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ENVIRONMENTAL ENRICHMENT SELECTIVELY INCREASES GLUTAMATERGIC RESPONSES IN LAYER II/III OF THE AUDITORY CORTEX OF THE RAT

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Abstract—Prolonged exposure to environmental enrichment (EE) induces behavioral adaptation accompanied by detectable morphological and physiological changes. Auditory EE is associated with an increased auditory evoked potential (AEP) and increased auditory gating in the primary auditory cortex. We sought physiological correlates to such changes by comparing synaptic currents in control vs. EE-raised rats, in a primary auditory cortex (AI) slice preparation. Pharmacologically isolated glutamatergic or $GABA_A$-receptor-mediated currents were measured using perforated patch whole-cell recordings. Glutamatergic AMPA receptor (AMPAR)-mediated excitatory postsynaptic currents (EPSCs) displayed a large amplitude increase (64±11% in EE vs. control) accompanied by a rise-time decrease (−29±6% in EE vs. control) and decrease in pair pulse ratio in layer II/III but not in layer V. Changes in glutamatergic signaling were not associated with changes in the ratio between *N*-methyl-D aspartate-receptor (NMDAR)-mediated vs. AMPAR-mediated components, in amplitude or pair pulse ratio of GABAergic transmission, or in passive neuronal properties.

A realistic computational model was used for integrating *in vivo* and *in vitro* results, and for determining how EE synapses correct for phase error of the inputs. We found that EE not only increases the mean firing frequency of the responses, but also improves the robustness of auditory processing by decreasing the dependence of the output firing on the phase difference of the input signals.

We conclude that behavioral and electrophysiological differences detected *in vivo* in rats exposed to an auditory EE are accompanied and possibly caused by selective changes in cortical excitatory transmission. Our data suggest that auditory EE selectively enhances excitatory glutamatergic synaptic transmission in layer II/III without greatly altering inhibitory GABAergic transmission.

Key words: behavior, AI, patch-clamp, perforated patch, excitation/inhibition balance, neuronal modeling.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AEP, auditory evoked potential; AMPAR, AMPA receptor; APV, D-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EE, enriched environment; EPSC, excitatory postsynaptic current; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); HF, high-frequency; I_{AMPA}, AMPA current; I_{NMDA}, *N*-methyl-D-aspartate current; IPI, interpulse interval; IPSC, inhibitory postsynaptic current; LF, low-frequency; mEPSC, miniature excitatory postsynaptic current; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; PPR, paired pulse ratio; QX314, lidocaine *N*-ethyl bromide.

The anatomic connectivity and physiological properties of the CNS are determined by a combination of genetic programs and by the type and amount of sensory input (Bartoletti et al., 2004). Many studies have shown anatomical and cellular consequences of the exposure to a sensory enriched environment (EE, Diamond et al., 1964; Volkmar and Greenough, 1972).

While several studies reported the effects of EE on synaptic transmission in the hippocampus (Duffy et al., 2001; Artola et al., 2006; Irvine and Abraham, 2005; Foster and Dumas, 2001), scant information is available on the effects of EE on the synaptic properties of the neocortex. In a previously developed model of auditory EE, Engineer et al. (2004) demonstrated large increases in surface auditory evoked potentials (AEPs) and in the number of action potentials recorded at the auditory cortex. EE also increased the degree of auditory gating (paired pulse depression) recorded with both epidural and intracortical electrodes (Percaccio et al., 2005). Physiological differences detected in the auditory cortical responses might originate in the cortex itself, or might rather be the result of a different subcortical processing between control and EE animals. The purpose of this study was to identify a possible local, cortical source of differential processing between control and EE animals. We used the same behavioral paradigm of auditory EE reported above (Engineer et al., 2004; Percaccio et al., 2005) to investigate differences in pharmacologically isolated excitatory and inhibitory synapses in EE vs. control-raised animals in the auditory cortex of the rat.

EXPERIMENTAL PROCEDURES

Environmental conditions

Twenty-three control and 27 EE-raised Sprague–Dawley rats were used in this study. All rats were housed with their mothers and littermates until they reached an age of 21 days. They were then randomly separated and placed into either enriched or standard housing conditions. Rats were given a code of colored tail stripes in order to preserve their housing condition's confidentiality from experimenters and avoid any unintentional bias. All rats were provided with food and water *ad libitum*. A reverse 12-h light/dark cycle and constant humidity and temperature were maintained for both groups. All experimental procedures were performed in accordance with the NIH Ethical Treatment of Animals and were approved by the University Committee on Animal Research at the University of Texas at Dallas. The number of animals was kept to the minimum necessary to ensure statistical validity. The enriched environment did not induce any pain to the experimental animals, nor did the anesthesia before decapitation. Housing conditions were nearly identical to those described in previous studies (Per-

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caccio et al., 2005; Engineer et al., 2004). The EE exposure time for this study was 5–6 weeks.

Four to eight rats were housed together in the EE cage which was located in a separate room from the main rat colony at UTD. This cage (76×45×90 cm) had four levels accessible by ramps. The environment's complexity was augmented by bells, wind chimes, and chains. Tones at 2.1 or 4.0 kHz were sounded when touch plates (located at the bottom of two of the ramps) were depressed. Additionally a chime was sounded when an infrared beam was broken in front of the water source and each rotation of an exercise wheel activated a small green light emitting diode and a 3 kHz tone. These devices were designed and positioned in such a way that their sounds provided information about movement in a specific location within the cage at a particular time.

Other meaningful sounds were provided by a CD player. Every 2–60 s, a randomly selected sound was played, including simple tones, amplitude and frequency modulated tones, noise bursts, and other complex sounds (rat vocalizations, classical music, rustling leaves, etc.). Seven of the 74 sounds activated a pellet dispenser (Med Associates, St. Albans, VT, USA) that delivered a sugary treat intended to encourage attention to the sounds. The rewarded tracks included interleaved tones of different carrier frequencies (25 ms long and 4, 5, 9, 12, 14, and 19 kHz tones with inter-stimulus intervals ranging from 50 ms to 2 s) and frequency modulated sweeps (one octave up or down in a 140 or 300 ms sweep with inter-stimulus intervals ranging from 80 to 800 ms). All sounds were <75 dB SPL, provided 24 h a day and spanned the entire hearing range of the rat (1–45 kHz).

Standard environment cages were 26×18×18 cm and included one to two rats per cage. The standard housing environment exposed rats to vocalizations from 20–30 other rats housed in the same room, in addition to general sounds (which were also heard by rats in the EE) resulting from daily traffic, cleaning, and feeding while they were most active. Although rats housed in both environments heard approximately the same number of sounds each day, sounds in the EE condition were more diverse, and provided more behaviorally significant information than the sounds in the standard condition.

Slice preparation

We followed an auditory cortex slice preparation protocol similar to one previously described (Atzori et al., 2001). After exposure to enriched or standard environmental conditions (as described above), 6- to 9-week-old Sprague–Dawley rats were anesthetized (evaluated by toe-pinch response) in a chamber with vaporized isoflurane (0.2 ml/100 g) and immediately decapitated. The brains were carefully extracted and immersed in a solution (slicing artificial cerebrospinal fluid (ACSF)) chilled to approximately 0.5 °C containing (mM) 130 NaCl, 3.5 KCl, 10 glucose, 24 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$ and 1.5 $MgCl_2$, and saturated with a mixture of 95% O_2 and 5% CO_2 (pH ≈7.35 osmolarity 301±5 mOsm). The low concentration of calcium decreases the spontaneous release of glutamate prolonging viability of the preparation. After removal of the cerebellum, a vibratome (VT1000, Leica, Wetzlar, Germany) was used to cut 270 μm-thick coronal slices from the first sixth of the caudal part of the brain, corresponding to the primary auditory area (A1). Slices were then placed into an incubating chamber super-fused with the ACSF solution described above and incubated at 33 °C for approximately 1 h, and then maintained at room temperature until used for recording.

Electrophysiology

Slices were transferred to a recording chamber and immersed in a solution (recording-ACSF) similar to the slicing-ACSF solution described above containing 1.5 mM $CaCl_2$ rather than 0.5 mM. Pyramidal neuron selection procedures were adopted from those described previously (Atzori et al., 2005). Cells with an obvious apical dendrite located in layer II/III or V and dorsal to the ectosylvian region were visually selected using a Luigs and Neumann 380 FM Workstation (Luigs & Neumann, Ratingen, Germany) with Olympus BX51 WI optics and an infrared camera system (Olympus, Tokyo, Japan).

Perforated patch clamp recordings were performed using techniques similar to the whole cell patch clamp technique already described (Atzori et al., 2001), with an internal recording solution containing, in addition, the antibiotic amphotericin B (3.24 mM). Intracellular recording solution contained in mM 100 CsCl, 5 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid K (BAPTA-K), 1 lidocaine *N*-ethyl bromide (QX314), 1 $MgCl_2$, 10 Hepes, 4 glutathione, 1.5 $ATPMg_2$, 0.3 GTPNa2, 8 biocytin (pH ≈7.35 osmolarity 270±10 mOsm). Amphotericin B was used to form pores in the neuronal membrane layer allowing electrical access (perforated-patch) without the intracellular dialysis normally associated with whole cell patch clamping. The pulled glass electrode tips (5–8 MΩ) were backfilled with the intracellular recording solution after the most distal 200 μm were filled with the amphotericin B free intracellular solution in order to prevent tip clogging during electrode-membrane seal formation. Holding current and input resistance (Ω_{in}) were continuously monitored with a 2 mV negative pulse delivered before the paired pulse protocols. Recording was delayed until the voltage-gated sodium channel blocker lidocaine (QX314) reached an intracellular concentration high enough to prevent action potentials and input resistance stabilized (15–20 min).

Electrically evoked postsynaptic currents were measured by delivering two electric stimuli (90–180 μs) either 20, 40, 50, 100, 500, or 1000 ms apart every 8 s, in the order stated, with a stimulus isolator (A365 triggered by a DS8000-82112 Digital Simulator, both from World Precision Instruments, Sarasota, FL, USA) through a glass stimulation mono-polar electrode filled with recording-ACSF, and always placed at the same distance (approximately 120 μm) from the recording electrode, and averaged over 4 to 10 responses. The intensity of the stimulation was standardized as the one determining a postsynaptic response corresponding to 80% of the maximal response.

Excitatory postsynaptic currents (EPSCs) were measured in a bath of bicuculline (10 μM) at a holding potential of −60 mV for inward currents and +60 mV for outward currents and reversibly blocked by DNXQ (10 mM) and kynurenic acid (2 mM) indicating a glutamatergic composition. EPSC's amplitude was measured at the peak of inward current as AMPA current (I_{AMPA}), and current amplitude 45 ms after the outward excitatory current peak was taken as the estimate of *N*-methyl-D-aspartate (NMDA) current (I_{NMDA}). Similar methods were previously described (Duffy et al., 2001). We selected the ratio between *N*-methyl-D-aspartate receptor (NMDAR)-mediated currents and AMPA receptor (AMPAR)-mediated currents (I_{NMDA}/I_{AMPA}) as an indicator of postsynaptic function. Paired pulse ratio (PPR) was defined as the ratio between the mean of the peak of the second inward current response and the mean of the peak of the first inward current response (P_2/P_1).

Inhibitory postsynaptic currents (IPSCs) were measured at a holding potential $V_h = -60$ mV in a bath solution containing 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 2 mM kynurenic acid for blocking glutamate receptor-mediated currents. The intracellular recording solution provided a reversal potential for Cl^- of approximately 0 mV. IPSCs were blocked by bicuculline (10 μM), indicative of their GABAergic origin.

Signals were acquired via a Digidata 1322A 16 bit data acquisition system controlled by Clampex 9.2 and Multiclamp 700B software (Axon Instr., Foster City, CA, USA) and filtered at 3200 Hz (low pass) with a Frequency Devices 900 tunable active filter (Frequency Devices, Haverhill, MA, USA). The recording chamber was situated within a 1 m^3 Faraday cage on an anti-vibration table (Technical Manufacturing Corporation, Peabody,