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Behavioral training reverses global cortical network dysfunction induced by perinatal antidepressant exposure

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Abnormal cortical circuitry and function as well as distortions in the modulatory neurological processes controlling cortical plasticity have been argued to underlie the origin of autism. Here, we chemically distorted those processes using an antidepressant drug-exposure model to generate developmental neurological distortions like those characteristics expressed in autism, and then intensively trained altered young rodents to evaluate the potential for neuroplasticity-driven renormalization. We found that young rats that were injected s.c. with the antidepressant citalopram from postnatal d 1–10 displayed impaired neuronal repetition-rate following capacity in the primary auditory cortex (A1). With a focus on recovering grossly degraded auditory system processing in this model, we showed that targeted temporal processing deficits induced by early-life antidepressant exposure within the A1 were almost completely reversed through implementation of a simple behavioral training strategy (i.e., a modified go/no-go repetition-rate discrimination task). Degraded parvalbumin inhibitory GABAergic neurons and the fast inhibitory actions that they control were also renormalized by training. Importantly, antidepressant-induced degradation of serotonergic and dopaminergic neuromodulatory systems regulating cortical neuroplasticity was sharply reversed. These findings bear important implications for neuroplasticity-based therapeutics in autistic patients.

autism | behavioral training | cortical network | antidepressant exposure | recovery of function

Recently, extensive efforts have been made to understand better the etiology of pervasive developmental disorders (PDDs), such as autism spectrum disorders (ASDs), with an ultimate goal of identifying preventive and more effective treatment strategies. At present, a general consensus from both human and animal studies is that a variety of genetic and environmental factors play an integrated role in the establishment of neurobehavioral abnormalities marking these disorders (1–5). Given the complexity of its origins and of the expressions of neurological abnormalities in ASD, it has been generally concluded that no single drug or therapy can be expected to provide effective treatment for the core and associated symptoms of ASDs (6–9).

Interestingly, early behavioral intervention has been associated with significant improvements in intelligence quotient, language acquisition, and adaptive behavior (10). Furthermore, positive outcomes of individuals with an unequivocal history of moderate-severe ASD (11) provide hope that the strong behavioral deficits expressed in the disorder might, on some corrective neurobehavioral path, be reversible. What is that path? A critical question to answer is whether and to what extent the cortical network dysfunction and the subcortical machinery that regulates it, repeatedly described as distorted in humans with autism and in animal models of autism (12–15), can be reversed, either through drug treatment or via behavioral approaches.

Cortical circuit miswirings and synaptic malformations are characteristic neuropathological markers for ASDs (14, 16, 17). Recent studies have clearly implicated that early exposure to selective serotonin reuptake inhibitors (SSRIs) could have long-term consequences on neurodevelopment (18). Specifically, rodent studies have shown that perinatal exposure to SSRIs like citalopram (CTM) induce abnormal autistic-like behaviors and cortical network disorganization, including degraded topographic organization and callosal connectivity (19, 20). Exposure to SSRIs also alters speech perception in infants (21). A potential link between prenatal SSRI exposure and subsequent ASDs in children has been indicated recently by studies of Harrington et al. (22). Interestingly, most of these children with ASDs also display deficient auditory temporal processing (23). In addition, a series of auditory behavioral training studies in rodents have shown that auditory cortical network miswirings can potentially be reversed at any postnatal age (24–27). All of these studies prompted us to investigate further whether intensive auditory behavioral training could result in a reversal in auditory system network function and in global forebrain network miswiring in this rat autism (SSRI-exposed) model.

Results

Effects of CTM Exposure on Behavioral Performance. We first examined the consequences of CTM exposure on an auditory temporal rate discrimination task. Rats from different groups (experimental timelines are provided in Fig. 1*A*) were trained to

Significance

Cortical circuit miswiring underlying dysfunctional networks and aberrant behavior and distortions in modulatory control nuclei contributing to the regulation of learning, memory, and mood are hallmarks of autism. No available treatment effectively addresses these different dimensions of neurological distortion expressed in this disorder. Here, we show that a simple form of intensive auditory behavioral training in young adult rats resulted in a reversal of autistic-like neurological distortions expressed in the primary auditory cortex arising in an early postnatal epoch and also broadly improved the status of modulatory control processes expressing serotonin, dopamine, and noradrenaline.

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The authors declare no conflict of interest.

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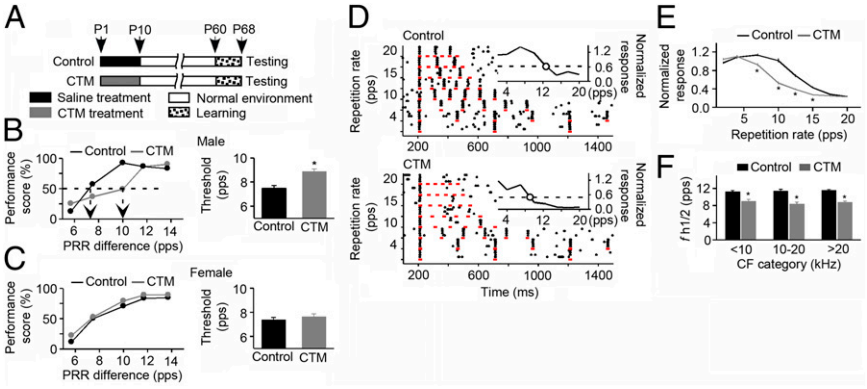

Fig. 1. Effects of perinatal CTM exposure on behavioral and neuronal discrimination of the temporal rate of sound. (*A*) Experimental timelines. Rat pups were injected s.c. with CTM (2 mg/mL) at a dose of 10 mg/kg of body weight or saline (control) twice daily from postnatal d 1 (P1) to P10. (*B*) Examples of psychometric curves (*Left*) and average discrimination thresholds (*Right*) obtained from male CTM-exposed and control rats. Thresholds (i.e., rate differences corresponding to 50% performance score in psychometric curves) are marked by arrows. Values shown are mean ± SEM. *$P < 0.00005$, Bonferroni-corrected *t* tests. (*C*) Examples of psychometric curves (*Left*) and the average discrimination thresholds (*Right*) obtained from female CTM-exposed rats vs. same-sex control rats. Note that discrimination thresholds for female CTM-exposed rats did not differ from controls ($P = 0.38$, Bonferroni-corrected *t* test). (*D*) Dot raster plots of cortical responses to pulse trains of different repetition rates obtained from male CTM-exposed and same-sex control rats. The red lines indicate pulse durations. (*Inset*) RRTF for each raster plot example. ○, $f_{h1/2}$ for each RRTF; dashed lines, 50% of the maximal normalized response for each RRTF. (*E*) Average RRTFs for all recordings obtained from male CTM-exposed and control rats. *$P < 0.00001$, Bonferroni-corrected *t* tests. (*F*) Average $f_{h1/2}$s for all recordings from male CTM-exposed and control rats. *$P < 0.00001$, Bonferroni-corrected *t* tests.

discriminate a pulse train of 6.3 pulses per second (pps; nontarget) from one of 20 pps (target) to receive food rewards. The detection of this large temporal rate difference was perceptually unchallenging; all rats responded selectively (>80%) to the target within 1 wk of training. In a following test phase, the nontarget for each trial was randomly chosen from identical-length pulse trains that had varying repetition rates (6.3, 8.3, 10, 12.5, or 14.3 pps); the target was always a 20-pps train. A psychometric curve (Fig. 1 *B*, *Left*, and *C*, *Left*) was constructed, and the discrimination threshold (i.e., the rate difference corresponding to a 50% performance score in the psychometric curve; arrows in Fig. 1*B*, *Left*) was derived, to assess the temporal rate discrimination abilities of animals from different groups. By this simple measure, discrimination thresholds for male CTM-exposed rats ($n = 15$) were higher than in saline-exposed control rats ($n = 12$; Fig. 1*B*, *Right*; $P < 0.00005$). Discrimination thresholds for female CTM-exposed rats ($n = 14$), however, did not differ from controls ($n = 15$; Fig. 1*C*, *Right*; $P = 0.38$). These studies show that CTM exposure had a significant effect on the auditory temporal rate discrimination performance of male but not female rats.

Altered Cortical Responses After CTM Exposure. Because behavioral deficits were seen only in male CTM-exposed rats, we next investigated the effects of CTM on cortical responses in male rats, using conventional extracellular unit response-recording methods. Responses were recorded from neurons in the middle cortical layers at 247 primary auditory cortex (A1) sites in six CTM-exposed rats and at 278 A1 sites in six saline-exposed controls, again at approximately postnatal d 68 (Fig. 1*A*). Unless otherwise specified, all subsequent quantitative analyses in this section are based on these samples.

Cortical responses evoked by a variable rate of tone pulses presented at recorded units' characteristic frequencies (CFs) are shown for both rat groups in Fig. 1*D*. Whereas most cortical neurons in controls could follow repeated stimuli at repetition rates up to 10 pps, most cortical neurons in CTM-exposed rats only followed stimuli at rates up to about 7 pps (Fig. 1*D*). These effects were further documented by constructing repetition-rate transfer functions (RRTFs) for neurons at each site (Fig. 1*D*, *Insets*). As shown in Fig. 1*E*, the average normalized responses at higher temporal rates (i.e., 7–15 pps) for CTM-exposed rats were weaker than in controls (all $P < 0.00001$). We also determined the repetition rate at which the normalized response was at half of its maximum ($f_{h1/2}$) for each RRTF (○ in Fig. 1*D*, *Insets*) to quantify the cortical capacity for processing high-rate stimuli. The average $f_{h1/2}$s for CTM-exposed rats, as shown in Fig. 1*F*, were consistently lower than for controls at all CFs (all $P < 0.00001$). These data show a degraded cortical auditory temporal rate following ability resulting from perinatal CTM exposure in male rats.

Consistent with an earlier study (20), the average frequency bandwidths of tuning curves measured 20 dB above the threshold (BW20s) for male CTM-exposed rats were significantly larger than in controls (Fig. S1 *A* and *B*; $P < 0.005$–0.00001), indicating decreased spectral response selectivity after perinatal CTM exposure. Importantly, response thresholds of cortical sites did not differ for CTM-exposed vs. control rats (Fig. S1*C*; all $P > 0.16$).

We also compared temporal cortical unit responses for five female CTM-exposed rats (200 A1 recording sites) and six female saline-exposed rats (277 recording sites). Average $f_{h1/2}$s for CTM-exposed rats (Fig. S2) were comparable to controls at all CFs (all $P > 0.34$). These data show that perinatal CTM exposure has little effect on the A1 unit temporal responses in young female rats, consistent with their normal behavioral performance (Fig. 1*C*). Thus, sex-specific behavioral deficits in male CTM-exposed animals are likely related to impaired temporal rate following of cortical neurons resulting from perinatal CTM exposure.

Behavioral Training Restores Cortical Temporal Deficits. Our next goal was to investigate whether intensive training could restore degraded cortical temporal responses induced by perinatal CTM exposure in male rats, using a modified go/no-go operant training procedure (*SI Materials and Methods*). CTM-exposed male rats ($n = 7$) were trained to identify a target auditory stimulus [pulse train with a specific pulse repetition rate (PRR)], when presented with a set of distractor auditory stimuli (pulse trains with different nontarget PRRs), to receive food rewards. The target pulse train rate changed daily on a random schedule. In the early days of training, rats continuously nose-poked in each trial such that the nose-poke responses in each block were equally distributed over all pulse trains presented (Fig. 2*A*, *Top*; $P = 0.08$–0.41, ANOVA). That strategy resulted in low performance scores for all training

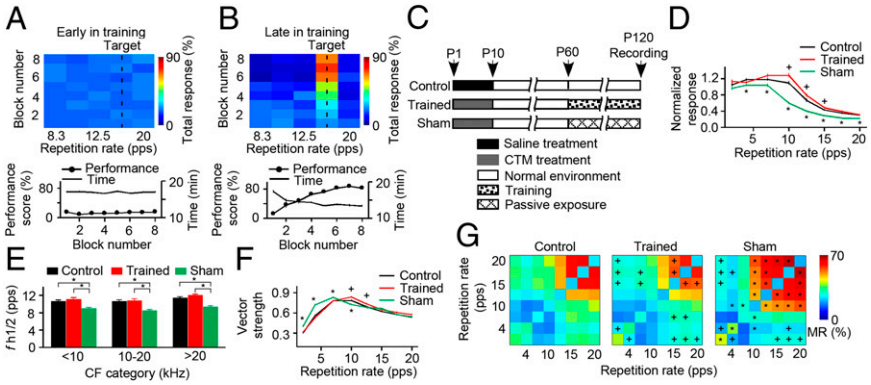

Fig. 2. Degraded middle-layer A1 temporal responses in male CTM-exposed rats restored by training. Behavioral performance of CTM-exposed rats on the temporal rate discrimination task on early (*A*) and late (*B*) training days. (*Top*) Distribution of total nose-poke responses for each block during a single training day. Values shown are the means of all animals. Target stimuli are marked with dashed lines. (*Bottom*) Performance score for each block. The time required for rats to complete the block is also shown (right ordinate). Values shown are mean ± SEM. (*C*) Experimental timelines for different groups of rats. Trained, CTM-treated followed by intensive auditory training; Sham, CTM-treated followed by passive auditory exposure. (*D*) Average RRTFs of cortical responses to repetitive stimuli of different PRRs. $*P < 0.05$, sham vs. controls; $+P < 0.05$, trained vs. controls (ANOVA with post hoc Student–Newman–Keuls tests). (*E*) Average $f_{h1/2}$s for all recording sites in the different groups. $*P < 0.001$, ANOVA with post hoc Student–Newman–Keuls tests. (*F*) Average vector strengths for the different groups. $*P < 0.001$, sham vs. controls; $+P < 0.05$, trained vs. controls (ANOVA with post hoc Student–Newman–Keuls tests). (*G*) Average MRs for all combinations of repetition rates for the different groups. MRs were significantly larger (*) or smaller (+) compared with control rats ($P < 0.05$, ANOVA with post hoc Student–Newman–Keuls tests).

blocks (Fig. 2*A*, *Bottom*). It took these animals a long time (an average of 16.6–17.2 min) to complete a training block due to frequent "time-outs." After several weeks of training, rats had learned to identify each new randomly set target during the initial block on each day, as demonstrated by their progressively more selective responses to it in following training blocks (Fig. 2*B*, *Top*; $P < 0.04$–0.0001, ANOVA); performance scores increased on successive training blocks in every session (Fig. 2*B*, *Bottom*; all $P < 0.001$, ANOVA with post hoc Student–Newman–Keuls tests). The time required to complete a training block also significantly decreased across the session (all $P < 0.001$, ANOVA with post hoc Student–Newman–Keuls tests).

After approximately 2 mo of training, all CTM-exposed rats had mastered the behavior. Cortical unit modulation rates in CTM-exposed and then trained rats (281 A1 recording sites) were again reconstructed and compared with cortical unit modulation rates of (*i*) age-matched, passively-stimulated, CTM-treated rats (i.e., a sham training group; 236 A1 recording sites) and (*ii*) naive saline-injected controls (219 A1 recording sites; experimental timelines are provided in Fig. 2*C*).

As shown in Fig. 2*D*, normalized cortical responses in RRTFs in sham rats fell off rapidly above 7 pps compared with controls. By contrast, cortical responses for trained rats were significantly increased at these higher repetition rates (i.e., 7–20 pps) compared with sham rats (all $P < 0.05$), and were now stronger than those cortical responses recorded from controls at some rates (i.e., 10–15 pps; all $P < 0.05$). We again compared $f_{h1/2}$s obtained from the different groups of rats (Fig. 2*E*). As expected, whereas average $f_{h1/2}$s were smaller for sham compared with control rats across all CFs (all $P < 0.001$), average $f_{h1/2}$s for trained rats were now similar to control rats (all $P > 0.26$).

We then calculated vector strengths to evaluate the degree of phase locking of cortical responses to repetitive stimuli. Overall, vector strengths as a function of temporal rate for CTM-exposed sham rats shifted leftward and peaked at lower rates compared with the vector strengths from controls (Fig. 2*F*; all $P < 0.001$). The vector strengths of trained rats again matched the vector strengths of control rats at most repetition rates (all $P > 0.1$) but were actually greater at some middle rates (i.e., 10 and 12.5 pps; both $P < 0.05$).

To examine further the reliability and precision of cortical temporal responses to repetitive stimuli, we calculated the misclassification rate (MR) for all possible combinations of pulse trains using the van Rossum spike train distance metric (Fig. 2*G*). The MR provides an index of similarity between spike trains responding to different stimulus trains, or the difference between spike trains responding to identical stimulus trains, taking into account both spike timing and spike number. A smaller MR value indicates more reliable and precise spiking (more reliable coding) in response to repetitive stimuli. MRs of sham rats for some combinations of dissimilar high pulse rates (10–20 pps) or identical low rates (2 or 4 pps) were significantly larger compared with control rats [denoted with asterisks (*) in Fig. 2*G*, *Right*; all $P < 0.05$]. Sound rate discrimination training reduced the MRs; values for trained rats were comparable to, or even smaller than, values in control rats [denoted with crosses (+) in Fig. 2*G*, *Center*; all $P < 0.05$]. It is interesting to note that the MRs for some combinations of low and high rates for sham (passive sound-exposed, CTM-treated) rats were also smaller compared with control rats (denoted with crosses in Fig. 2*G*, *Right*; all $P < 0.05$).

The above data show that perceptual training broadly renormalized A1 temporal responses that were degraded by perinatal CTM exposure in these male rats. However, BW20s of frequency tuning curves for trained rats were comparable to sham rats (Fig. S3; all $P > 0.21$) but significantly differed compared with controls ($P < 0.05$–0.001). Not surprisingly, temporal rate discrimination training did not restore frequency response selectivity of cortical neurons to normal (26).

Reversal of Serotonergic and Dopaminergic Systems. The earlier discovery of selective loss of serotonin transporter (SERT) immunoreactive fiber density in the neocortex and reduced expression of tryptophan hydroxylase (TPH) among the midline subgroups of serotonergic raphe neurons after perinatal exposure to CTM (19, 20) led us to examine whether such changes in the serotonergic system were affected by auditory behavioral training (Fig. 3 *A*–*F*). Immunohistochemical analyses confirmed a statistically significant decrease in TPH staining density within the dorsal raphe (DR) nuclear complex after CTM exposure compared with control animals (Fig. 3*G*; $P < 0.05$–0.01) and a statistically significant recovery within the rostral DR compared with sham rats after auditory training ($P = 0.027$). Interestingly, this restoration was recorded in every subregion of

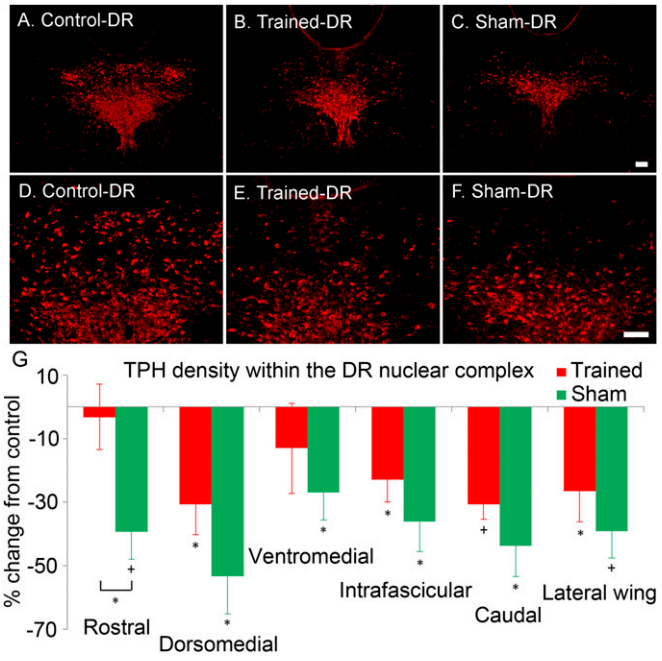

Fig. 3. Color photomicrographs of lower (*A–C*) and higher (*D–F*) magnifications showing TPH immunoreactivity in the DR nuclear complex from control (*Left*), trained (*Center*), and sham (*Right*) rats. (Scale bars: *Upper*, 100 μm; *Lower*, 60 μm.) (*G*) Quantitative analysis within each subregion of the DR verified that TPH density significantly decreased in CTM-exposed sham rats compared with saline-exposed control rats. This impairment was partially recovered by auditory training in nearly every subregion of the DR nuclear complex. Values shown are mean ± SEM. $*P < 0.05$, $+P < 0.01$; one-sample or independent sample *t* tests.

the DR nuclear complex, consistent with a global recovery of serotonergic circuit function. We next examined whether SERT immunoreactive fiber density was affected by training (Fig. 4 *A–C*). Our semiquantitative immunohistochemical data showed that in the limbic area of the medial prefrontal cortex (mPFC), SERT immunoreactive fiber density was significantly reduced in sham rats compared with saline-exposed controls (Fig. 4*G*; $P = 0.023$). SERT fiber density returned to near control levels after training ($P = 0.086$). These findings indicate that behavioral training reverses both serotonergic DR nuclear complex enzymatic expression and the serotonergic substrate broadly across the neocortical target zone.

It is well known that neuromodulator circuits supporting serotonin, dopamine, and norepinephrine are reciprocally interconnected (28, 29). In particular, dopamine and serotonin can be coreleased upon stimulation (30). Further, dopaminergic neurons in the ventral tegmental area and substantia nigra (SN) are known to take up 5-hydroxytryptamine (5-HT; via dopamine transporter) transiently during normal early development (31–33). It therefore seemed likely that recovery of neuromodulatory circuit function may extend to the dopaminergic system (Fig. 4 *D–F*). Indeed, dopaminergic tyrosine hydroxylase (TH) immunoreactive fiber density was lower in the mPFC of CTM-exposed sham rats compared with saline-exposed controls ($P = 0.008$), and partially recovered as a result of training (Fig. 4*H*). Moreover, the intensity of TH immunoreactive fibers within the striatal complex (e.g., caudate-putamen and nucleus accumbens) was lower in CTM-exposed sham rats compared with saline-exposed controls (Fig. S4; $P = 0.003$ for caudate-putamen, $P = 0.009$ for nucleus accumbens core, $P = 0.013$ for nucleus accumbens shell). Training resulted in a partial reversal of that index of dopamine expression.

Serotonin is also known to take up (via norepinephrine transporter) 5-HT transiently in the noradrenergic locus coeruleus (LC) system during early development (31, 33), and it is again likely that noradrenergic circuitry may be affected by CTM exposure. Our semiquantitative TH immunohistochemical data in the cerebellum (the sole source of which comes from noradrenergic LC neurons) revealed patchy-like fibers in control rats (Fig. S5). A more widespread distribution of TH immunoreactive fibers was seen in CTM-exposed sham rats compared with saline-exposed controls ($P = 0.056$), and an even greater density was seen in CTM-exposed trained rats ($P = 0.038$).

To our knowledge, the above findings are the first to demonstrate that recovery of global brain function after behavioral training involves a substantial renormalization of neuromodulatory systems. These neuromodulators are well known to play a key role in monitoring normal behavior, and are especially related to cognitive processing of information, emotion, anxiety, sensory perception, learning and memory, and motor coordination (34). Our studies thus provide data on the potential cellular mechanism(s) and offer future specific therapeutic treatment strategies for patients with ASDs, including both the core and associated symptoms, such as mood and sensory deficits (13, 35–37).

Recovery of GABAergic Inhibitory Interneuron Expression. Appropriate information processing in the neocortex requires a balance between excitation and inhibition (12). Currently, dysfunction of GABAergic neuronal circuitry has been suggested as one of the pathological events in autistic patients, leading to degraded integration and synchronization among cortical neurons (38–40). In particular, parvalbumin-positive (PV^+) cortical neurons are known to play a central role in cortical inhibitory action and synaptic plasticity, not only during early development but throughout the life span (27, 41, 42). Does perinatal exposure to CTM induce

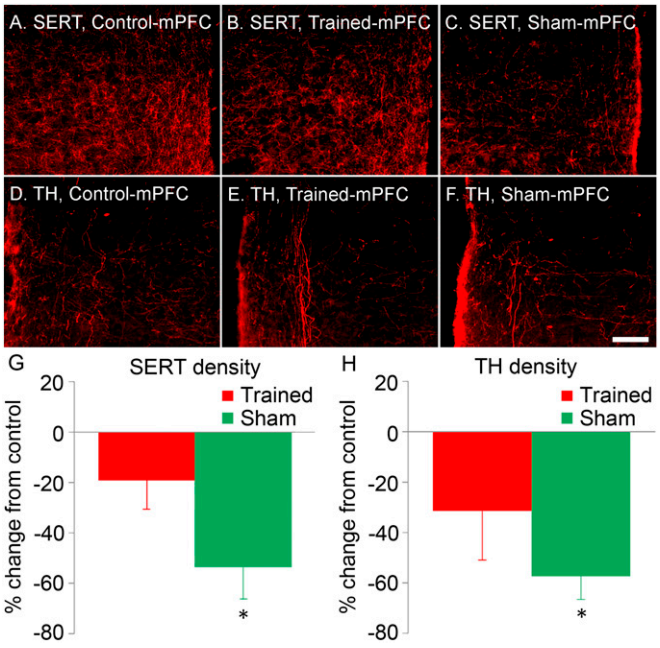

Fig. 4. Color photomicrographs showing SERT (*A–C*) and TH (*D–F*) immunostaining patterns in the mPFC in control (*Left*), trained (*Center*), and sham (*Right*) rats. (Scale bar: 60 μm.) Quantitative analysis of SERT (*G*) and TH (*H*) expression within the mPFC verified a reduction in fiber density in CTM-exposed sham rats compared with saline-exposed controls that could be partially recovered by auditory training. Values shown are mean ± SEM. $*P < 0.05$ (*G*), $*P < 0.01$ (*H*); one-sample *t* tests.

changes in PV^+ interneurons indexing their (and cholinergic) functional integrity and power? Can training reverse them?

A reduction in the density of PV^+ neurons was noted in CTM-exposed sham rats compared with saline-exposed controls within the A1 (Fig. 5 *A*, *D*, *G*, and *J*; $P = 0.017$), the primary somatosensory cortex (S1; Fig. 5 *B*, *E*, *H*, and *J*; $P = 0.014$), and the mPFC (Fig. 5 *C*, *F*, *I*, and *J*; $P = 0.060$). Behavioral training completely reversed the decreased expression of PV^+ neurons in trained rats within the A1 ($P = 0.018$) and, interestingly, partially reversed PV expression in the S1 and mPFC. The recovery of PV immunoreactivity within the A1 is likely driven by electrophysiologically documented changes in temporal responding, which are believed to be PV^+ neuron-dependent (43, 44). Together, these data point to the likely source of the imbalance of excitation and inhibition within the neocortex in autism and other disorders, and indicate that this key aspect of global brain function is recoverable via auditory behavioral training.

Discussion

Our results show that early-life SSRI exposure leads to sex-specific deficits in auditory cortical information processing and alterations in neuromodulatory systems expressing serotonin, dopamine, and norepinephrine, as well as ACh-modulated PV inhibitory GABAergic processes. These data indicate that a global circuit dysfunction results from perinatal exposure to antidepressants, such as CTM. Importantly, extensive auditory behavioral training in juveniles at least substantially reverses most of the deficits, and modest elaboration in training (e.g., extending training to recovery spectral response selectivity) could be expected to reverse changes not addressed by this single, simple training task. Perhaps the most intriguing aspect of these findings is the magnitude of the reversals of these physical and functional deficits, achieved via a very simple and time-limited form of behavioral therapy.

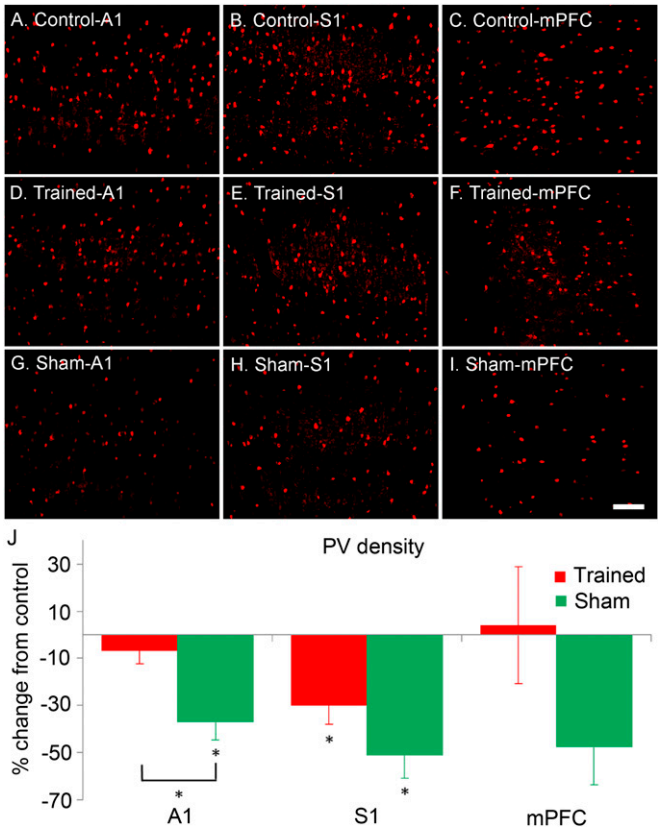

Fig. 5. (*A–I*) Color photomicrographs showing PV^+ neurons in the A1 (*Left*), S1 (*Center*), and mPFC (*Right*) of control (*Upper*), trained (*Middle*), and sham (*Lower*) rats. Pial surface on the top is for A1 and S1; pial surface on the right is for mPFC. (Scale bar: 60 μm.) (*J*) Quantitative analysis of PV immunoreactivity verified a reduced expression density within the A1, S1, and mPFC in CTM-exposed sham animals compared with saline-exposed controls. This reduction was partially recovered after auditory training in the S1 and mPFC, with an apparently complete reversal recorded in the A1. Values shown are mean ± SEM. $*P < 0.05$, one-sample or independent sample *t* tests.

It should be noted that observed deficits after early exposure to CTM seen only in males were reported in other studies after similar drug applications (20, 45, 46). For example, it has been shown that impaired social behavior and response to novelty are more obvious in CTM-exposed male compared with female rats (20). Drug exposure also selectively alters both spontaneous and stimulus-driven activity of LC neurons in male rather than female rats (45). All of these results suggest a sexually dimorphic response in our CTM-exposed rodent model. Interestingly, earlier studies have also reported that boys are approximately fivefold more likely to exhibit ASDs than girls (47). In this study, CTM-induced behavioral deficits were observed in male animals only, and the subsequent anatomical experiment was therefore carried out on male rats. Whether drug-induced anatomical impairment also occurs in female rats needs further study.

Extensive behavioral training applied in this study renormalized PV expression in the cortical field A1 degraded by perinatal CTM exposure, which paralleled the recovery of cortical temporal processing. PV^+ neurons in auditory cortex are believed to be involved in regulating the temporal precision of cortical responses instead of shaping frequency tuning, because they usually have faster response latencies and are well tuned for frequency (48). This result may explain the observation that, although training renormalized A1 temporal responses of CTM-exposed rats, it did not restore cortical frequency selectivity to normal.

A potential limitation to our study was that only one time course of only one form of auditory training (i.e., ∼2 mo in the rodent model) was investigated. Obvious next steps are to identify dose–response relationships and the breadth of behavioral training needed to reverse ASD-related neurobehavioral distortions arising in early development most completely and to determine whether more extended and broader training regimes result in the expected, still more complete neurological renormalization. It is also interesting to examine further whether the observed neurological renormalization after training is persistent or only transitory. Finally, the precise cellular and molecular mechanisms underlying the distortions induced here by CTM and the recoveries attributed to training remain to be elucidated, and shall, of course, require a combination of anatomical and physiological approaches at various levels of analysis.

Taken together, our results describe how intensive auditory behavioral training can substantially recover global cortical network dysfunction caused by perinatal exposure to antidepressants, such as CTM. To our knowledge, these previously unidentified findings provide additional support for the application of neurologically targeted forms of behavior training as a part of treatments for improving clinical outcomes in individuals with ASDs and related PDDs. The reversal of changes in global cortical networks involving neuromodulatory and inhibitory systems has significant additional implications for our clinical potential for reversing abnormal human behavior for auditory- and language-related impairments, as well as for other higher cognitive and emotional/social disabilities, such as phobia, fear, mood, and anxiety, which are also often exhibited in patients with ASDs.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committees at the East China Normal University and the University of Mississippi and complied with NIH standards.

Animal Preparation. Both male and female offspring of timed-pregnant Sprague–Dawley rats were used in the experimental procedures. The basic preparation of the CTM (Toronto Research Chemicals) and animal care were performed as described in *SI Materials and Methods*.

Sound Temporal Rate Discrimination Testing. After rats learned to discriminate a pulse train of four noise bursts (i.e., nontarget) from an 11-burst train of the same duration (target), their temporal rate discrimination abilities were tested by randomly delivering nontarget pulse trains with various pulse rates, as described in *SI Materials and Methods*.

Cortical Mapping. Cortical responses were recorded with tungsten microelectrodes under pentobarbital anesthesia (50 mg/kg of body weight). Data were collected and analyzed as described in *SI Materials and Methods*.

Immunohistochemistry and Quantitative Analysis. Rats received a lethal dose of pentobarbital (85 mg/kg of body weight) and were perfused intracardially with paraformaldehyde. Details of immunohistochemical procedures have been described in *SI Materials and Methods*.

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