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SEROTONERGIC MODULATION OF LTP AT EXCITATORY AND INHIBITORY SYNAPSES IN THE DEVELOPING RAT VISUAL CORTEX

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Abstract—The stability and efficacy of neuronal circuits are achieved through a detailed balance between pyramidal cell and interneuron activities. Interestingly, the neocortical excitatory–inhibitory (E–I) balance is actively maintained at the soma of Layer 5 pyramidal neurons which receive 20% of excitation and 80% of inhibition after dendritic integration, and this is not affected by changes in synaptic strength. To infer the role of serotonergic neuromodulation on the activity-dependent maintenance of the E–I balance, we performed continuous voltage clamp measurements of stimulation-locked conductance dynamics in Layer 5 pyramidal neurons before and after long-term potentiation (LTP) induction, together with chronic or acute manipulation of serotonin function. When a theta-burst stimulation was applied in Layer 2/3 of 5-HT depleted cortical slices (after *in vivo* treatment with the tryptophan hydroxylase inhibitor p-chlorophenylalanine (pCPA)), or after *in vitro* perfusion of the potent 5-HT$_{1A}$ receptor antagonist WAY-100,635, we observed a persistent shift of the ratio between excitation and inhibition toward more inhibition. This was due to a strong LTP of inhibition co-aligned with a weak LTP of excitation, whereas the same protocol caused a similar potentiation of excitatory and inhibitory inputs when applied in control slices. In contrast, neither excitatory nor inhibitory postsynaptic currents were potentiated when LTP protocols were delivered in the presence of either the selective serotonin reuptake inhibitor citalopram or the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. This is the first demonstration that serotonergic neuromodulation is crucial for the maintenance of the neocortical E–I balance during high-frequency regimes.

Key words: serotonin, 5-HT$_{1A}$ receptor, synaptic plasticity, neocortical circuits, SSRI, excitatory inhibitory balance.

INTRODUCTION

Maintaining a tight interplay between excitation and inhibition is crucial for the maturation of neocortical circuits (Hensch, 2005) and for sensory information processing (Wehr and Zador, 2003; Mariño et al., 2005; Yizhar et al., 2011). The E–I balance controls the stability and responsiveness of neocortical circuits (Economo and White, 2012) and its alteration has been shown to generate behavioral impairments (Yizhar et al., 2011) as well as epileptiform states (Treiman, 2001; Cossart et al., 2005; Dudek and Sutula, 2007). Within individual neurons the ratio between incoming excitation and inhibition can change rapidly (Higley and Contreras, 2006) allowing the fine tuning of synaptic inputs integration and neuronal output to specific stimuli (Isaacson and Scanziani, 2011). It is thus critical that changes in the number and strength of synapses that normally occur during learning and development are matched by compensatory homeostatic effects triggered in both excitatory and inhibitory circuits (Davis, 2006; Wenner, 2011). Such a phenomenon has been reported in Layer 5 pyramidal cells (L5PCs) of the rodent visual cortex, where the ratio between excitatory and inhibitory inputs remains close to 20/80 (E/I) under high-frequency stimulation regimes (Le Roux et al., 2006, 2008).

Serotonin is a pleiotropic modulator of neuronal activity (Bockaert et al., 2006) and of synaptic plasticity in both developing and adult visual cortex (Kirkwood, 2000; Maya Vetencourt et al., 2008; Jang et al., 2012; Park et al., 2012). Transient alterations in the serotonin homeostasis is also known to produce long-lasting modification of neuronal wiring in several sensory areas including the visual cortex (Durig and Hornung, 2000; Gaspar et al., 2003). We previously reported that serotonin finely tunes the E–I balance around its control set-point through the recruitment of distinct 5-HT receptor subtypes on neocortical pyramidal cells and interneurons (Moreau et al., 2010). However, whether 5-HT neuromodulation is important for the maintenance of the E–I balance during high-frequency activities remains unknown. Strikingly, 5-HT function during the critical

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Abbreviations: aCSF, artificial cerebrospinal extracellular solution; E–I, excitatory–inhibitory; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol tetraacetic acid; e-LTP, excitatory LTP; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; i-LTP, inhibitory LTP; L5PC, Layer 5 pyramidal cell; LTP, long-term potentiation; PBS, phosphate-buffered saline; pCPA, p-chlorophenylalanine; RT-PCR, reverse transcription polymerase chain reaction; sEPSCs, spontaneous Excitatory Postsynaptic Currents; sIPSCs, spontaneous inhibitory currents; SSRI, selective serotonin reuptake inhibitor; TBS, theta-burst stimulation.

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period of early postnatal life determines the adult behavior (Grosse et al., 2000; Ansorge et al., 2004; Andolina et al., 2011). In the present study we thus focused on the role of serotonin on the plasticity of excitation and inhibition during the critical period for plasticity. In 3-week-old rats, we monitored under various neuromodulatory states, the E–I balance changes following the induction of LTP at excitatory and inhibitory synapses embedded in cortical microcircuits. To achieve this goal, we manipulated endogenous 5-HT function using *in vivo* chronic injection of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (pCPA), *in vitro* application of the selective serotonin reuptake inhibitor citalopram, or agonist and antagonist of the 5-HT_{1A} receptor. Effects of these drugs were determined on the simultaneous potentiation of evoked excitatory and inhibitory conductances that we recorded in L5PCs of the rat visual cortex.

Our results show that chronic pCPA-pretreatment decouples the LTP of excitatory and inhibitory inputs onto L5PCs following the theta-burst stimulation of Layer 2/3 and consequently disrupts the E–I balance. This was mimicked by blocking the 5-HT_{1A} receptor with WAY-100,635. In contrast, both the acute 5-HT_{1A} activation by 8-0H-DPAT and the citalopram-induced increase in endogenous 5-HT tone abolished the potentiation of excitation and inhibition in L5PCs. We concluded that serotonergic neuromodulation is required to maintain a normal E–I balance during plasticity induction in sensory microcircuits.

EXPERIMENTAL PROCEDURES

Cortical 5-HT depletion

Wistar rats (P15-18) were pretreated with either physiological serum (isotonic NaCl 0.9%, sham-pretreated group) or with the tryptophan hydroxylase inhibitor pCPA. pCPA, at a dose of 200 mg/kg, was injected intraperitoneally once a day during 4 consecutive days. Pretreated animals were sacrificed (see "*Slices preparation*" for animal procedures) 1 day after the fourth injection in order to obtain the best depletion efficacy (see the comparative study of Kornum et al. (2006)).

HPLC quantification

Primary visual cortices of individual sham- or pCPA-pretreated rats were quickly dissected (see "*Slices preparation*" for animal procedures) and were homogenized in ice-cold 10^{-3} M chlorhydric acid containing sodium metabisulfite (10 μM), EDTA (10 μM) and ascorbic acid (10 μM). After centrifugation, the supernatant was passed through a 10,000 MW filter (Nanosep 10K, Pall). Then, a 20-μl aliquot of the sample was analyzed for 5-HT content by HPLC coupled to fluorometric detection as previously described (Kema et al., 1993). The results were expressed as fmoles per milligram of fresh tissue.

Immunostaining and imaging

One brain hemisphere of each rat pretreated with pCPA or isotonic NaCl and not used for electrophysiological recordings (see "*Slices preparation*") was fixed in a 4% paraformaldehyde/15% saccharose solution during 1 week at 4 °C, cut in 30-μm slices and stored at 4 °C in a 0.1 M phosphate-buffered saline (PBS), 0.02% sodium azide solution for further immunostaining controls of 5-HT depletion. Fixed brain slices of pCPA or isotonic NaCl-injected rats were permeabilized during 30 min (glycine 10 mM, triton 0.4 mM), saturated during 60 min (bovine albumin serum 2%, sheep serum 3%, in PBS), and incubated (overnight at 4 °C) with polyclonal antibodies against 5-HT (1:750; Immunostar Incorporated, Hudson, USA) or against the serotonin reuptake transporter (1:1000; Calbiochem, La Jola, USA). The latter antibody was used in order to label serotonergic fibers as described previously (Zhou et al., 1996). Bound antibodies were detected with fluorescein isothiocyanate (FITC)-conjugated (1:750) or Alexa-Fluor-488-conjugated (1:500) goat anti-rabbit secondary antibodies (Invitrogen, Saint Aubin, France) incubated for 2 h at room temperature. Immunostained sections were mounted on Superfrost Plus slides (Microm International, Walldorf, Germany) using Vectashield mounting medium (Vector Laboratories, Peterborough, UK) and conserved at 4 °C until imaging. Mounted slides were visualized under upright epifluorescence (Leica DMRXA2) or confocal (Leica SP2, 488 nm argon laser) microscopes with 10× or 40× (oil-immersed) objectives. For confocal microscopy, laser intensity and PMT gain were optimized in order to minimize photobleaching. Offset and gain settings were the same for the different conditions observed (e.g. sham- and pCPA-pretreated slides). Immunostaining specificity was verified by replacing the primary antibody by nonimmune serum, without changing the rest of the procedure. In all cases, no labeling was observed (data not shown). Images were processed in ImageJ. Brightness adjustment was applied equally across the entire image and equally across images gathered from different experimental conditions.

Slices preparation and electrophysiology recordings

In accordance with the European and Institutional guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC and French National Research Council), 19- to 25-day-old Wistar rats were decapitated and their brains quickly removed. All efforts were made to minimize animal suffering and to reduce the number of animals used. After hemisection one hemisphere was removed, attached to the stage of a tissue slicer (WPI NVSLM1, UK) and immersed in cold (roughly 4 °C) artificial cerebrospinal extracellular solution (aCSF) containing (in mM): 126 NaCl, 26 $NaHCO_3$, 10 Glucose, 2 $CaCl_2$, 1.5 KCl, 1.5 $MgSO_4$ and 1.25 KH_2PO_4 (pH 7.5, 310–320 mOsm). Two hundred and fifty micrometers parasagittal slices containing primary visual cortex were cut and transferred to a holding chamber filled with aCSF, bubbled with 95% O_2/5% CO_2 and maintained at room temperature after an initial 1 h incubation at 36 °C. For experiments, slices were perfused (2–3 ml/min) at 31 °C (inline heater TC 324B WARNER) with bubbled aCSF. Neurons were patched at 40× magnification using an upright microscope (Zeiss Axioscop 2 FS+) with video-enhanced DIC. Patch pipettes (of 4–5 MΩ resistance) contained the following intracellular solution (in mM): 140 K-gluconate, 10 HEPES, 4 ATP, 2 $MgCl_2$, 0.4 GTP and 0.5 EGTA (pH 7.3 adjusted with KOH, 280–290 mOsm). Stable whole-cell stable voltage-clamp recordings were obtained from Layer 5 pyramidal neurons (identified by the shape of their soma and main apical dendrite and from their current-induced firing profile to 1-s depolarizing steps ranging from −100 to 200 pA) with a Multiclamp 700A amplifier (Axon Instruments, USA). Data were sampled at 2 kHz using a Digidata 1322A acquisition board (Axon Instruments, USA). Voltage data were corrected off-line for a measured liquid junction potential of −10 mV.

After capacitance neutralization, bridge balancing was done on-line under current clamp to make initial estimations of the access resistance (Rs). The later procedure was repeated before every voltage clamp recording. The membrane input