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Layer-Specific Noradrenergic Modulation of Inhibition in Cortical Layer II/III

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Norepinephrine (NE) is released in the neocortex after activation of the *locus coeruleus* of the brain stem in response to novel, salient, or fight-or-flight stimuli. The role of adrenergic modulation in sensory cortices is not completely understood. We investigated the possibility that NE modifies the balance of inhibition acting on 2 different γ-aminobutyric acid (GABA)ergic pathways. Using patch-clamp recordings, we found that the application of NE induces an α_1 adrenergic receptor–mediated decrease of the amplitude of inhibitory postsynaptic currents (IPSCs) evoked by stimulation of layer I (LI-eIPSCs) and a β and α_2 receptor–mediated increase in the amplitude of IPSCs evoked by stimulation of layer II/III (LII/III-eIPSCs). Analysis of minimal stimulation IPSCs, IPSC kinetics, and sensitivity to the $GABA_A$ receptor subunit–selective enhancer zolpidem corroborated the functional difference between LI- and LII/III-eIPSCs, suggestive of a distal versus somatic origin of LI- and LII/III-eIPSCs, respectively. These findings suggest that NE shifts the balance between distal and somatic inhibition to the advantage of the latter. We speculate that such shift modifies the balance of sensory-specific and emotional information in the integration of neural input to the upper layers of the auditory cortex.

Keywords: auditory cortex, cortical circuitry, dendritic inhibition, norepinephrine, patch clamp, somatic inhibition

Introduction

The auditory cortex displays large variability in response to biologically or otherwise relevant stimuli (Jääskeläinen et al. 2007; Pantev et al. 2009). Acute changes of the topographic representation of auditory stimuli and other types of short-term synaptic plasticity contribute to experience-dependent modifications and auditory cortical map reorganization (Buonomano and Merzenich 1998; Chowdhury and Suga 2000; Ma and Suga 2001). A large body of results has implicated the brain stem noradrenergic system in learning, attention, and integrative functions in the neocortex (Berridge et al. 1993; Berridge and Waterhouse 2003; Arnsten and Li 2005; Ramos and Arnsten 2007). In particular, the presence of prominent projections from the "locus coeruleus" to the temporal regions (Freedman et al. 1975; Fuxe, Hamberger, and Hokfelt 1968; Fuxe, Hokfelt, et al. 1968) prompts at the central norepinephrine (NE) system as a good candidate for the induction of short-term as well as long-term plasticity in the auditory cortex (Foote et al. 1975; Manunta and Edeline 1997, 1999), where NE exerts an overall inhibitory action on baseline neuronal activity (Foote et al. 1975; Manunta and Edeline 1997, 1998) and induces frequency-selective changes in the tuning curves (Manunta and Edeline 2004).

Studies by our group and others have shown that the activation of NE receptors alters both cortical glutamatergic (Nowicky et al. 1992; Ji, Cao, et al. 2008; Ji, Ji, et al. 2008; Dinh et al. 2009) as well as γ-aminobutyric acid (GABA)ergic synaptic transmission (Kawaguchi and Shindou 1998; Lei et al. 2007). A puzzling issue is that NE has been associated with both increases and decreases in cortical excitability (Foote et al. 1975; Armstrong-James and Fox 1983; Videen et al. 1984; Mueller et al. 2008). Despite anatomical studies suggesting a layer-specific action of NE at sensory cortical synapses (Levitt and Moore 1978; Morrison et al. 1978, 1979), the possibility of a lamina-selective modulation that might explain this dual action of NE has not been fully investigated. While our previous work suggests that NE modulation of excitatory synapses is not lamina specific (Dinh et al. 2009), no information is available concerning the layer specificity of NE modulation of GABAergic fibers, which can project to different cortical targets with remarkable cell type specificity and spatial selectivity (Miles et al. 1996; Ascoli et al. 2008). The goal of the present study was to determine whether and how NE modulates inhibitory synaptic transmission in the auditory cortex. We addressed this question using whole-cell recordings from primary auditory cortex pyramidal neurons and examining NE modulation of GABAergic synaptic drive elicited by layers I and II/III stimulation.

Materials and Methods

Preparation

We used an auditory cortex slice preparation similar to the one previously described (Atzori et al. 2001, 2003). Sprague–Dawley rats, 23–35 days old (Charles River), were anesthetized with isoflurane (Baxter) and sacrificed according to the National Institutes of Health Guidelines (UTD IACUC number 04-04) and their brains sliced with a vibrotome (VT1000, Leica) in a cold solution (0–4 °C) containing (mM) 126 NaCl, 3.5 KCl, 10 glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 1.5 $CaCl_2$, 1.5 $MgCl_2$ and 0.2 ascorbic acid, at pH 7.4 and saturated with a mixture of 95% O_2 and 5% CO_2 (ACSF). Coronal slices (270 μm thick) from the most caudal fourth of the brain were retained after removing the occipital convexity (caudal end of the brain after removal of the cerebellum) and subsequently incubated in ACSF at 32 °C before being placed in the recording chamber. The recording area was selected dorsally to the rhinal fissure corresponding to the auditory cortex (Rutkowski et al. 2003). The recording solution also contained 6,7-dinitroquinoxaline-2,3-dione (10 μM) and kynurenate (2 mM) or (2*R*)-amino-5-phosphonovaleric acid; (2*R*)-amino-5-phosphonopentanoate (100 μM) for blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor– and *N*-methyl-D-aspartate receptor–mediated currents, respectively.

Electrophysiology

Slices were placed in an immersion chamber, where cells with a prominent apical dendrite, suggestive of pyramidal morphology, were

visually selected using an upright microscope (BX51, Olympus) with a ×60 objective and an infrared camera system (DAGE-MTI). Whole-cell voltage-clamp recordings from layer II/III pyramidal neurons of the auditory cortex were performed under visual guidance. Neurons were selected by their pyramidal shape and by their pronounced apical dendrite. Inhibitory postsynaptic currents (IPSCs) were recorded in the whole-cell configuration, in voltage-clamp mode, at a holding membrane potential V_h = –60 mV, with 3–5 MΩ electrodes filled with a solution containing (mM) 100 CsCl, 5 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid K, 1 lidocaine *N*-ethyl bromide (QX314), 1 $MgCl_2$, 10 *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), 4 glutathione, 3 $ATPMg_2$, 0.3 $GTPNa_2$, 8 biocytin, and 20 phosphocreatine. The holding voltage was not corrected for the junction potential (<4 mV). The intracellular recording solution was titrated to pH 7.2 and had an osmolarity of 275 mOsm.

Electrically evoked IPSCs (eIPSCs) were measured by delivering 2 electric stimuli (90–180 μs, 10–50 μA) 50 ms apart every 10 s, with an isolation unit, through a glass stimulation monopolar electrode filled with ACSF, or with a concentric bipolar electrode (FHC Inc.), placed at about 100–200 μm from the perpendicular axis connecting the recorded neuron to the cortical neuropil, and layer II/III, lateral from the recorded cell. Synaptic responses were monitored at different stimulation intensities prior to baseline recording. "Normal" stimulation was defined as a stimulation reliably evoking a synaptic current in the range 100 pA to 1 nA. "Minimal" stimulation was defined by a percentage of failures in the range between 15% and 30% and a correspondingly lower response amplitude compared with "normal" stimulation. For each recording, a detection threshold was set at 150% of the standard deviation of the noise (typically around 4–5 pA, threshold around 7–8 pA). Evoked responses lower than the threshold level were counted as failures.

A 2-mV voltage step was applied at the beginning of every episode in order to monitor the quality of the recording. Access resistance (10–20 MΩ) was monitored throughout the experiment. Recordings displaying >20% change in input or access resistance were discarded from the analysis. All signals were filtered at 2 kHz and sampled at 10 kHz. We calculated the reversal potential for our postsynaptic currents through current–voltage (I-V) relationships for the eIPSCs (peak amplitude of 20 events at each of 5 holding potentials V_h in the range from V_h = –60 mV to V_h = +60 mV). The eIPSCs reversed polarity near 0 mV (–2.4 ± 0.3 mV, n = 3, data not shown), near the theoretical reversal potential of –4.6 mV. All experiments were performed at room temperature (22 °C).

Biocytin Injections

Recorded neurons were injected with 8 mM biocytin in the intracellular solution for post hoc identification. Following recording, slices were immediately transferred to a 24-well plate and fixed in a phosphate buffer containing 80 mM Na_2HPO_4, 80 mM NaH_2PO_4, and 4% paraformaldehyde. Biocytin staining was then processed using diaminobenzidine as chromogen, using a standard ABC kit (Vector Labs). A light cresyl violet Nissl counterstain was used to identify the cortical layers.

Drugs and solutions

All drugs were purchased from Sigma or Tocris. After recording an initial baseline for 10–15 min, drugs were bath-applied for 10 min or longer, until reaching a stable condition (as defined below in Statistical Analysis). NE, isoproterenol, clonidine, and phenylephrine were prepared immediately before experiments and their exposure to intense light was avoided to prevent oxidation.

Statistical Analysis

We defined a statistically stable period as a time interval (5–8 min) along which the IPSC mean amplitude measured during any 1-min assessment did not vary according to Mann–Whitney U test. All data are expressed as mean ± standard error of the mean. Pair pulse ratio (PPR) was calculated as the mean of the second response divided by the mean of the first response, according to Kim and Alger (2001). The effects of drug application on the IPSC amplitude changes were reported as $R \equiv 100 \times (1 - A_{treat}/A_{ctrl})$, where A_{treat} and A_{ctrl} are the mean IPSC amplitude (including failures) in treatment and control, respectively, or simply as percentage change between A_{treat} and A_{ctrl}. Drug effects were assessed by measuring and comparing the different parameters (R, IPSC mean amplitude, or other parameters as indicated) of baseline (control) versus treatment with a Mann–Whitney U test. One-way analysis of variance (ANOVA) with Tukey's post hoc test was used for comparisons between different groups of cells. Wilcoxon test was used for comparing between PPRs, and paired Student's t-test was used to compare slow and fast kinetics from eIPSCs. Data were reported as different only if $P < 0.05$ unless indicated otherwise. Single asterisk (*) indicates $P < 0.05$ and double asterisk (**) indicates $P < 0.01$.

Results

NE Differentially Modulates GABAergic Currents in the Auditory Cortex

Synaptic currents were recorded from 230 pyramidal cells, 84 stimulating layer I, 143 stimulating layer II/III, and 3 stimulating either layer in sequence, in the same recording.

Bath application of NE (20 μM) decreased the amplitude of layer I eIPSCs (LI-eIPSCs) by 43.8 ± 6.5% in 16/19 (84%) of cells tested (178.4 ± 26.2 pA in control vs. 100 ± 23 pA after NE, P < 0.03, Mann–Whitney U test; Fig. 1*A*). On the contrary, NE increased layer II/III eIPSCs (LII/III-eIPSCs) from 245 ± 27 pA (baseline) to 413 ± 41 pA in 27/31 cells tested (corresponding to 68.5 ± 7.1% calculated on the set of responsive cells, P < 0.002, Mann–Whitney U test; Fig. 1*B*). The percentage of NE-induced change in eIPSCs is shown in the histogram in Figure 1*C* (n = 19 for LI-eIPSCs and n = 31 for LII/III-eIPSCs) and in the bar graph in Figure 1*D*. Both effects of NE were reversed within 20–25 min of drug washout (Fig. 1*A*,*B*). A similar result was obtained when LI- and LII/III-eIPSCs were elicited in the same cell by placing the 2 stimulating electrodes in the same slice. Three cells were tested using this configuration, and LI- and LII/III-eIPSCs were delivered with a 300-ms interval. Again, bath application of NE selectively attenuated LI-eIPSCs by 32.7 ± 4.6% (P < 0.04, paired t-test) but enhanced LII/III-eIPSCs by 63.1 ± 7.5% (P < 0.001, paired t-test; Fig. 1*E*–*G*). These results indicate that NE-dependent modulation of GABAergic inputs in the auditory cortex is layer specific.

Analyses of the evoked synaptic response revealed that LI-eIPSCs exhibited a significantly slower rise time (r.t., 10–90%) and decay time constant (d.t., τ) compared with LII/III-eIPSCs (Fig. 1*H*): typical LI-eIPSCs r.t. and d.t. were >4 and >40 ms, respectively (r.t. = 6.2 ± 0.1 ms and d.t. = 43.8 ± 0.8 ms) versus LII/III-eIPSCs r.t. and d.t. <3 and <30 ms (r.t. = 2.7 ± 0.03 ms and d.t. = 29.5 ± 0.3 ms), respectively. Differences between LI- and LII/III-eIPSCs could be associated to different $GABA_A$ receptor ($GABA_AR$) subunit composition and/or could be due to electrotonic filtering. We tested whether LI-eIPSCs or LII/III-eIPSCs contain α_5 GABA receptor subunits by examining the impact of zolpidem, which, at concentrations <1 μM, is known to bind preferentially to α_1 and α_2/α_3 subunits agonist but not to α_5 (EC_{50} = 20 and 400 nM for α_1 and α_2/α_3, respectively, and EC_{50} = 5 μM for α_5; Vicini et al. 2001; Bosman et al. 2002; Goldstein et al. 2002; Heinen et al. 2004; Ortinski et al. 2004). Bath application of zolpidem (500 nM) did not change LI-eIPSCs amplitude (R = 2 ± 6%, n = 6, NS, paired t-test; Fig. 2*A*–*C*). In contrast, LII/III-eIPSCs were selectively enhanced by zolpidem application (R = –55 ± 13%, n = 6, P < 0.002, paired t-test; Fig. 2*D*–*F*). These results indicate that supragranular pyramidal neurons of the auditory cortex (example in Fig. 3*A*)

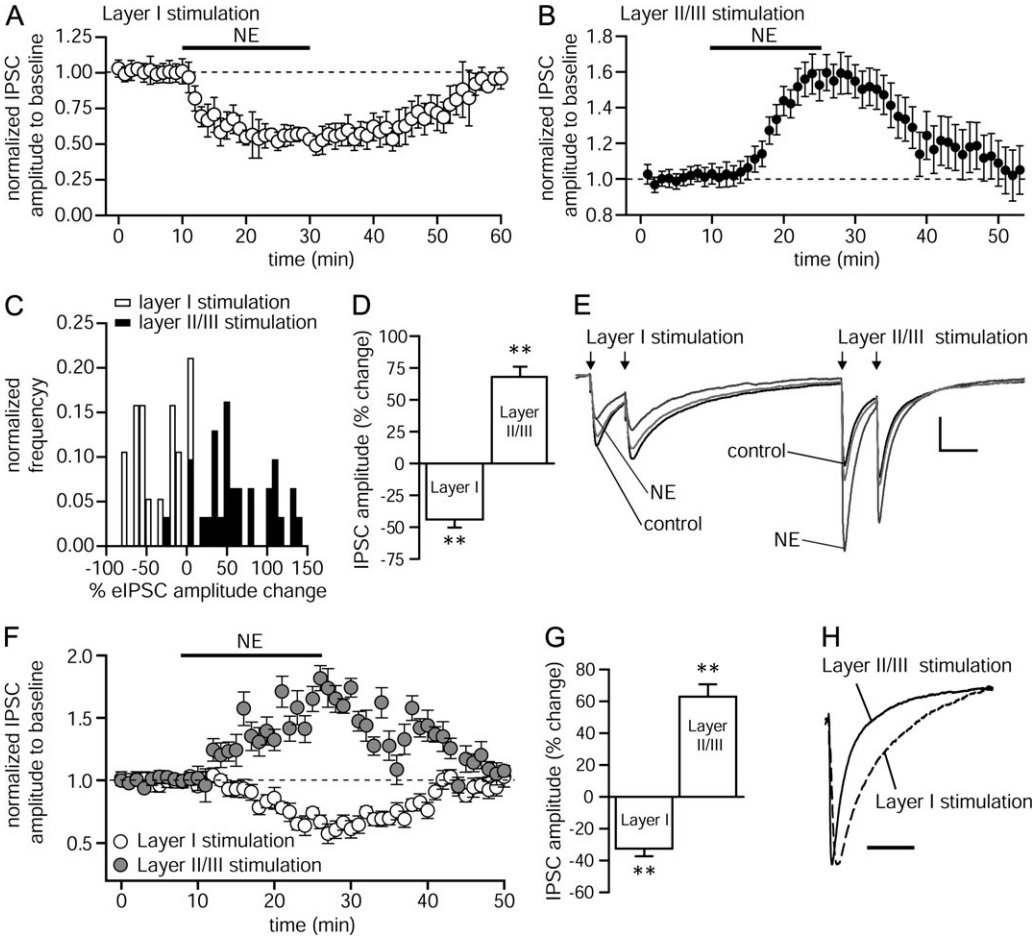

Figure 1. Differential eIPSCs modulation by NE. (*A*) Bath application of NE (20 μM) showing a decrease in amplitude of LI-eIPSCs. (*B*) Time course showing that NE (20 μM) increased amplitude of LII/III-eIPSCs. (*C*) Histogram of the percentage changes in the mean amplitude of LI- ($n = 19$ cells) and LII/III-eIPSCs (31 cells). (*D*) Percentage change in eIPSCs mean amplitude after NE application. (*E–G*) Trace, time course, and percentage amplitude change illustrating the differential effect of NE on LI- versus LII/III-eIPSCs on the same pyramidal cell, respectively. NE reversibly decreased the amplitude of LI-eIPSCs ($n = 3$) but increased the amplitude of LII/III-eIPSCs. Control (black), NE (dark gray), and recovery (light gray). Calibration bar: 100 pA, 50 ms. (*H*) LI- and LII/III-eIPSCs have different kinetics: LI-eIPSCs display a markedly slower kinetic compared with LII/III-eIPSCs. Each trace is the average of 50 traces from the same recorded cell.

receive GABAergic input from 2 types of synapses differing in kinetic properties and subunit composition: LI-eIPSCs synapses, with slow kinetics and $GABA_AR$ containing α_5 subunits, and LII/III-eIPSCs, with fast kinetics and $GABA_AR$ containing α_1 and/or α_2/α_3 subunits.

We also examined the impact of NE on LI- and LII/III-eIPSCs PPR (S_2/S_1) to determine whether the effects of NE reflect a presynaptic modulation of GABA release. Bath application of NE significantly increased the PPR in LI-eIPSCs from 0.83 ± 0.04 (baseline) to 1.06 ± 0.08 (NE; $P < 0.05$, Wilcoxon test; Fig. 3*B*) but decreased the PPR in LII/III-eIPSCs from 1.12 ± 0.03 (baseline) to 0.86 ± 0.04 ($P < 0.05$, Wilcoxon test; Fig. 3*C*), indicating a presynaptic component of the NE effect in both LI- and LII/III-eIPSCs (Fig. 3*D*).

To further examine the differential modulation by NE on LI- and LII/III-eIPSCs, a "minimal stimulation" protocol (described in Materials and Methods) was chosen to activate a single or a small number of release sites and to distinguish them from synaptic failures. Typically, the intensity of the stimulation (i.e., 10–70 μA) was set to elicit eIPSCs with approximately 15–30% of synaptic failures. Both fast ($n = 9$) and slow ($n = 6$) eIPSCs were obtained by stimulating layers I and II/III, respectively, with a concentric bipolar electrode. As summarized in Figure 4, bath application of NE (20 μM) slightly reduced the peak amplitude of LI-eIPSCs obtained with minimal stimulation, from 23.3 ± 0.3 pA (baseline) to 20.4 ± 0.3 pA ($n = 9$, $P < 0.05$, Mann–Whitney *U* test; Fig. 4*A–B*), effect that was accompanied by an increase of IPSCs failure rate (baseline: 25 ± 3% vs. NE: 35 ± 6%; $P < 0.05$, Mann–Whitney *U* test; insert in Fig. 4*A*). On the contrary, NE increased the peak amplitude of eIPSCs obtained with minimal stimulation of LII/III, from 26.3 ± 0.8 pA (baseline) to 41.8 ± 0.9 pA ($n = 6$, $P < 0.05$, Mann–Whitney *U* test; Fig. 4*C–D*), and reduced the failure rate from 21 ± 6% (baseline) to 5 ± 2% ($P < 0.05$, Mann–Whitney *U* test; insert in Fig. 4*C*). These results support the hypothesis that the GABA-mediated inhibitory drives originating from layers I and II/III are functionally segregated. We also assessed the effect of NE on eIPSCs evoked by stimulation of layer V. We found that applications of NE increase the amplitude of eIPSCs by less than 25% (22.7 ± 6.1%, $n = 8$, data not shown), suggesting that axons stemming from cortical layer V do not yield a major contribution to NE modulation in supragranular layers.

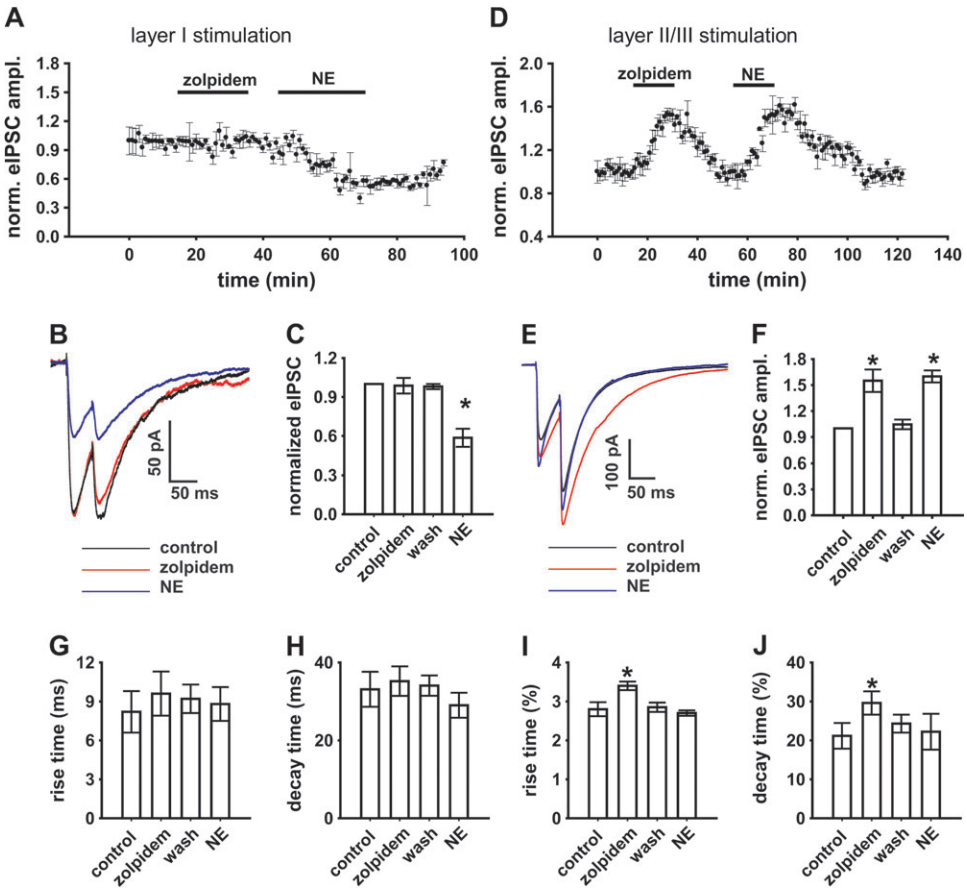

Figure 2. Zolpidem exerts a different effect on LI- and LII/III-eIPSCs. (*A*) Average of the time course of the effect of zolpidem (500 nM) on LI-eIPSCs ($n = 6$). (*B*, *C*) Representative trace and mean eIPSCs amplitude average showing that zolpidem application did not change eIPSCs amplitude following LI stimulation, while NE did. Control: black, zolpidem: red, NE: gray lines. (*D*–*F*) As above, but on LII/III-eIPSCs ($n = 6$); notice the zolpidem-induced amplitude increase. The response to NE application, following zolpidem application, confirmed the NE sensitivity of the fiber. (*G*, *H*) zolpidem did not affect kinetic parameters of LI-eIPSCs (*G*, r.t.; *H*, d.t.), but selectively increased r.t. and d.t. of the IPSC evoked from LII/III (*I*, *J*).

Pharmacological Properties of LI- and LII/III-eIPSCs

α_1 Receptor Activation Decreases LI-eIPSCs

We examined the role of α_1 adrenergic receptors in mediating NE inhibitory effect on LI-eIPSCs, as this receptor subtype has been reported to reduce the probability of release for a wide range of neurotransmitters at various central and peripheral synapses (see review by Madison and Nicoll 1988). NE-induced attenuation of LI-eIPSCs was selectively blocked by the α_1 receptor antagonist prazosin ($n = 10$; Fig. 5*A*), while simultaneous blockade of α_2 (with 1 μM yohimbine) and β (1 μM propranolol) receptors failed to antagonize such a depressing effect (Fig. 5*B*). Furthermore, bath application of the selective α_1 receptor agonist phenylephrine (1 μM) decreased LI-eIPSCs amplitude in all cells of the sample (35.6 ± 4%, $n = 10$; Fig. 5*C*), albeit to a slightly lesser extent when compared with that obtained with NE (46.7 ± 6.5%, $n = 19$; Fig. 5*E*), probably due to different potency at the dose used. The inhibitory action of phenylephrine was completely reversed by the α_1 receptor antagonist prazosin (Fig. 5*D*). Together, these results indicate that NE attenuation of LI-eIPSCs is mediated by activation of α_1 and not by α_2 or β receptors.

α_2 and β Receptor Activation Potentiates LII/III-eIPSCs

Similarly, the nature of NE-induced facilitation of LII/III-eIPSCs was determined pharmacologically. We examined the impact of selective α_1, α_2, and β adrenergic receptor antagonists on the noradrenergic modulation of LII/III-eIPSCs. We found that the facilitatory action of NE on LII/III-eIPSCs was blocked by simultaneous application of the β (propranolol) and α_2 (yohimbine) receptor antagonists ($n = 10$; Fig. 6*A*). On the contrary, in the presence of the α_1 receptor antagonist prazosin (1 μM, $n = 12$), bath application of NE elicited an even larger increase of LII/III-eIPSCs amplitude compared with application of NE alone (90 ± 10% in NE + prazosin vs. 68.2 ± 7.1% in NE alone, $P < 0.05$, ANOVA with Tukey's post hoc test; Fig. 6*B*). Accordingly, NE elicited a small but significant (24 ± 5%) reduction of LII/III-eIPSCs in the presence of propranolol and yohimbine in all cells of the sample ($n = 10$, $P < 0.001$, ANOVA with Tukey's post hoc test; Fig. 6*A*). To further determine the relative roles of adrenergic β, α_2, and α_1 receptors in mediating the modulatory action of NE, the impact of selective adrenergic receptor agonists on LII/III-eIPSCs amplitude was examined. Bath application of the β receptor agonist isoproterenol (50 μM) increased LII/III-eIPSCs amplitude by 70.8 ± 16% but only in 66% (8/12) of the cells tested (Fig. 6*C*; $P < 0.001$, ANOVA with Tukey's post hoc test). This effect was completely blocked by the selective β receptor antagonist propranolol (1 μM; Fig. 6*D*). Similarly, bath application of the selective α_2 receptor agonist clonidine (1 μM) enhanced LII/III-eIPSCs by 98 ± 26% ($P < 0.005$; Fig. 6*E*) but only in 27% (6/22) of the cells

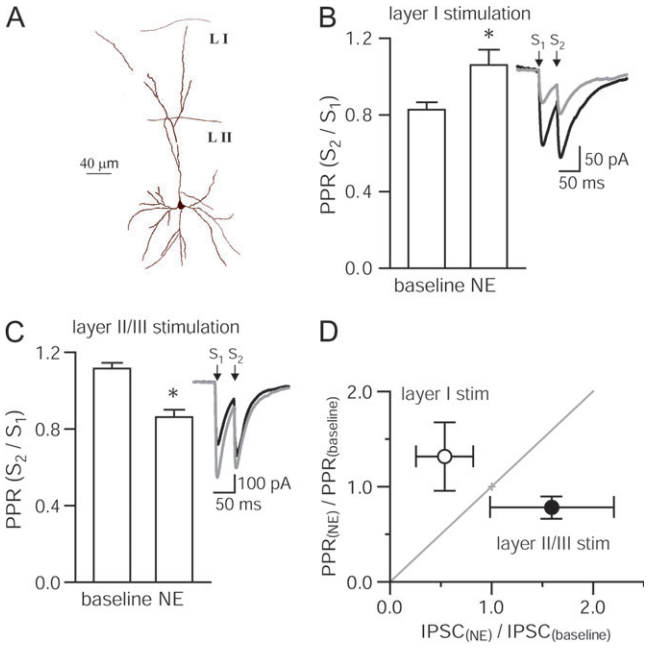

Figure 3. NE modifies differentially PPR. (A) *camera lucida* drawing of a biocytin-injected neuron displaying the typical basal and apical dendritic arborization of a neocortical pyramidal cell. (*B*, *C*) Effects of NE on eIPSCs PPR. (*B*) NE facilitated PPR in LI-eIPSCs ($n = 19$), while (*C*) depressed it in LII/III-eIPSCs. (*D*) Variation of the pair pulse ratio $PPR_{(NE)}/PPR_{(control)}$ for LI- and LII/III-eIPSCs; the NE-induced change in PPRs do not overlap, suggesting that 2 types of presynaptic GABAergic terminals are differentially modulated by NE.

tested. The α_2 receptor antagonist yohimbine (1 µM) attenuated the facilitatory action of clonidine, suggesting that the effect is mediated by activation of α_2 receptors (Fig. 6*F*). On the contrary, bath application of the α_1 receptor agonist phenylephrine (1 µM) decreased LII/III-eIPSCs by 32 ± 8% in all cells tested ($n = 10$, $P < 0.05$, ANOVA with Tukey's post hoc test; Fig. 6*G*). Together, these results reveal that despite a critical role for β and α_2 receptors in mediating the facilitatory effect of NE, coactivation of α_1 receptors limited the magnitude of NE-mediated increase of LII/III-eIPSCs.

Second Messengers: α_1 Receptor—PLC Modulation

The previous results suggest the existence of 2 pharmacologically segregated GABAergic inputs onto pyramidal neurons of LII/III of the auditory cortex. To examine whether such a segregation is associated with specific NE receptor signaling cascade, we tested the effects of the selective phospholipase C (PLC) blocker U73122 (10 µM) on the noradrenergic modulation of LI- and LII/III-eIPSCs. We found that preincubation of brain slices in the presence of U73122 blocked the decrease induced by NE on LI-eIPSCs amplitude ($n = 3$; example in Fig. 7*A*, average effect in Fig. 7*B*), whereas the NE-induced facilitation of LII/III-eIPSCs was even enhanced in the presence of U73122 (97.5 ± 20%, $n = 3$; example in Fig. 7*C*, average effect in Fig. 7*D*), similar to the effect observed on LII/III-eIPSCs in the presence of the α_1 receptor blocker prazosin (Fig. 6*B*).

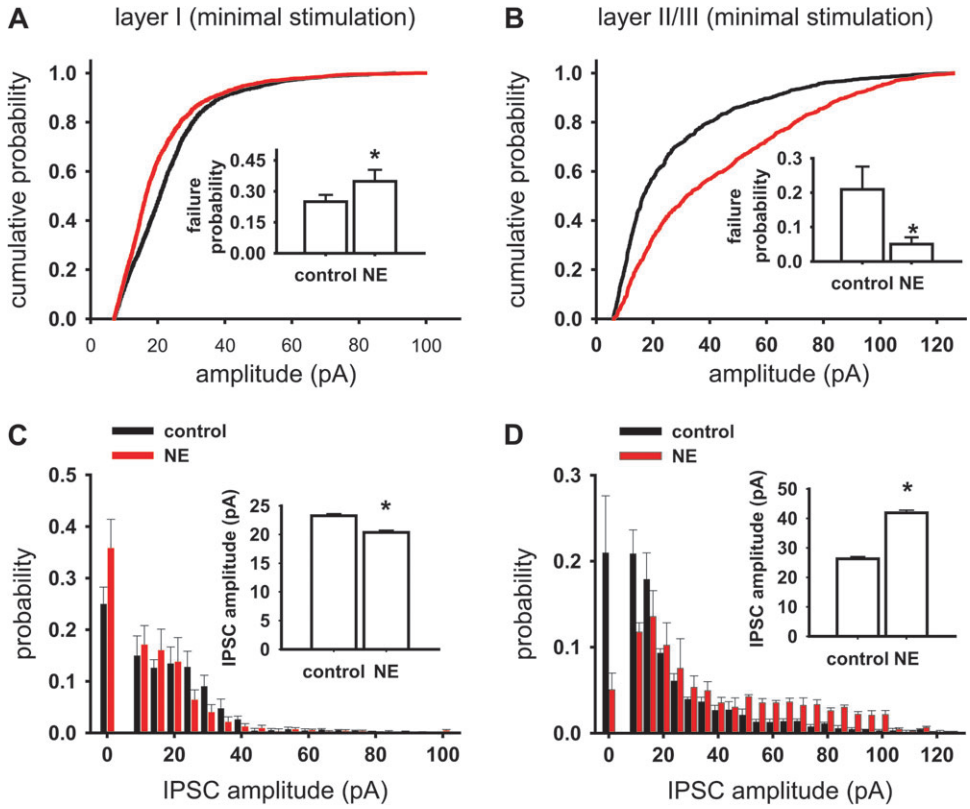

Figure 4. Minimally evoked GABAergic currents are modulated by NE. (*A*, *B*) Cumulative probability of minimally evoked LI- or LII/III-eIPSCs amplitude ($n = 9$ and $n = 6$, respectively) in control and NE. NE application reduced the average amplitude of LI-eIPSCs but increased LII/III-eIPSCs amplitude. The inserts show changes in eIPSCs failure rates. (*C*, *D*) Cumulative probability of the minimally evoked LI- and LII/III-eIPSCs amplitudes, respectively. The inserts display an opposite effect of NE on the average of minimally evoked IPSCs amplitude. Black: control; red: NE.

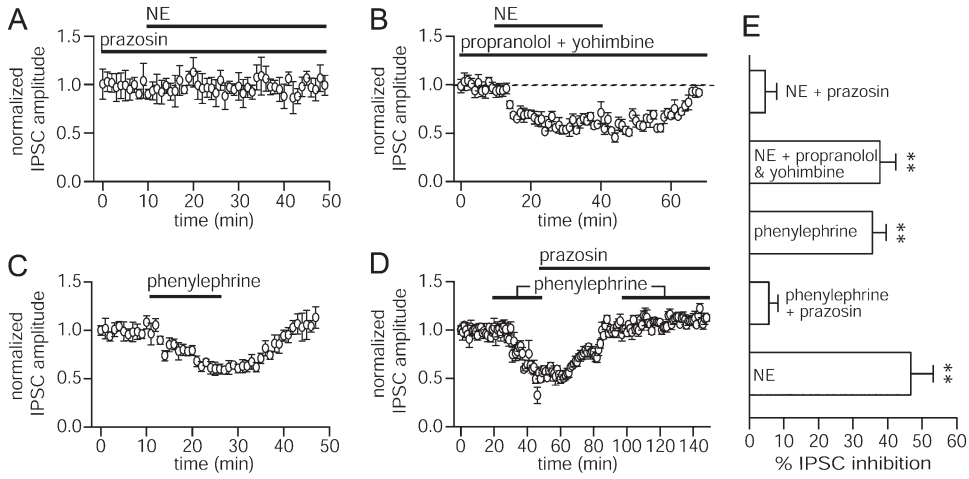

Figure 5. α_1 Receptors produce a decrease in LI-eIPSCs amplitude. Averaged time course of the effect of adrenergic agonists and antagonists on eIPSCs. (*A*) Temporal course showing that the α_1 adrenoceptor blocker prazosin (1 μM) blocks the effects of NE. (*B*) The antagonists for β and α_2 receptors (propranolol and yohimbine, 1 μM) did not block the NE action on LI-eIPSCs, suggesting that α_1 receptors activation modulated LI-eIPSCs. (*C*) Phenylephrine (1 μM) decreases LI-eIPSCs amplitude. (*D*) Previous application of prazosin blocked LI-eIPSCs reduction by the selective α_1 noradrenergic receptor (phenylephrine 1 μM). (*E*) Summary of results with agonists and antagonists indicating that α_1 receptors are responsible for NE-induced reduction in LI-eIPSCs amplitude.

Discussion

Our results provide for the first time evidence for a layer-specific noradrenergic modulation of inhibition in the auditory cortex. LI- and LII/III-eIPSCs differed in kinetics, subunit composition, and direction and pharmacology of their noradrenergic modulation. We will discuss our results first at the synaptic and then at the cortical microcircuit level.

Synaptic Nature of the Noradrenergic Modulation

The NE-induced increase in LII/III-eIPSCs was due, at least in part, to presynaptic modulation of GABAergic terminals, as suggested by the NE-induced decrease in PPR and by the failure rate decreases and amplitude increase of minimal stimulated IPSCs, suggesting that NE increased the probability of release from GABAergic axons in layer II/III. The NE-induced enhancement of LII/III-eIPSCs took place despite the presence of α_1 antagonist, was mimicked by application of α_2 and β agonists, and was blocked by β and α_2 antagonists, indicating that NE increases the release of GABA acting via α_2 and β adrenergic receptors.

On the contrary, pharmacological analysis of the adrenergic-induced reduction of LI-eIPSCs was mediated by α_1-type adrenoceptors. In fact, 1) phenylephrine (but not clonidine or isoproterenol) mimicked the effect of NE, 2) preapplication of the α_1-type antagonist prazosin completely blocked the effect of either NE or phenylephrine, and 3) the application of the α_2- or β-type adrenergic receptors yohimbine or propranolol did not block the NE-induced amplitude decrease of LI-eIPSCs. A presynaptic contribution of α_1 receptors was corroborated by the phenylephrine-induced increase in PPR and failure rate for minimally stimulated LI-eIPSCs. The effectiveness of a PLC inhibitor in blocking the NE modulation confirmed the involvement of α_q-type G-protein/PLC pathway, consistent with activation of classic α_1 receptors.

The larger NE-induced amplitude increase of LII/III-eIPSCs obtained in the presence of an α_1 blocker suggests that the prevalent effect of NE on LII/III is an α_2R- and/or βR-mediated enhancement of inhibition that masks a less effective α_1R-mediated inhibition. Consistent with the previous results, NE has been shown to depress eIPSCs in the developing hippocampus (Madison and Nicoll 1988), while in the entorhinal and perirhinal cortices elicits both pre- and postsynaptic α_1R-mediated effects (Lei et al. 2007; Hillman et al. 2009).

The different kinetics of LI- and LII/III-eIPSCs might indicate that the synapses corresponding to the 2 stimulation sources contacted different regions of the somatodendritic membrane (Sceniak and Maciver 2008), similar to those observed in other studies in the neocortex and hippocampus, and/or might be due to a different subunit composition of the corresponding $GABA_A$Rs (Pearce 1993; Banks et al. 1998, 2002; Banks and Pearce 2000; Prenosil et al. 2006). The analysis of minimally stimulated eIPSCs strongly supports the hypothesis that synapses generating slow and fast synaptic events are spatially segregated, similar to the results of Miles et al. (1996) in the hippocampus, where preferential activation of dendritic or perisomatic inhibitory synapses follows extracellular stimulation of *stratum pyramidale* or *stratum lacunosum-moleculare*. On the other hand, the sensitivity of LI- versus LII/III-eIPSCs to zolpidem is indicative of a different composition of the corresponding postsynaptic $GABA_A$R subunits.

IPSC kinetics from fast-spiking (FS) pyramidal cell synapses appear to be faster than IPSC kinetics in low-threshold–spiking (LTS) pyramidal cell synapses (Hajos and Mody 1997; Banks et al. 1998) indicating their prevalent somatic innervation of the former. On the contrary, LTS interneurons, which express numerous neuropeptides (Cauli et al. 1997; Bacci et al. 2004; Wang et al. 2004), regulate the excitability of distal dendritic input (Freund and Gulyas 1997; Xiang et al. 1998; Bacci et al. 2003; Wang and Zhang 2004). Although our data do not supply conclusive information about the source of LII/III- and LI-eIPSCs, we speculate that their difference in kinetics (fast vs. slow), subunit composition (zolpidem sensitive vs. zolpidem insensitive), and pharmacology (α_2 and β vs. α_1 adrenoceptor sensitivity) are associated with inputs at the basal versus apical dendritic arborizations, respectively. NE would thus favor proximal-somatic versus distal-apical inhibitory input to supragranular neurons by specifically modulating local circuit

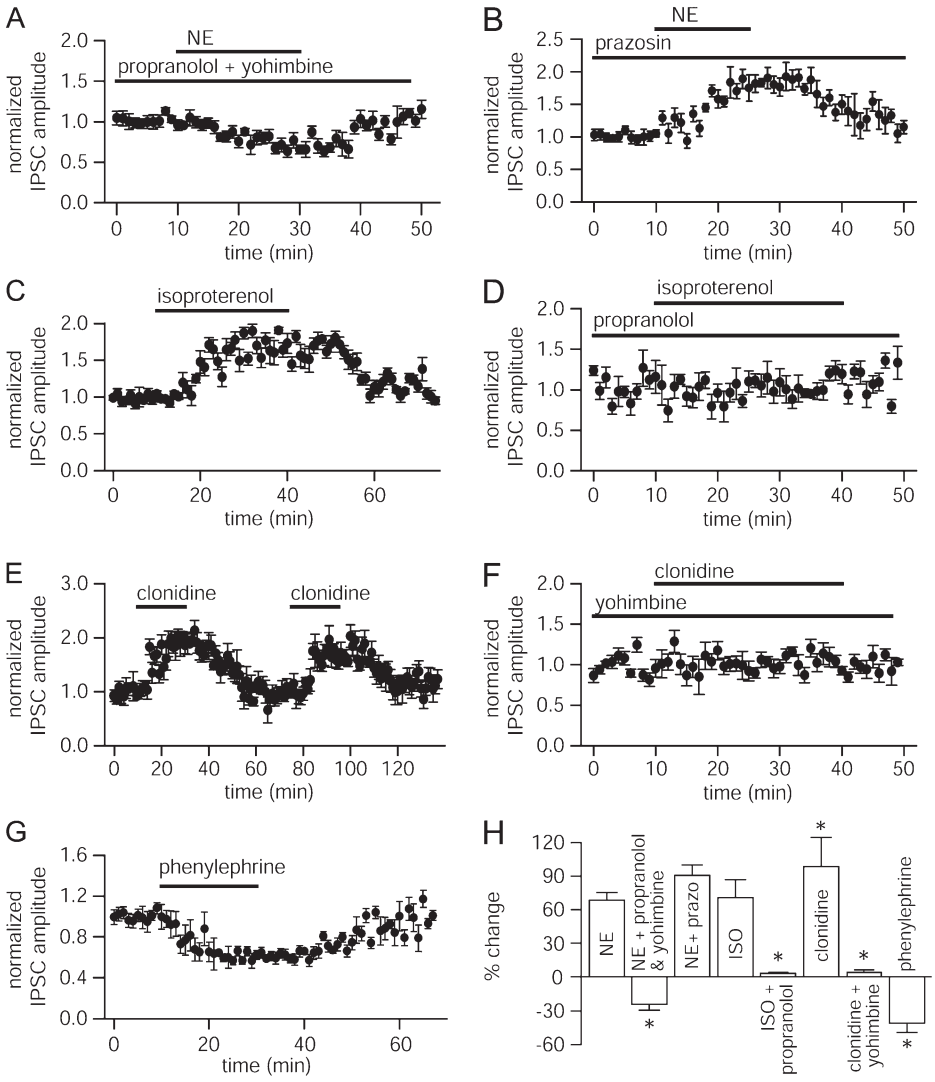

Figure 6. β and α_2 receptors activation increase GABA release in LII/III-eIPSCs. LII/III-eIPSCs amplitude time courses and averages showing the effects of adrenergic agonists and antagonists. (*A*) The antagonists for β and α_2 receptors (propranolol and yohimbine) blocked the enhancement of the LII/III-eIPSCs by NE, unmasking a NE-induced amplitude decrease. (*B*) Prazosin does not block the enhancement of the eIPSCs by NE. (*C*) The selective β noradrenergic receptor agonist (isoproterenol, iso 50 μM) increases LII/III-eIPSCs amplitude. (*D*) The β antagonist propranolol (1 μM) blocks the effect of isoproterenol. (*E*) The α_2 agonist clonidine (1 μM) mimics the NE effects on eIPSCs in 28% of the cells studied. (*F*) Effects of clonidine were blocked by previous application of the α_2 antagonist yohimbine (1 μM). (*G*) Application of the α_1 agonist phenylephrine (1 μM) decreases eIPSCs amplitude. Complete statistics are reported in the text. (*H*) Summary of the experiment with adrenergic agonists and antagonists on eIPSCs evoked from LII/III stimulation. These results suggest that β and α_2 receptors are responsible for NE–induced eIPSCs enhancement.

GABAergic interneurons. A nonspecific adrenergic modulation of postsynaptic $GABA_A$Rs is unlikely because 1) during simultaneous stimulation in layers I and II/III, NE produced both decrease in LI- and increase in LII/III-eIPSCs amplitude (Fig. 1), 2) NE changed the PPR ratio, and 3) NE altered the failure rates during minimal stimulation. These forms of inhibition are triggered by the activation of adrenergic receptors at different types of presynaptic interneurons and have the potential to induce unique spatial and temporal properties on synaptic integration onto pyramidal cells (Kapfer et al. 2007).

Inhibitory Circuit Underlying Layer-Specific Adrenergic Modulation

The morphological and electrophysiological diversity of inhibition suggests that different interneuron types have layer-specific roles and targets in the cortical circuitry (Gupta et al. 2000). For example, Martinotti cells of LII/III send abundant projections to layer I and contact apical and basal dendrites in multiple neocortical layers. In particular, Martinotti cells of layer II/III target mostly layer I and to a lesser degree layer II/III (Miles et al. 1996; Somogyi et al. 1998; Sceniak and Maciver 2008), while Martinotti cells of layers V and VI target mostly layers IV and I and to a lesser degree their own somatic layer. These cells (also called LTS neurons) are positive for somatostatin and negative for parvalbumin (PV), while perisomatic inhibition is mainly provided by PV-positive FS cells. We speculate that electrical stimulation activates LTS- or FS-presynaptic axons that mediate LI- and LII/III-eIPSCs, respectively, although we cannot exclude additional contributions from neurons in different cortical layers. Our pharmacological data suggest that an even finer interneuron classification might be necessary to exhaustively describe the details of adrenergic modulation, at least for LII/III-eIPSCs.

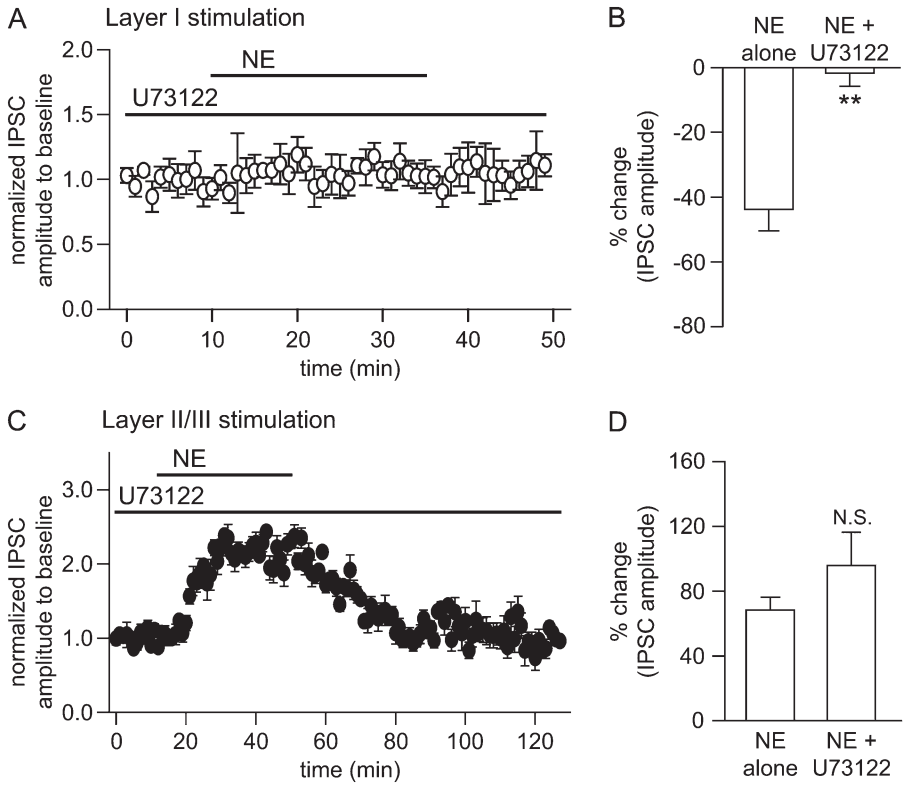

Figure 7. α_1 - PLC is responsible for NE-induced modulation of LI-eIPSCs. NE effects after slice preincubation with the PLC blocker U73122 (10 μM) for >3 h. (A) Time course showing that U73122 preincubation blocks all effects of NE on LI-eIPSCs. (*B*) Bar graph summarizing NE effects. Mean ± standard error of the mean of NE-induced depression of LI-eIPSCs. (*C*) Time course of NE effects on LII/III-eIPSCs after preincubation in U73122. PLC blockage does not prevent the modulation of NE in LII/III. (*D*) Bar graph summarizing the effects of NE. Mean ± standard error of NE-induced enhancement on eIPSCs. These data suggest that α_1 receptors are associated with PLC pathway.

Functional Implications

The impact of NE on the cortical network is complex. NE modulation of GABA release has been proposed to alter signal-to-noise ratio as reported in early experiments (Foote et al. 1975), together with the ability to extract sensory information and, consequently, to differentiate between behaviorally relevant and irrelevant input. Synaptic inhibition plays an important role in shaping auditory receptive fields, temporal patterns, as well as frequency tuning (Suga 1995; Oswald et al. 2006). An increased inhibition of intracortical inputs has been suggested to contribute to a selective amplification of behaviorally relevant signals by tuning the optimal stimulus response of cortical cells (Liu et al. 2007). In vitro and in vivo studies (Sato and Kayama 1983; Devilbiss and Waterhouse 2004) have demonstrated that while α_1 receptors promote cortical excitability, activation of α_2 or β receptors is more likely to suppress cortical signals (Devilbiss and Waterhouse 2000). Our results corroborate those findings and supply a potential explanation to the phenomenon in terms of a specific increase in perisomatic versus a decrease in apical inhibition by different adrenergic receptors. NE might lead to simultaneous "apical" hyperexcitability and "proximal" interneuron-gated synchronization of sensory input, possibly through the enhancement of γ-oscillations (Gire and Schoppa 2008), which could in turn open a spatiotemporal window of synaptic integration for signal propagation to the next computational stage. A possibility is that during complex sound processing, adrenergic modulation of GABAergic function could promote the initial processing of corticocortical, non-tonotopic, or nonlemniscal distal inputs, allowing tonotopic information to be subsequently processed in layer II/III after being "primed" by the transiently hyperexcitable layer I. This process might contribute to auditory attention by enabling the extraction of sensory information to differentiate behaviorally relevant from irrelevant stimuli (Ramos and Arnsten 2007).

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