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Pairing with enriched sound exposure restores auditory processing degraded by an antidepressant

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34

Abstract

Antidepressants, while effective in treating depression and anxiety disorders, also induce deficits in sensory (particularly auditory) processing, which in turn may exacerbate psychiatric symptoms. How antidepressants cause auditory signature deficits remains largely unknown. Here, we found that fluoxetine-treated adult female rats were significantly less accurate when performing a tone-frequency discrimination task compared with age-matched control rats. Their cortical neurons also responded less selectively to sound frequencies. The degraded behavioral and cortical processing was accompanied by decreased cortical perineuronal nets (PNNs), particularly those wrapped around parvalbumin-expressing inhibitory interneurons. Furthermore, fluoxetine induced critical period-like plasticity in their already mature auditory cortices; therefore, a brief rearing of these drug-treated rats under an enriched acoustic environment renormalized auditory processing degraded by fluoxetine. The altered cortical expression of PNNs was also reversed as a result of enriched sound exposure. These findings suggest that the adverse effects of antidepressants on auditory processing, possibly due to a reduction in intracortical inhibition, can be substantially alleviated by simply pairing drug treatment with passive, enriched sound exposure. They have important implications for understanding the neurobiological basis of antidepressant effects on hearing and for designing novel pharmacological treatment strategies for psychiatric disorders.

Keywords: auditory cortex, cortical plasticity, frequency tuning, inhibition, antidepressant

Significance Statement

Clinical experience suggests that antidepressants adversely affect sensory (particularly auditory) processing, which can exacerbate patients' psychiatric symptoms. Here, we show that the antidepressant fluoxetine reduces cortical inhibition in adult rats, leading to degraded behavioral and cortical spectral processing of sound. Importantly, fluoxetine induces a critical period-like state of plasticity in the mature cortex; therefore, a brief rearing under an enriched acoustic environment is sufficient to reverse the changes in auditory processing caused by the administration of fluoxetine. These results provide a putative neurobiological basis for the effects of antidepressants on hearing and indicate that antidepressant treatment combined with enriched sensory experiences could optimize clinical outcomes.

Introduction

Antidepressants, such as fluoxetine, are widely used to treat depression and anxiety disorders. However, it has been clinically reported that chronic exposure to antidepressants has adverse side effects on sensory (particularly auditory) processing, eliciting hearing disorders, including tinnitus and auditory hallucinations (Oranje et al., 2011; Pattyn et al., 2016; Zhong et al., 2021). These negative effects of antidepressants on hearing are acknowledged factors for psychiatric disorders and are argued to exacerbate psychiatric symptoms (Pattyn et al., 2016; Blazer and Tucci, 2019). While recent studies in rodent models have also demonstrated antidepressant-induced auditory signature deficits (Simpson et al., 2011; Dringenberg et al., 2014; Ampuero et al., 2019; Pan et al., 2021), their neurological basis remains largely unknown.

Previous studies on the auditory system have documented an early, several day-long postnatal epoch (i.e., a critical period), during which cortical representation and response selectivity can be remodeled on a large scale merely by passive exposure to sound stimuli (de Villers-Sidani et al., 2007; Insanally et al., 2009; Pysanenko et al., 2018; Nakamura et al., 2020; Svobodová Burianová and Syka, 2020). Beyond this period, cortical modification requires sound input that carries behavioral significance (i.e., behavioral context with attention or reward; Recanzone et al., 1993; Polley et al., 2006; Zhou and Merzenich, 2009; Zhang et al., 2013; Cheng et al., 2017,2020). Accordingly, our earlier studies have shown that developmentally degraded cortical processing can be restored in adulthood by auditory training rather than passive sound exposure (Zhou and Merzenich, 2009; Zhu et al., 2016; Liu et al., 2019). While studies have reported that antidepressants reactivate critical period-like plasticity in the adult visual cortex of rodents (Maya

Vetencourt et al., 2008; Steinzeig et al., 2019), no similar investigations have been conducted so far on the auditory cortex, which is also a paradigmatic model for plasticity studies of cortical networks. Thus, whether degraded auditory processing can be renormalized through passive sound exposure in the antidepressant-treated mature brain remains to be tested.

Using adult rats as a research model, we first documented the physiological changes in the primary auditory cortex (A1) following chronic fluoxetine treatment and their possible effects on behavioral outcomes. We then investigated whether fluoxetine might induce juvenile-like plasticity in the mature cortex and therefore renormalize drug-altered behavioral and cortical processing, if any, by simply pairing the treatment with enriched sound exposure. Finally, we quantified changes in cortical inhibition accompanying these post-exposure effects to elucidate the mechanisms underlying fluoxetine exposure.

Materials and Methods

All procedures complied with NIH standards and were approved by the Institutional Animal Care and Use Committee of East China Normal University. Efforts were made to minimize animal suffering and the number of animals employed in the experiments.

Animal preparation

Female Sprague-Dawley rats, aged eight weeks, were randomly assigned to either the fluoxetine or water condition. As described previously (Maya Vetencourt et al., 2008), fluoxetine (fluoxetine hydrochloride, Sigma) was dissolved in the drinking water at 200 mg/L and was available ad libitum. The control animals received drinking water without fluoxetine. All rats were given free access to food and drinking water under a 12 h

light/12 h dark cycle. The researcher remained blind to the group identity of the animals.

Sound exposure

Rats in a cage (35×22×20 cm, length×width×height) were placed in a sound-shielded test chamber for passive sound exposure. The stimuli for tone exposure were pulsed 7 kHz tone pips (50 ms duration with 5 ms ramps) that were delivered at 5 pulses per second (pps) with an intensity of 65 dB sound pressure level (SPL) measured at the center of the cage. There was a 1 s interval of silence between every 5 pulses to minimize adaptation effects. The stimuli for enriched sound exposure were pulse trains with a duration of 1 s that contained 2, 5, 10, or 15 tone pips (50 ms duration with 5 ms ramps). The frequency of each tone pip in the pulse train was set at 1.5, 2.3, 3.5, 5.3, 8.1, 12.3, 19, or 29 kHz. These pulse trains with different frequencies and repetition rates were randomly delivered at 65 dB SPL. Again, there was a 1 s interval of silence between each train presented.

Open field test

The apparatus for the open field test was a rectangular box (42×42×37 cm, length×width×height). During the test, the rats were placed at the center of the box facing the wall and were left to freely explore over a 10 min period. The total distance traveled was analyzed using a True-Scan System (Coulbourn Instruments). The box was wiped clean with 75% ethanol after each test.

Elevated zero-maze test

The elevated zero-maze is an annular platform (5.5 cm width) with an outer diameter of 92 cm divided into two open arms and two opposite closed arms. While the closed arms were enclosed by side walls 20 cm in height, there were no walls for the open arms.

The apparatus was elevated 50 cm above the floor. During the test, the rats were placed into one of the open arms facing the closed arm and allowed to freely explore the maze. The behavior of each rat was recorded with a digital camera and analyzed using an ANY-maze system (Stoelting). The maze was wiped clean with 75% ethanol after each test.

Sound frequency discrimination test

The behavioral task was conducted in a wire-mesh cage (20×20×18 cm, length×width×height) enclosed within a sound-attenuating chamber. The acoustic stimuli used were 50 ms tone pips with a 5 ms rise-decay time at 60 dB SPL. The rats were first trained to discriminate a target tone (8 kHz) from a non-target tone (4 kHz; i.e., one-octave separation from 8 kHz). Only one tone with a specific frequency was presented in each trial. The rats were rewarded for making a go response within a limited time window after the presentation of a target tone. When the animals achieved steady performance scores, sound frequency discrimination was tested on the next day by randomly setting the frequency of the non-target tone to 7, 6.1, 5.3, 4.6, or 4 kHz (i.e., 0.2, 0.4, 0.6, 0.8 or 1 octave separation from 8 kHz, respectively). The target tone was always 8 kHz during the test.

The behavioral test and data acquisition were controlled by an input and output system consisting of a speaker, photo-beam detector, food dispenser, sound card, and house light (Med Associates). In each trial, a rat's behavioral state could be classified as either a go or a no-go response. The rats were in a go state when the photo-beam of the nose-poke device was interrupted (i.e., a nose-poke response). All other states were considered no-go. For a given trial, the rats could elicit one of five reinforcement states.

The first four states were given by the combinations of responses (go or no-go) and stimulus properties (target or non-target). A go response within 3 s of a target was scored as a hit; a failure to respond within this time window was scored as a miss. A go response within 3 s of a non-target was scored as a false-positive, and the absence of a response was scored as a withhold. The fifth state, false-alarm, was defined as a go response that occurred 3 s or more after stimulus presentation. While a hit triggered the delivery of a 45 mg food pellet (BioServe) as a reward, a miss, false-positive, or false-alarm initiated a 9 s time-out during which the lights were turned off and no stimuli were presented. A withhold, however, resulted in neither a reward nor a time-out.

The behavioral discrimination of each rat was quantified as a performance score: H-F×H (expressed as a percentage), where H is the hit rate=the number of hits/the number of target trials and F is the false-positive rate=the number of false-positives/the number of non-target trials (Rybalko et al., 2010; Zhu et al., 2014; Tang et al., 2022).

Auditory brainstem response (ABR) and cortical response recording

As described in our earlier studies (Cheng et al., 2020, 2022; Tang et al., 2022), the recording was conducted in a shielded, double-walled sound room. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). A state of areflexia was maintained with supplemental doses of 8 mg/ml dilute pentobarbital injected intraperitoneally during the recording. The animal's body temperature was monitored using a rectal probe and maintained at ~37 ℃ using a feedback-controlled heating blanket.

For recording the ABR, tone pips (3, 10, 15, or 20 kHz) at different intensities were delivered to the ear through a calibrated earphone with a sound tube positioned inside the

external auditory meatus. The ABR was measured by placing three electrodes subdermally at the scalp midline, posterior to the stimulated ear, and on the midline of the back 1-2 cm posterior to the neck of the animal. The ABR threshold was defined as the lowest sound intensity capable of eliciting a response pattern characteristic of that seen at higher intensities.

For cortical recording, the animal was anesthetized and the skull was secured in a head holder, leaving the ears unobstructed. The auditory cortex was surgically exposed and the dura was resected. The cortical responses were recorded using parylene-coated tungsten microelectrodes (1-2 MΩ at 1 kHz; FHC). Acoustic stimuli were delivered to the contralateral ear relative to the recording site through a calibrated earphone with a sound tube positioned inside the external auditory meatus. At each recording site, the microelectrode was lowered orthogonally into the cortex to a depth of ~450-550 μm (layers 3/4) (Games and Winer, 1988; Roger and Arnault, 1989) to record the evoked spikes of a neuron or a small cluster of neurons.

The frequency tuning curves were reconstructed by presenting pure tones (25 ms duration) of 50 frequencies (1-30 kHz) at eight sound intensities (0-70 dB SPL in 10 dB increments) in a random, interleaved sequence at a rate of 2 pps. The characteristic frequency (CF) of a cortical site was defined as the frequency at the tip of the V-shaped tuning curve. For flat-peaked tuning curves, the CF was defined as the midpoint of the plateau at the threshold. For tuning curves with multiple peaks, the CF was defined as the frequency at the most sensitive tip (i.e., the tip with the lowest threshold). The response bandwidths that were 20 dB above the threshold of the tuning curves (BW20s) were measured for all recording sites.

As previously described (Rutkowski et al., 2003; Polley et al., 2006,2007), the A1 was identified based on the unique rostral-to-caudal tonotopy and reliable neuronal responses to tone pips of selective frequencies. To generate A1 maps, Voronoi tessellation (a MATLAB routine; MathWorks) was performed to create tessellated polygons with electrode penetration sites at their centers. Each polygon was assigned the characteristic (i.e., the CF) of the corresponding penetration site. In this way, every point on the surface of the auditory cortex was linked to the characteristic that was experimentally derived from a sampled cortical site closest to this point.

A software packages SigCal, SigGen, BioSig, and Brainware (Tucker-Davis Technology) were used to calibrate the earphone, generate acoustic stimuli, monitor the response properties online, and store the data for offline analysis, respectively.

Immunohistochemistry

As in our earlier studies (Cheng et al., 2020,2022), the rats that received a lethal dose of pentobarbital (85 mg/kg body weight) were perfused intracardially with saline solution followed by 4% paraformaldehyde in 0.1 M potassium phosphate-buffered saline (pH 7.2). The brains were removed and placed in the same fixative containing 20% sucrose for 12-24 h. The fixed material was sliced in the coronal plane on a freezing microtome (Leica CM3050 S, Leica Microsystems) at a thickness of 40 μm. The free-floating sections were preincubated in a blocking solution to suppress nonspecific binding. The sections were then incubated at 4 °C for 12 h with anti-parvalbumin (anti-PV; 1:1000, Sigma) and fluorescein wisteria floribunda lectin (1:400, Vector), and at 37 °C for 1 h with the secondary antibody (1:400, Invitrogen). Samples from the different groups of rats were always processed together during the immunostaining procedures to limit

variations related to antibody penetration, incubation time, and the post-sectioning condition of the tissue.

Fluorescence images were acquired using a Leica DM4000 B (Leica Microsystems) epifluorescent microscope equipped with a Leica DFC450 C digital camera (Leica Microsystems). Image J (NIH) was utilized for the quantitative analysis of neuron densities in the different cortical layers. To quantify the fluorescence intensity, a horizontal line was drawn in the middle of an identified neuron. Image J was then used to analyze the fluorescence intensity along that line to obtain a fluorescence-distance function. The highest intensity on this curve was chosen as the fluorescence intensity of the neuron.

Experimental design and statistical analysis

Behavioral, electrophysiological, and immunohistochemistrical data were recorded as described above and compared between different groups of rats to evaluate the fluoxetine effects on cortical processing. In addition, a basic index of critical period plasticity (i.e., tone-specific enlargement in A1 representation resulting from passive sound exposure) was determined to investigate the plasticity status of A1 following fluoxetine treatment. Finally, fluoxetine treatment was paired with enriched sound exposure to study the reversal effect on drug-degraded cortical processing.

Data are presented as the mean±SEM. Statistical analysis was conducted using unpaired Student's t test or ANOVA with Student-Newman-Keuls post hoc test. In addition, the cumulative distributions of data from different groups were compared with the Kolmogorov-Smirnov test. Differences were considered statistically significant at $p<0.05$.

254

255 **Results**

256 The rats were exposed to fluoxetine dissolved in drinking water over a four-week
257 period beginning at postnatal week 8 (**Fig. 1A**). These rats were thereafter referred to as
258 the fluoxetine-treated (Flx) rats. In concordance with earlier studies (Thompson et al.,
259 2004; Guirado et al., 2012), chronic fluoxetine treatment produced a moderate decrease in
260 weight gain when compared with the age-matched control rats reared under standard
261 housing conditions (240.8±3.3 g for the Flx rats vs. 251.8±3.8 g for the control rats;
262 unpaired t test, $t(22)=2.18$, $p=0.04$). In addition, the average distances traveled by the Flx
263 rats for locomotor activities during the different test periods were all longer than those
264 traveled by the control rats in the open field test, although the differences did not reach
265 statistical significance (**Fig. 1B** and **C**; two-way ANOVA, $F(1,48)=3.65$, $p=0.062$). The
266 elevated zero-maze test also revealed that the distance traveled by the Flx rats was longer
267 than that traveled by the control rats (**Fig. 1D** and **E**, left; unpaired t test, $t(15)=2.95$,
268 $p=0.0099$). The number of transitions between the open and closed arms was also greater
269 for the Flx rats than for the control rats (**Fig. 1E**, middle; unpaired t test, $t(15)=2.43$,
270 $p=0.028$). However, no significant difference in the time spent in the open arm of the
271 elevated zero-maze was found between the two groups (**Fig. 1E**, right; unpaired t test,
272 $t(15)=0.26$, $p=0.8$). These data reflect slightly increased locomotor activities but normal
273 anxiety-related behaviors in the Flx rats compared with the controls.

274 Next, we evaluated the possible consequences of fluoxetine treatment on sound
275 frequency discrimination performance for the Flx rats vs. the control rats (**Fig. 2A**). All
276 the rats were first trained to detect a large frequency difference (i.e., a one-octave

separation) between a target tone and a non-target tone. After they achieved steady performance scores, the animals underwent a behavioral test in which the frequency difference between the target and the non-target was varied from 0.2 to 1 octave in each trial by randomly setting the frequency of the non-target tone. The psychometric curve for each animal was then obtained by plotting the performance score as a function of the frequency difference between the target and the non-target (**Fig. 2B**). As shown in **Fig. 2C**, while the performance scores for both groups of rats increased as the frequency difference increased (two-way ANOVA, $F(4,80)=118.17$, $p<0.001$), the values were significantly different between the Flx and control rats (two-way ANOVA, $F(1,80)=47.28$, $p<0.001$); that is, the performance scores at frequency differences of 0.6, 0.8, and 1 octaves were significantly lower for the Flx than the control rats (Student-Newman-Keuls post hoc test, all $p<0.001$). The discrimination threshold, defined as the frequency difference corresponding to 50% of the maximal performance score on the psychometric curve, was significantly higher in the Flx group than in the control group (**Fig. 2D**; unpaired t test, $t(16)=3.91$, $p=0.0012$). Further analysis revealed that the hit rates during the test were comparable between the two groups of rats (90.6±1.9% for the Flx rats vs. 93.8±1.5% for the controls; unpaired t test, $t(16)=1.55$, $p=0.14$). However, the fluoxetine treatment had a significant effect on the false-positive rates (**Fig. 2E**; two-way ANOVA, $F(1,80)=22.86$, $p<0.001$) such that the values at frequency differences of 0.6, 0.8, and 1 octaves were significantly higher for the Flx than for the control rats (Student-Newman-Keuls post hoc test, $p=0.005$ at a frequency difference of 0.6 octaves, $p=0.012$ at a frequency difference of 0.8 octaves, and $p<0.001$ at a frequency difference of 1 octave). These results indicate that fluoxetine treatment degrades the rats'

performance in a sound frequency discrimination task.

Earlier studies have shown that peripheral hearing alterations also affect auditory-related behavioral performance (Riley et al., 2021). The ABR is an evoked potential indicator of auditory activity in the auditory nerve and subsequent fiber tracts and nuclei within the auditory brainstem pathways. It provides information about peripheral hearing status and the integrity of brainstem pathways (Zhou et al., 2006; Cheng et al., 2022). To ensure that the degraded performance of the Flx rats in the sound frequency discrimination task could not simply be due to changes in hearing sensitivity after fluoxetine treatment, we recorded the ABRs for both the Flx and control rats (**Fig. 3A** and **B**). As shown in **Fig. 3C**, the ABR thresholds recorded from the Flx rats at different frequencies were all comparable to those recorded from the control rats (two-way ANOVA, $F(1,68)=0.04$, $p=0.84$). In addition, no significant differences in the ABR latencies of waves I and IV (**Fig. 3D**; two-way ANOVA, $F(1,68)=1.1$, $p=0.30$ for wave I; $F(1,68)=1.94$, $p=0.17$ for wave IV) or in the wave IV/I amplitude ratios (**Fig. 3E**; two-way ANOVA, $F(1,68)=1.93$, $p=0.14$) were found between the Flx and control rats. Thus, fluoxetine treatment has little impact on peripheral hearing sensitivity.

However, highly significant differences in frequency response selectivity between the Flx and control rats were documented in cortical field A1s. As shown in **Fig. 4A** and **B**, cortical frequency selectivity was examined by constructing frequency tuning curves for neurons in A1. The CFs and bandwidths 20 dB above the threshold (i.e., BW20s) were then determined. At first glance, cortical field A1s in both the Flx and control rats were relatively tonotopically organized, with the isofrequency bands oriented approximately orthogonal to a systematic rostrocaudal frequency representation gradient

(**Fig. 4C**). Further analysis confirmed that the distribution of all CFs recorded from the Flx rats was comparable to that recorded from the control rats (Kolmogorov-Smirnov test, $p=0.31$). However, neurons all across A1 in the Flx rats responded less selectively (i.e., were less sharply tuned) to sound frequencies than the neurons in the control rats, as evidenced by the systematically and significantly increased bandwidths of the tuning curves (**Fig. 4D**; two-way ANOVA, $F(1,461)=187.95$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p<0.001$ for all CF ranges). Interestingly, the response thresholds of A1 neurons recorded in the Flx rats were lower than those recorded in the controls (**Fig. 4E**; two-way ANOVA, $F(1,461)=45.84$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p=0.002$ in the low CF range but $p<0.001$ in both the middle and high CF ranges). However, the spontaneous firing rates of A1 neurons were higher in the Flx rats than in the controls (**Fig. 4F**; two-way ANOVA, $F(1,461)=55.16$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p=0.007$ in the low CF range but $p<0.001$ in both the middle and high CF ranges). All these results were further confirmed by comparing the data using the number of animals as the sample size (BW20: 2.59±0.06 oct. for the Flx rats vs. 1.68±0.03 oct. for the control rats, unpaired t test, $t(11)=15.4$, $p<0.0001$; threshold: 16.7±0.3 dB SPL for the Flx rats vs. 22.5±0.8 dB SPL for the control rats, unpaired t test, $t(11)=6.3$, $p<0.0001$; spontaneous firing rate: 4.1±0.13 spikes/s for the Flx rats vs. 2.9±0.14 spikes/s for the control rats, unpaired t test, $t(11)=6.5$, $p<0.0001$).

Fluoxetine has been shown to induce plasticity in the adult visual cortex, resulting in ocular dominance shifts of neurons after monocular deprivation which is otherwise restricted to the critical period (Maya Vetencourt et al., 2008; Steinzeig et al., 2019). We

346 next used a classical model of sound exposure-driven plasticity to determine the plasticity
347 status of A1 in the Flx rats. For this purpose, a subset of the Flx rats was exposed to
348 pulsed 7 kHz tones during the last week of their fluoxetine treatment epoch; these rats
349 were thereafter referred to as the Flx-treated plus tone-exposed rats (Flx+Te rats). In
350 addition, another group of age-matched control rats was exposed to pulsed 7 kHz tones
351 over the same epoch (known thereafter as the control+Te rats). The cortical fields of both
352 groups of rats were then mapped and compared with those of the Flx rats and the controls
353 (**Fig. 5A**). As shown in **Fig. 5B**, the A1 zone of the control+Te rats that selectively
354 responded to 7 kHz±0.25 octaves was comparable to the age-matched controls
355 (control+Te vs. control), confirming that their A1s had matured far beyond the normal
356 closure of the critical period window (de Villers-Sidani et al., 2007; Insanally et al., 2009;
357 Zhou et al., 2011). By contrast, in the Flx+Te rats, the A1 zone that selectively responded
358 to 7 kHz±0.25 octaves was enlarged by mere sound exposure compared with the control
359 rats (Flx+Te vs. control), as seen during the developmental critical period of the rat (de
360 Villers-Sidani et al., 2007; Insanally et al., 2009).
361 To quantitatively characterize the effects of fluoxetine on A1 frequency
362 representation, the percentages of A1 areas representing each frequency range were
363 averaged within the same experimental group and the differences between the exposed
364 and control animals were plotted. As shown in **Fig. 5C**, a one-way ANOVA
365 ($F(3,22)=48.47$, $p<0.001$) revealed that the average percentage of the A1 area tuned to 7
366 kHz±0.25 octaves in the Flx+Te rats was significantly higher than in the control rats
367 (Student-Newman-Keuls post hoc test, $p<0.001$). As expected, the average percentages of
368 A1 areas tuned to 7 kHz±0.25 octaves in the control+Te and Flx rats were both

369 comparable to those in the control rats (Student-Newman-Keuls post hoc test, p=0.70 for
370 control+Te rats vs. control rats and p=0.93 for Flx rats vs. control rats). Since
371 tone-specific enlargement in A1 representation resulting from transient exposure to sound
372 stimuli is a basic index of critical period plasticity, our results indicate that chronic
373 fluoxetine treatment induces critical period-like plasticity in the mature A1s of rats.
374 Passive sound exposure beyond the critical period does not appear to be sufficient to
375 generate persistent changes in mature A1s (Recanzone et al., 1993; Polley et al., 2006;
376 Zhou and Merzenich, 2009; Zhang et al., 2013; Cheng et al., 2017,2020). However,
377 inducing critical period-like plasticity in Flx rats makes it possible to remodel their
378 fluoxetine-degraded auditory processing by simply pairing the drug treatment with
379 enriched sound exposure. To test this hypothesis, a group of Flx rats was reared in an
380 enriched acoustic environment during the last week of their fluoxetine treatment epoch
381 (hereafter referred to as the Flx+Eae rats). Their behavioral and cortical processing of
382 sound frequency were then examined and compared with those of age-matched control
383 rats reared under identical conditions over the same epoch (these rats were thereafter
384 referred to as the control+Eae rats), as well as those of the Flx and control rats (**Fig. 6A**).
385 As shown in **Fig. 6B** and **C**, a one-way ANOVA ($F(3,28)=5.81$, $p=0.003$) revealed that
386 the average discrimination threshold of the psychometric curves obtained from the
387 Flx+Eae rats significantly decreased compared with the Flx rats (Student-Newman-Keuls
388 post hoc test, p=0.003) and was now comparable to that of the control rats
389 (Student-Newman-Keuls post hoc test, p=0.27). The average discrimination threshold of
390 the control+Eae rats, however, was substantially similar to that of the controls
391 (Student-Newman-Keuls post hoc test, p=0.79). In addition, extracellular recording

revealed that the average BW20s of the frequency tuning curves recorded in the Flx+Eae rats were significantly smaller than those in the Flx rats (**Fig. 6D** and **E**; two-way ANOVA, $F(3,936)=81.09$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p<0.001$ for all CF ranges). These values were again comparable to those recorded in the control rats (Student-Newman-Keuls post hoc test, $p=0.10$, 0.074, and 0.33 in the low, middle, and high CF ranges, respectively). As expected, the average BW20s of the control+Eae rats did not differ from those of the control rats (Student-Newman-Keuls post hoc test, $p=0.052$, 0.46, and 0.20 in the low, middle, and high CF ranges, respectively). These data show that a week of enriched sound exposure broadly renormalizes the behavioral and cortical processing of sound frequency degraded by fluoxetine treatment.

The status of PV-expressing inhibitory interneurons and the expression of perineuronal nets (PNNs) are both proposed to contribute to neural processing and the regulation of plasticity in cortical networks (Pizzorusso et al., 2002; de Villers-Sidani et al., 2008; Moore et al., 2013; Li et al., 2015; Lensjø et al., 2017; Nguyen et al., 2017; Cheng et al., 2022). To document the cellular and molecular changes at the cortical level that were induced by the fluoxetine treatment and their potential reversion to normal by enriched sound exposure, we quantified the expression levels of PV and PNNs in cortical field A1s for the Flx+Eae, Flx, and control rats (**Fig. 7A**).

Consistent with earlier studies (Nguyen et al., 2017; Fawcett et al., 2019; Reinhard et al., 2019; Cheng et al., 2020,2022; Aronitz et al., 2021), both PV and PNNs labeled neurons were distributed more in the middle layer than in the superficial and deep layers of A1. While the fluoxetine treatment had little effect on the densities of PV neurons in

the different cortical layers (**Fig. 7B**, top; two-way ANOVA, $F(2,489)=0.40$, $p=0.67$), it did decrease the densities of PNN neurons, particularly in layer 4 (**Fig. 7B**, middle; two-way ANOVA, $F(2,495)=7.81$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p=0.004$ for layer 4 but $p=0.33$ for layers 2/3 and $p=0.11$ for layers 5/6). Enriched sound exposure significantly increased the density of PNN neurons in layer 4 of the Flx+Eae rats compared with the Flx rats (Student-Newman-Keuls post hoc test, $p=0.003$); thus, the neuron density in layer 4 for the Flx+Eae rats was substantially similar to that for the control rats (Student-Newman-Keuls post hoc test, $p=0.92$). Since PNNs in the cortex typically form around PV neurons (Favuzzi et al., 2017; Aronitz et al., 2021), we also quantified the densities of PV/PNNs co-labeled neurons for the different groups of rats. As shown in the bottom of **Fig. 7B**, a two-way ANOVA ($F(2,489)=11.08$, $p<0.001$) revealed that the density of PV/PNN neurons for the Flx rats was significantly lower than that for the control rats in cortical layer 4 (Student-Newman-Keuls post hoc test, $p=0.001$). Once again, the density of PV/PNN neurons in the Flx+Eae rats was significantly higher than that in the Flx rats (Student-Newman-Keuls post hoc test, $p<0.001$) and was similar to that in the control rats (Student-Newman-Keuls post hoc test, $p=0.09$). The densities of PV/PNN neurons in both the superficial and deep cortical layers, however, did not differ among the various groups of rats (Student-Newman-Keuls post hoc test, all $p>0.22$).

For the PV/PNNs co-labeled neurons, we further quantified the intensities of expression for both the PV and PNNs (**Fig. 8A**). As shown in **Fig. 8B**, the intensities of PV expression for the PV/PNN neurons located in the different cortical layers were all comparable among the different groups of rats (two-way ANOVA, $F(2,378)=2.12$,

p=0.12). However, the intensities of PNN expression for the Flx rats were significantly lower than those for the controls, except for neurons in the deep cortical layer (two-way ANOVA, $F(2,378)=44.20$, $p<0.001$. The Student-Newman-Keuls post hoc test showed that $p<0.001$ for both layers 2/3 and layer 4 but $p=0.30$ for layers 5/6). The intensities of PNN expression for both layers 2/3 and 4 of the Flx+Eae rats were again significantly higher than those of the Flx rats (Student-Newman-Keuls post hoc test, both $p<0.001$) and were comparable to those of the control rats (Student-Newman-Keuls post hoc test, $p=0.73$ for layers 2/3 and $p=0.67$ for layer 4).

Discussion

In this study, two-month-old rats were chronically treated with antidepressant fluoxetine for four weeks. At this age, a rat is approaching sexual maturity, and its A1 has matured far beyond the normal closure of the critical period for passive sound exposure-driven plasticity (de Villers-Sidani et al., 2007; Insanally et al., 2009; Zhou et al., 2011). We found that the Flx rats were significantly less accurate when performing a tone-frequency discrimination task compared with the control rats. Their cortical neurons also responded less selectively to sound frequencies, as shown by the larger bandwidths of the frequency tuning curves. The degraded behavioral and cortical spectral processing was accompanied by decreased cortical PNNs, particularly those wrapped around PV-expressing interneurons, indicating a reduction in introcortical inhibition. More importantly, fluoxetine induced a period of sound exposure-driven plasticity in their auditory cortices. Thus, a week of rearing the Flx rats under an enriched acoustic environment substantially renormalized tone-frequency discrimination and cortical

spectral selectivity, which had been degraded by the fluoxetine treatment. The cortical expression of the PNNs was also reversed as a result of this passive, enriched sound exposure. Our study thus documents a strong capacity for driving "negative" cortical changes (i.e., decreased frequency tuning and reduced introcortical inhibition) which normally characterize a more immature cortex by chronically exposing adult rats to fluoxetine. The findings that cortical processing moved in the direction of a "more immature" status indicate that fluoxetine promotes cortical plasticity by inducing a critical period-like epoch in the A1s of adult rats.

Cortical inhibition plays an important role in shaping the response properties of neurons in A1, which determine behavioral and perceptual discrimination (Wang et al., 2000; Wehr and Zador, 2003; Wu et al., 2008; Li et al., 2014; Seybold et al., 2015; Natan et al., 2017; Liu and Kanold, 2021; Cheng et al., 2022). A mature, functionally differentiated cortex, with its strong and powerful selective inhibitory processes in place, responds with greater selectivity to the stimuli. Conversely, reduced introcortical inhibition, while promoting cortical plasticity (see discussion below), might decrease the frequency selectivity of neurons and thus degrade the tone-frequency discrimination performance, as observed in the Flx rats. This hypothesis is further strengthened by a positive change in cortical inhibition after enriched sound exposure, which parallels the recovery of cortical and behavioral spectral processing in the Flx+Eae rats. Of note, recent studies have also shown that fluoxetine decreases the complexity of the dendritic arbors and inhibits the long-term potentiation induced by theta-burst stimulation of the medial geniculate nucleus in the auditory cortices of adult rats (Dringenberg et al., 2014; Ampuero et al., 2019). These fluoxetine effects recorded in the auditory cortex plausibly

484 also contribute to the altered behavioral performance in the Flx rats. At the same time,
485 most current studies on fluoxetine action have focused on forebrain structures (Maya
486 Vetencourt et al., 2008; Karpova et al., 2011; Ohira et al., 2013; Mikics et al., 2018;
487 Steinzeig et al., 2019). Therefore, whether and how fluoxetine might alter neural
488 processing in the subcortical areas of the adult brain remains to be studied. Our ongoing
489 studies conducted on the auditory midbrain (i.e., the inferior colliculus) are expected to
490 shed light on this question in the near future.

491 Earlier studies on the auditory system have shown that cortical representation and
492 response selectivity can be substantially remodeled by passive exposure to sound stimuli
493 during the critical period of postnatal development (de Villers-Sidani et al., 2007;
494 Insanally et al., 2009; Pysanenko et al., 2018; Nakamura et al., 2020; Svobodová
495 Burianová and Syka, 2020). Beyond this period, cortical modification requires sound
496 input that carries behavioral significance (Recanzone et al., 1993; Polley et al., 2006;
497 Zhou and Merzenich, 2009; Zhang et al., 2013; Cheng et al., 2017,2020). In this study, a
498 subset group of Flx rats was passively exposed to enriched sound to further identify the
499 effects of fluoxetine on cortical plasticity. While transient sound exposure had little effect
500 on the behavioral or cortical processing of sound frequency in the control rats, it did
501 evoke significant changes in the fluoxetine-treated adult rats; juvenile-like immature
502 characteristics (i.e., degraded frequency discrimination, reduced cortical frequency
503 selectivity, and decreased cortical PNN expression) were all reversed after sound
504 exposure. Our results therefore again support the conclusion that chronic fluoxetine
505 treatment induces a critical period-like epoch in the auditory cortices of adult rats.

506 It has been proposed that maturation of inhibition in cortical networks serves as a

"brake" for developmental plasticity (Hensch, 2005). Thus, manipulations that reduce intracortical inhibition can promote cortical plasticity in mature brains. For example, a reduction in cortical GABAergic inhibition, either by pharmacological treatment or dark rearing in adult animals, has been shown to reopen a period of stimulus exposure-based plasticity in the visual cortex (Maya Vetencourt et al., 2008; Harauzov et al., 2010). Similarly, exposing adult rats to continuous white noise restores critical period-like plasticity in the auditory cortex, and again, changes in the GABAergic inhibitory processes have been recorded in parallel with enhanced cortical plasticity (Zhou et al., 2011). More recently, it has been reported that reducing cortical inhibition by the chemogenetic inactivation of PV neurons also reinstates plasticity in the adult mouse auditory cortex (Cisneros-Franco and de Villers-Sidani, 2019). In this study, we found that chronic treatment with fluoxetine decreased the expression of cortical PNNs, resulting in a reduced density of PV/PNNs co-labeled neurons, particularly in the middle layers of the auditory cortex. Similar results have previously been reported in the medial frontal cortex and the basolateral amygdala of adult animals after fluoxetine treatment (Karpova et al., 2011; Ohira et al., 2013). PNNs are reticular structures composed of extracellular matrix molecules (Wang and Fawcett, 2012). In the cortex PNNs typically accumulate around the soma and proximal dendrites of PV neurons and have been shown to mediate trophic support to these neurons and maintain mature excitatory synaptic contacts onto them (Favuzzi et al., 2017; Fawcett et al., 2019; Aronitz et al., 2021). Thus, fluoxetine-reduced expression of PNNs, particularly those that encapsulate PV neurons, indicates a disruption of the intrinsic properties of these PV neurons and, hence, the reduction of cortical inhibitory networks (as evidenced by reduced spectral tuning and

decreased response thresholds of neurons recorded in the fluoxetine-treated A1). We propose that reduced intracortical inhibition resulting from the decline in PNN expression following fluoxetine treatment at least partly contributes to enhanced A1 plasticity in adult rats.

Except for GABAergic inhibition, both glutamatergic excitation and brain-derived neurotrophic factor (BDNF) signaling are also argued to play important roles in regulating or enabling changes in plasticity that express the transition of the brain from its infantile to adult stage (Zhou et al., 2011; Bracken and Turrigiano, 2009). Earlier studies have shown that antidepressant administration regulates glutamatergic transmission and BDNF signaling in the adult brain (Maya Vetencourt et al., 2008; Mikics et al., 2018; Ampuero et al., 2019; Van Dyke et al., 2019). Thus, we cannot rule out the possibility that altered glutamatergic excitation and/or BDNF signaling as a result of fluoxetine treatment also contribute to enhanced plasticity in the mature auditory cortex. Further studies are needed to answer questions as to whether and how these different mechanistic pathways are related to each other while inducing critical period-like plasticity in the adult auditory cortex.

Typical perceptual deficits often manifest as a lateral shift of the psychometric curve. In this study, the Flx rats showed significant behavioral deficits even at the largest frequency difference between the target and non-target we tested (i.e., at a difference of one octave). This result indicates that more large frequency differences that are perceptually unchallenging for the Flx rats to discriminate may need to be tested in future studies. Furthermore, the discrimination task applied in this study also requires top-down motivation and learning and memory processes to achieve target recognition. While the

hit rates of the Flx rats in the behavioral test were comparable to those of the control rats, the false-positive rates were significantly higher at large frequency differences. In addition to degraded auditory processing, these higher false-positive rates may reflect an over-motivated state for food rewards, impulsivity, or decreased attention. Further studies therefore are needed to distinguish the effects of fluoxetine exposure on auditory discrimination sensitivity versus other non-sensory factors. Finally, how long the post-exposure effects of fluoxetine on behavior and cortical processing last and whether the reversal effects of fluoxetine pairing with enriched sound exposure on auditory processing persist also require further study.

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795 **Figure Legends**

796 **Fig. 1** Open field and elevated zero-maze tests. (**A**) Experimental timelines for the Flx
797 and age-matched control rats. Pw, postnatal weeks. (**B**) Sample movement traces of an
798 Flx rat and a control rat in the open field test. (**C**) Comparisons of the average distances
799 traveled during the 10, 20, and 30 min periods between the Flx (n=9) and control (n=9)
800 rats in the open field test. Error bars represent SEM. (**D**) Sample movement traces of an
801 Flx rat and a control rat in the elevated zero-maze test. (**E**) Comparisons of the average
802 distance traveled (left), number of transitions between the open and closed arms (middle),
803 and time spent in the open arm (right) between the Flx (n=8) and control (n=9) rats in the
804 elevated zero-maze test. * or o, $p<0.05$ or 0.01.

805

806 **Fig. 2** Behavioral performance in the sound frequency discrimination task. (**A**) Schematic
807 of the sound frequency discrimination task. Only one tone with a specific frequency was
808 presented in each trial during the test. Each animal needed to identify a target tone with a
809 frequency of 8 kHz from a set of distracter tones with frequencies of 0.2, 0.4, 0.6, 0.8 or 1
810 octave separation from the target tone to obtain a food reward. (**B**) Individual
811 psychometric curves obtained from the Flx (n=7) and control (n=11) rats. The dashed line
812 shows 50% of the maximal score. (**C**) Average psychometric curves for both groups of
813 rats. Error bars represent SEM. +, $p<0.001$. (**D**) Comparison of the discrimination
814 thresholds (determined at a 50% performance score on the psychometric curve) for both
815 groups of rats. o, $p<0.01$. (**E**) Average false-positive rates for both groups of rats. *,
816 $p<0.05$.

817

818 **Fig. 3** Thresholds, wave latencies, and relative wave amplitudes of the ABRs. (**A**)
819 Schematic of the experimental setup for ABR recording. The acoustic stimuli were
820 delivered to the ear through a calibrated earphone with a sound tube positioned inside the
821 external auditory meatus. The ABR was recorded by placing three electrodes (indicated
822 by the arrows) subdermally at the scalp midline, posterior to the stimulated ear, and on
823 the midline of the back 1-2 cm posterior to the neck of the animal. (**B**) ABR patterns of an
824 Flx rat and a control rat determined with tone pips of 10 kHz. The ABR threshold (arrows)
825 was defined as the lowest sound intensity capable of eliciting a response pattern
826 characteristic of that seen at higher intensities. (**C**) ABR thresholds tested at different
827 frequencies for the Flx (n=8) and control (n=11) rats. Error bars represent SEM. (**D**) ABR
828 latencies of waves I (left) and IV (right) for the Flx and control rats. (**E**) Relative wave
829 amplitudes (wave IV/I amplitude ratios) for the Flx and control rats.
830
831 **Fig. 4** Cortical frequency response selectivity. (**A**) Schematic of the experimental setup
832 for cortical extracellular recording. (**B**) Representative examples of frequency tuning
833 curves of neurons recorded in cortical field A1s of an Flx rat and a control rat. (**C**)
834 Representative cortical CF maps of an Flx rat and a control rat. The color of each polygon
835 in these maps indicates the CF of the neurons recorded at that site (see the color scales).
836 A, anterior; D, dorsal. (**D**) Average BW20s of cortical neurons recorded in the Flx rats
837 (232 recording sites from 6 animals) and control rats (235 recording sites from 7 animals)
838 for each of the three CF ranges. Error bars represent SEM. +, $p<0.001$. (**E**) Average
839 response thresholds of cortical neurons recorded in the Flx and control rats. o, $p<0.01$. (**F**)
840 Spontaneous firing rates of cortical neurons recorded in the Flx and control rats.

841

842 **Fig. 5** Fluoxetine induces critical period-like plasticity in the A1s of adult rats. (**A**)
843 Experimental timelines for the Flx+Te, control+Te, Flx, and age-matched control rats.
844 The Flx+Te or control+Te rats, the Flx or control rats were passively exposed to pulsed 7
845 kHz tones for one week. (**B**) Representative CF maps obtained from the different groups
846 of rats. The outlined polygons indicate recording sites with CFs of 7 kHz±0.25 octaves.
847 (**C**) Differences in the percentages of A1 areas tuned to the different frequency ranges for
848 the Flx+Te (n=6), control+Te (n=7), and Flx (n=6) rats vs. control rats (n=7). Error bars
849 represent SEM. +, $p<0.001$ compared to the controls.

850

851 **Fig. 6** Enriched sound exposure renormalizes behavioral and cortical processing degraded
852 by fluoxetine treatment. (**A**) Experimental timelines for the Flx+Eae, control+Eae, Flx,
853 and age-matched control rats. The Flx+Eae or control+Eae rats, the Flx or control rats
854 were reared in an enriched acoustic environment for one week. (**B**) Individual
855 psychometric curves obtained from the Flx+Eae (n=7), control+Eae (n=7), Flx (n=7), and
856 control (n=11) rats. The dashed line shows 50% of the maximal score. (**C**) Comparison of
857 discrimination thresholds (determined at a 50% performance score on the psychometric
858 curve) for the different groups of rats. Error bars represent SEM. o, $p<0.01$ compared to
859 the controls. (**D**) Representative examples of frequency tuning curves of neurons
860 recorded from an Flx+Eae rat, a control+Eae rat, an Flx rat, and a control rat. (**E**) Average
861 BW20s of cortical neurons recorded in the Flx+Eae (n=255 from 7 animals), control+Eae
862 (n=226 from 6 animals), Flx (n=232 from 6 animals), and control (n=235 from 7 animals)
863 rats for each of the three CF ranges. +, $p<0.001$ compared to the controls.

Fig. 7 Cortical densities of PV labeled, PNNs labeled, and PV/PNNs co-labeled neurons. (**A**) Representative photomicrographs of cortical sections for an Flx+Eae rat, an Flx rat, and a control rat. See **Fig. 6A** for the experimental timelines of the different groups of rats. The cortical layers are indicated on the left, and sample PV labeled (top), PNNs labeled (middle), and PV/PNNs co-labeled neurons (bottom) are shown on the right. L1, layer 1; L2/3, layers 2/3; L4, layer 4; L5/6, layers 5/6. (**B**) Densities of PV labeled (top), PNNs labeled (middle), and PV/PNNs co-labeled neurons (bottom) in different cortical layers of the Flx-Eae rats, Flx rats, and control rats. n=49 for PV labeled neurons, 49 for PNNs labeled neurons, and 49 for PV/PNNs co-labeled neurons from 5 Flx+Eae rats, n=58 for PV labeled neurons, 59 for PNNs labeled neurons, and 58 for PV/PNNs co-labeled neurons from 6 Flx rats, and n=59 for PV labeled neurons, 60 for PNNs labeled neurons, and 59 for PV/PNNs co-labeled neurons from 6 control rats. Error bars represent SEM. o, $p<0.01$ compared to the controls.

Fig. 8 Expression intensities of PV and PNNs for the PV/PNNs co-labeled neurons. (**A**) Pixel intensities along the horizontal dashed line centered on a PV/PNNs co-labeled neuron were measured for PV and PNN expressions. (**B**) Expression intensities of PV and PNNs for the Flx+Eae rats, Flx rats, and control rats. n=120 (40 for each of layers 2/3, 4, and 5/6) from 5 Flx+Eae rats, n=130 (40 for both layers 2/3 and 4, and 50 for layers 5/6) from 6 Flx rats, and n=137 (46 for layers 2/3, 47 for layer 4, and 44 for layers 5/6) from 6 control rats. +, $p<0.001$ compared to the controls.