophylactic target for the pathological development of schizophrenia. The timeline of treatment also suggests that the pathological sequelae of oxidative stress in the TRN of adult MAM rats, and perhaps in schizophrenia patients (21), might have a developmental origin in childhood and/or adolescence.

The TRN contains predominantly inhibitory PV neurons, estimated to account for 50-90% of the total neuron population (21, 72, 73). In principle, the TRN is the major inhibitory system for corticothalamic circuits, providing strong feedforward inhibition to thalamic relay nuclei (74). The TRN is topographically organized, and the aTRN receives monosynaptic projections from the ilPFC (75) and in turn innervates the RE (76), forming parallel ilPFC-TRN-RE and ilPFC-RE pathways (77, 78). To our knowledge, there is no specific data relevant to Guillery and Sherman's distinction between "driver" and "modulator" input in ilPFC-RE pathways. However, as the RE receives dense ilPFC projections from layer V, presumably ilPFC projections are driver inputs to the RE (79). We recently demonstrated that the ilPFC acts through parallel pathways to dynamically regulate RE neuron activity (38), which by itself is sufficient to affect both the baseline and the ilPFC control of VTA dopamine neuron activity (39). While the exact circuit mechanism warrants a more comprehensive future study, we speculate that the significant aTRN PV/PNN impairments in MAM rats (Figure 1) might limit TRN inhibitory output to the RE, and thereby disrupt prefrontal-hippocampal interaction to affect afferent control of dopamine neurons (Figure 5). Specifically, in control animals with an intact TRN, the ilPFC-RE circuit is proposed to control downstream dopamine neurons primarily via the indirect pathway (39), allowing TRN inhibitory neurons to overcome direct excitation from the ilPFC (Figure 5A). At a system level, such organization would enable the ilPFC to provide tonic inhibition to the ventral subiculum (vSub) of the vHipp, therefore preventing hippocampal hyperactivation (32). This dominance of the ilPFC-TRN-RE indirect pathway would also be consistent with the findings that the primary action of ilPFC NMDA stimulation on RE is inhibitory (39). Furthermore, potent inhibitory TRN pathways appeared to be common in corticothalamic communication (80), allowing powerful feedforward TRN inhibition to overwhelm the direct yet weaker corticothalamic projections (81). The biological basis of such circuit dominance has been attributed to stronger synaptic connections formed in the TRN (82), possibly related to abundant TRN PNN expression known to enhance the excitability of fast-spiking interneurons (49). Consequently, in SAL rats with an intact TRN ilPFC inactivation would primarily attenuate TRN-mediated feedforward inhibition, and therefore disinhibit the RE-vSub pathway, leading to hippocampal hyperactivity and disinhibition of midbrain dopamine neuron via a well-characterized pathway involving vSub, NAc, and VP (Figure 5C) (32). In contrast, in MAM rats with substantial TRN PV decrease, TRN-mediated feedforward inhibition would be compromised, causing the direct ilPFC-RE glutamatergic projections to predominate (Figure 5B). Such circuit imbalance, when combined with hippocampal inhibitory PV neuron impairments (53), could contribute

to hippocampal hyperactivity and thereby dopamine neuron hyperresponsivity in MAM rats (52), consistent with functional impairments in the medial temporal lobe in schizophrenia (40). A compromised TRN would also be consistent with the emerging finding of PFC/thalamus dysconnectivity in schizophrenia (83) In addition to dopamine baseline firing, TRN defects could also impact prefrontal control of dopamine activity. In MAM rats, due to the lack of TRN-mediated feedforward inhibition, ilPFC inactivation would act predominantly upon the direct, glutamatergic ilPFC-RE projection, leading to decreased activity of the RE-vSub pathway and subsequently a decreased VTA dopamine output (Figure 2B, 5D), which is opposite to the PFC regulation of dopamine neurons in SAL controls. Unexpectedly, we also observed a small decreased burst firing in MAM rats responding to ilPFC inactivation (Figure 3C). This effect could be due to multiple alternate pathways through which the ilPFC can impact the dopamine phasic firing, which may include amygdala, a region that also shows oxidative stress-related alterations in pathological states such as schizophrenia (84).

Since NAC treatment prevented TRN PV/PNN pathology (Figure 1), we examined whether it can also prevent dopamine dysregulation in MAM rats (Figure 3). We found that both the baseline hyperdopaminergic activity and the abnormal prefrontal control of dopamine neurons in MAM rats were prevented by juvenile NAC treatment (Figure 3A). Together with the morphological findings, these data indicate clear prophylactic effects of NAC on TRN PV neuron impairments and dopamine dysfunction. Furthermore, this prevention also provides insights into the pathophysiology of schizophrenia. The primary action of NAC is to increase and restore GSH levels in the nervous system (45). Since brain GSH level is stringently regulated (69), NAC treatment is posited to be effective only in conditions with significantly altered redox balance (85) and is largely ineffective in subjects with intact redox systems (45, 56). Given this selective action, current findings with NAC treatment support the hypothesis that regional redox dysregulation might mediate the pathophysiological development of schizophrenia-related endophenotypes.

While this study primarily focused on the TRN, the extent of oxidative-stress induced impairments to PV/PNNs in MAM rats likely extends beyond thalamic networks. Thus, MAM rats also display decreased levels of PNN components in the vHipp (86). Therefore, to pinpoint the precise role of TRN PV/PNN abnormalities in dopamine-related pathophysiology, we acutely digested PNNs with ChABC and recorded from VTA dopamine neurons (Figure 4). We found that intra-TRN ChABC infusion resulted in a rapid and long-lasting PNN degradation (Figure 4), which is sufficient to elevate baseline population activity and reverse prefrontal control of dopamine neurons (Figure 4A), closely resembling those of MAM rats (Figure 3A). This result provided a direct mechanistic link between oxidative stress-associated PNN deficits and key schizophrenia pathophysiology. Previous electrophysiological studies reported that cortical (49) and TRN (48) PV inhibitory neurons lacking PNNs were unable to maintain high-frequency bursting, thereby diminishing the normally robust inhibition of their synaptic targets. Thus, the TRN PNN loss, as observed in schizophrenia and MAM rats, may decrease TRN inhibitory output to thalamic relay nuclei projecting to “downstream” afferent structures (e.g. the vHipp) involved in dopamine control.

Beyond thalamic abnormities, PV-associated deficits in the BLA and the vHipp were also found in MAM rats (63), suggesting extensive oxidative pathology. A key question is the spatio-temporal patterns of oxidative stress accumulation and the associated PV impairments, as this can potentially explain the progressive development of schizophrenia-related phenotypes in MAM rats (43). Translating to patients, as structural alterations of specific thalamic nuclei connected to the PFC were recently observed in early psychosis and chronic schizophrenia patients (87), such early TRN impairments might be central to a circuit-based development of the disease pathophysiology. Noteworthy, the early time course of the treatment in this study might implicate age-dependent mechanisms of NAC's efficacy, which warrants future studies to further elucidate optimal windows for prevention.

In summary, this study complements the growing knowledge of TRN-related abnormalities in schizophrenia pathophysiology. Our data indicate excessive TRN oxidative stress in adult MAM rats associated with significant impairments in PV/PNNs and neurophysiological deficits in the prefrontal control of dopamine neurons. We also found that early antioxidant treatment can prevent TRN PV/PNN deficits and dopamine dysfunction in MAM rats. In normal rats, ChABC-induced TRN PNN degradation recapitulates MAM-like dopamine dysregulation. Together, this study suggests that thalamic redox dysregulation could represent both a pathophysiological mechanism and a promising prophylactic target for schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure

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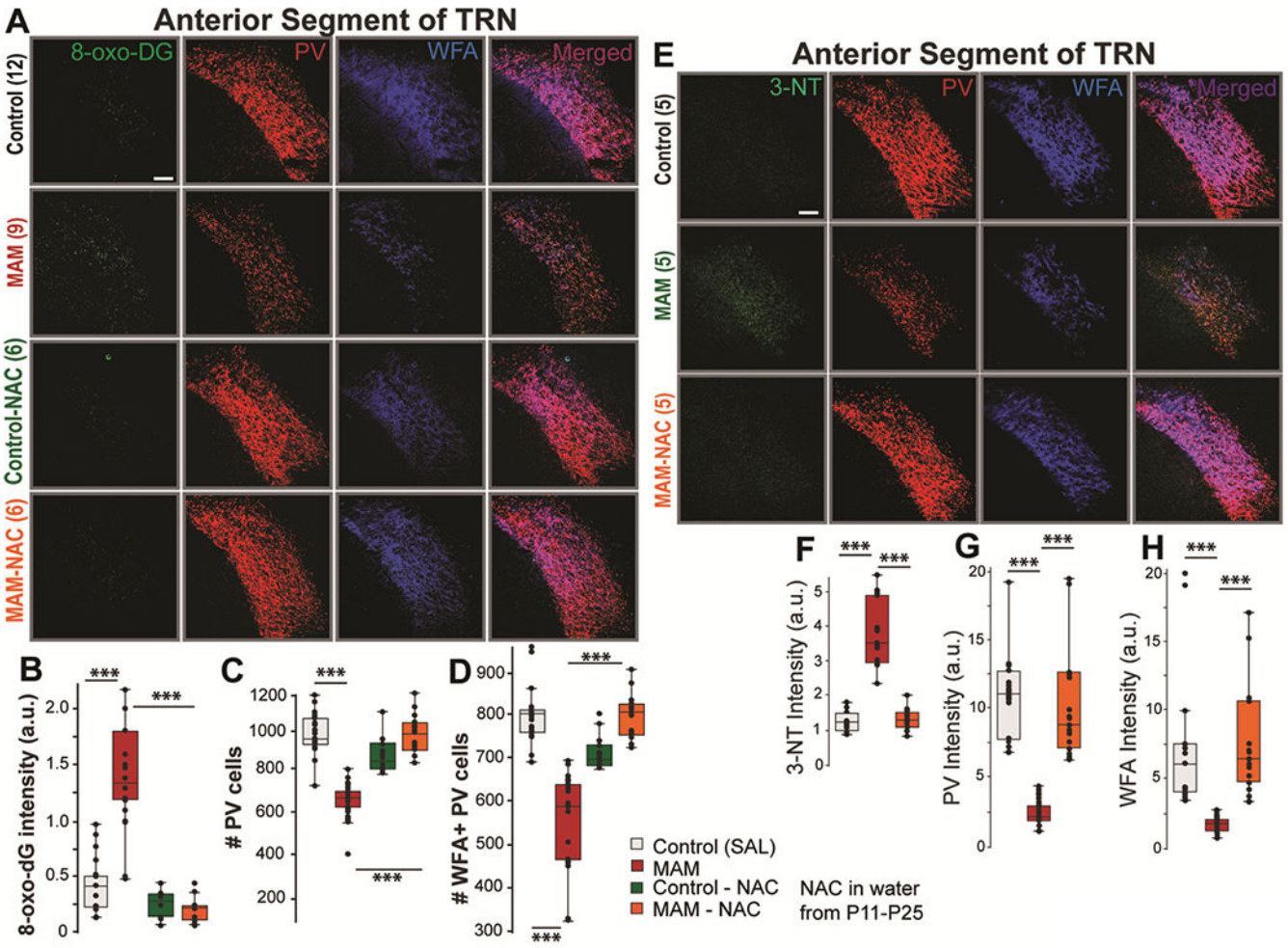

Figure 1. Oxidative stress-induced PV and PNN circuitry impairment in the anterior segment of TRN in adult MAM rats was prevented by juvenile N-acetylcysteine (NAC) treatment.

(A) Micrographs show labeling for 8-oxo-dG (green), PV (red), and WFA (labels PNN; blue) in the TRN of Control and MAM rats in the absence or presence of juvenile NAC treatment. Scale: 40 μm. (B) NAC prevents the increase in 8-oxo-dG labeling in MAM rats. (C) Stereological quantification reveals that NAC prevents oxidative stress-induced decrease of PV and (D) WFA+ PV number, in MAM rats. (E) Micrographs show labeling for another oxidative marker, 3-NT (green), PV (red), and WFA (labels PNN; blue) in the aTRN of Control and MAM rats in the absence or presence of juvenile NAC treatment. Scale: 40 μm. (F) NAC prevents the increase in 3-NT fluorescent intensity (a.u.). (G and H) NAC also prevents the loss of PV and WFA labeling intensity (a.u.,) in MAM rats. Scatter and box plots showing medians (horizontal line) and quartiles (the bottom and top of the box indicate the first (Q1) and third (Q3) quartiles) values; the upper-limit equals Q3 plus 1.5 times interquartile range (IQR), and the lower-limit equals Q1 minus 1.5 times interquartile range. For each group A-D: n=12 (Control), 9 (MAM), 6 (Control-NAC) and 6 (MAM-NAC), E-H: n=5 (Control), 5 (MAM) and 5 (MAM-NAC) ***p <0.0001 (pair-wise Dunnett tests). NAC treatment was added to drinking water from P11-P25.

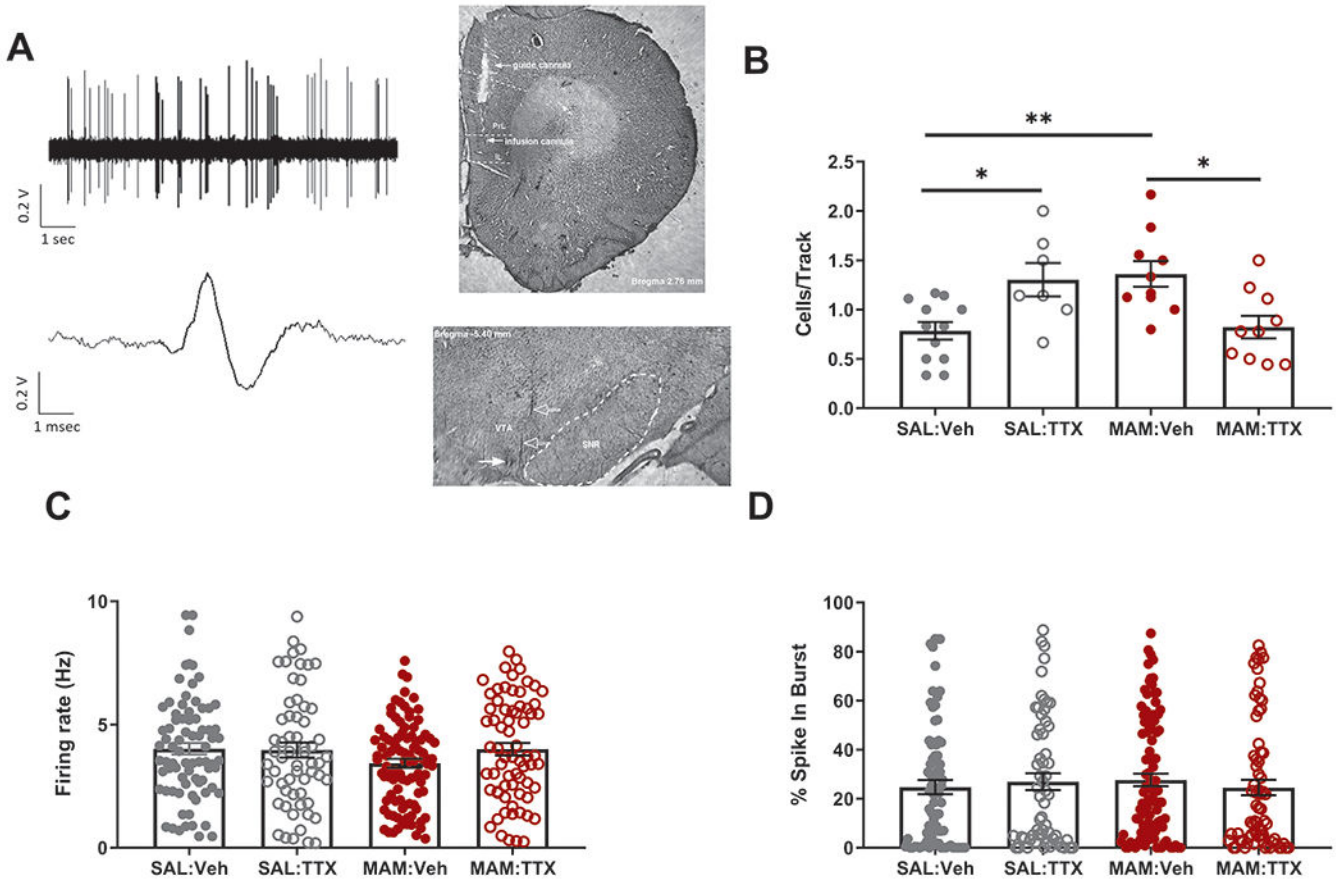

Figure 2. Population activity and ilPFC control of VTA dopamine neurons were altered in MAM rats.

(A) Left, Representative recording of a dopamine neuron from SAL:Veh rats. Right, representative images of histological placements of infusion and recording sites in the ilPFC and the VTA (solid arrow: electrode tip; open arrow: electrode track). **(B)** In vehicle (Veh) treated groups (solid circles), MAM rats (red circles) display heightened VTA dopamine population activity compared to SAL controls (grey circles). In addition, TTX-mediated ilPFC inactivation (open circles) induced opposite effects on VTA dopamine neurons in SAL vs. MAM rats. n(rat)=7-12; n(litter)=4-6 **(C, D)** No effect of either exposure to MAM or TTX infusion was observed on firing rate or burst firing of dopamine neurons. n(rat)=7-12; N(cell)=60-98. *p<0.05; **p<0.01; Data are presented as mean ± SEM. PrL: prelimbic PFC; IL: infralimbic PFC; SNR: substantia nigra, reticular part.

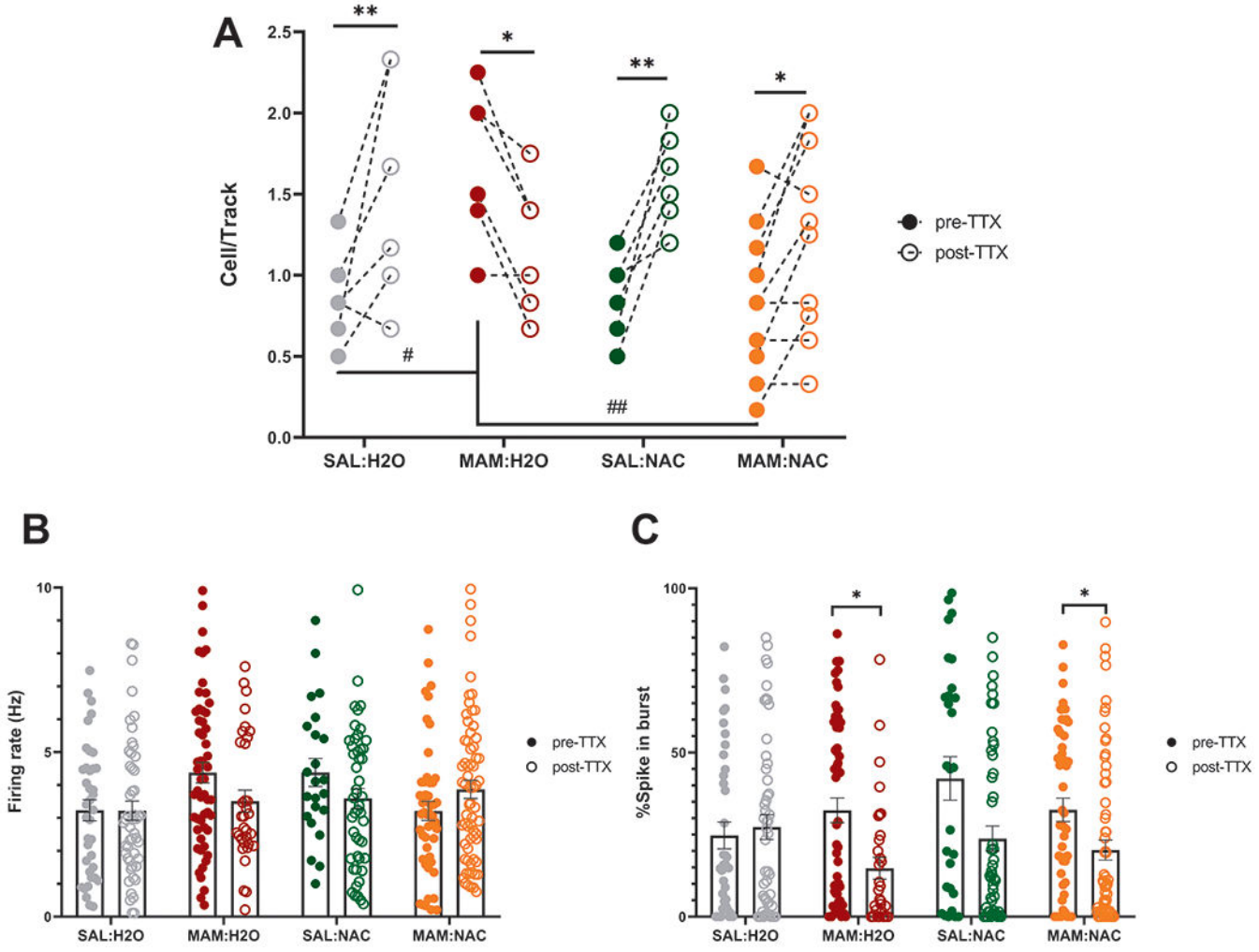

Figure 3. Enhanced baseline activity and abnormal prefrontal control of VTA dopamine neurons were prevented by P11-25 NAC oral treatment.

(A) Before ilPFC inactivation (i.e., solid circle; pre-TTX), MAM rats displayed heightened dopamine population activity, which was prevented by early NAC treatment. In response to ilPFC inactivation (i.e., open circle; post-TTX), SAL rats displayed upregulated dopamine population activity, which is affected by early NAC treatment. In contrast, MAM:H_2O rats displayed downregulated dopamine population activity in response to ilPFC inactivation, which is reversed in MAM:NAC group. n(rat)=6-10; n(litter)=4-6. **(B)** The firing rate of dopamine neurons was not affected by any manipulation (H=13.45, p>0.05, Kruskal–Wallis test, Dunn's *post hoc*). n(rat)=6-10; N(cell)=25-55 **(C)** Burst firing of dopamine neurons significantly differed across groups (H=16.90, p < 0.05), Dunn's *post hoc* analysis revealed that the percentage of cells firing in burst was significantly lower after TTX infusion in MAM:H_2O and MAM:NAC groups. Data are presented as mean ± SEM. *p or #p<0.05; **p or ##p<0.01; * indicates difference related to TTX infusion; # indicates difference in baseline dopamine population activity before TTX infusion.

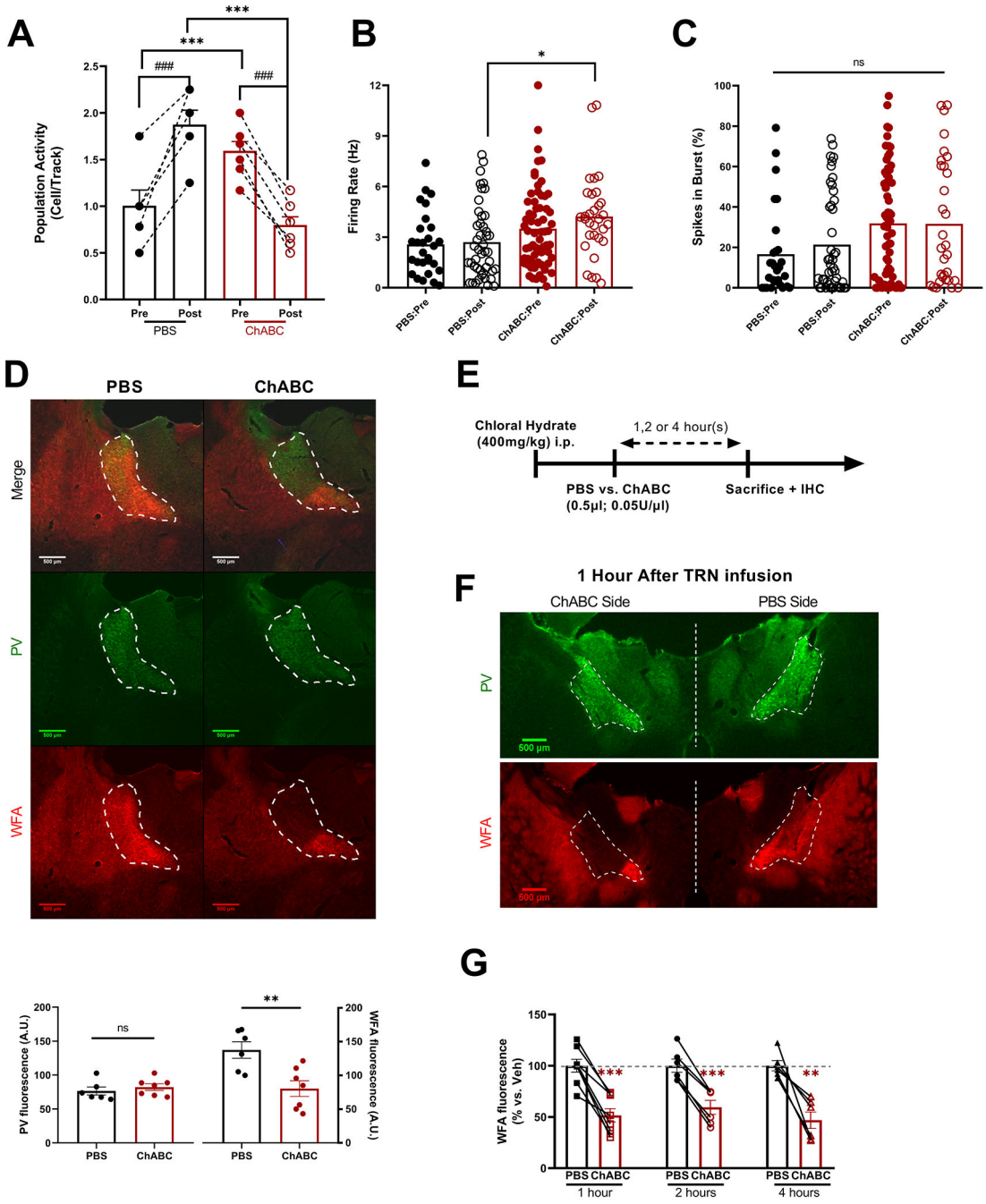

Figure 4. ChABC-induced PNN degradation in the TRN recapitulated MAM-like phenotypes in the prefrontal control of VTA dopamine neurons.

(A) PBS vehicle or ChABC was infused bilaterally into the aTRN. After one hour of PNN digestion, animals were tested for VTA dopamine population activity before and after TTX-induced inactivation of the ilPFC. ChABC-treated animals displayed increased baseline dopamine population activity and an inverse response to ilPFC inactivation, resembling those observed in MAM rats. n(rat)=6-7; n(litter)=4. **(B)** After ilPFC inactivation, the dopamine neurons recorded from ChABC pretreated rats display a slightly increased firing

rate, compared to those of PBS pretreated rats. n(rat)=6-7; N(cell)=25-66 **(C)** No change in dopamine neuron burst firing was observed. **(D, top)** Immediately after recordings, animals were euthanized for immunohistochemistry to verify PNN degradation. Note that thalamic PV is almost exclusively located to the TRN (marked by dashed lines), where the WFA staining was markedly decreased. (D, bottom) At the end of recording experiments, the intensity of WFA immunoreactivity was significantly reduced, whereas that of parvalbumin was unaffected. (E) Design of a separate experiment characterizing WFA intensity after aTRN ChABC injection. (F) One hour after the injections, the aTRN on the ChABC-treated sides already displayed visibly decreased WFA intensity compared to the PBS-treated side, indicative of rapid PNN digestion. (G) Quantification of ChABC-induced PNN degradation. N(brain section)=6-8; n(rat)=2-3. Measurements were normalized to the PBS control group mean at each time point, and a paired t-test was used. *p or #p <0.05; **p or ##p <0.01; ***p or ###p < 0.001; * or # indicates significant effects of ChABC or TTX, respectively. Data are presented as mean ± SEM. PBS: 0.5μl of Dulbecco's PBS. ChABC: chondroitinase ABC, 0.05U/μl, 0.5μl.

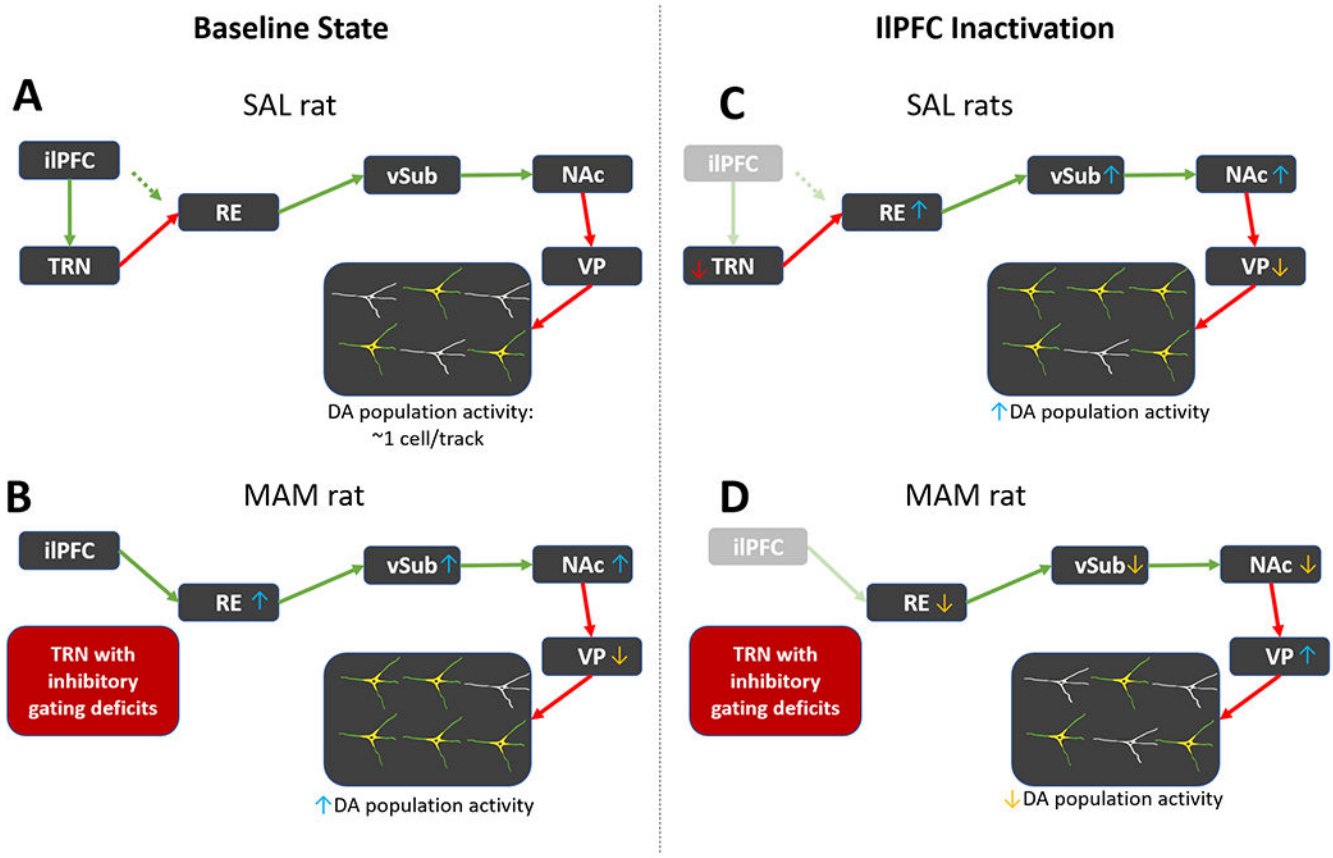

Figure 5. Schematics of the proposed pathways mediating the differential effects of ilPFC inactivation on VTA dopamine neurons in SAL vs. MAM rats.

(A) in SAL rats, at baseline ilPFC output is primarily mediated by TRN-driven feedforward inhibition of the RE. This pathway maintains normal vSub activation and hence the normal dopamine population activity in the VTA (green/yellow colored neurons indicate spontaneously active cells), through a multisynaptic pathway involving the nucleus accumbens (NAc) and the ventral pallidum (VP) (32). **(B)** In MAM rats, oxidative stress-induced impairments of the TRN PV neurons inactivate TRN-mediated feedforward inhibitory gating and hence shifts pathway dominance. As a result, the ilPFC output is mainly mediated by direct excitatory pathways to RE; this can contribute to the vSub hyperactivity and the consequent dopamine hyperactivity in MAM rats (52). **(C)** In SAL rats, given the predominant feedforward inhibition from the TRN, the effect of ilPFC inactivation is mainly mediated by the TRN-driven pathway, resulting in a net effect of increased vSub activity and consequently disinhibition of VTA dopamine neurons (36). **(D)** In MAM rats, due to the impairment in the TRN, ilPFC inactivation is primarily mediated by a multisynaptic excitatory pathway to vSub, potentially leading to attenuation of vSub activity and the consequent downregulation of VTA dopamine activity. Green arrow: glutamatergic projection; red arrow: GABAergic projection. Blue ↑: increase of activity; orange ↓: decrease of activity.