The Stress-Induced Cytokine Interleukin-6 Decreases the Inhibition/Excitation Ratio in the Rat Temporal Cortex Via Trans-Signaling

Supplemental Information

Preparation

One-hundred and sixty six, 25-50-day-old (mean ≈ 35 day old) Sprague Dawley rats (Charles River, Wilmington, MA) were anesthetized with isoflurane (Baxter, Round Lake, IL), sacrificed according to the National Institutes of Health Guidelines (UTD IACUC number 04-04), and their brains sliced with a vibrotome (VT1000, Leica, Germany) 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 $CaCl_2$ and 1.5 $MgCl_2$, 2 mM kynurenic acid, titrated at pH 7.4 and saturated with a mixture of 95% O_2 and 5% CO_2 (artificial cerebrospinal fluid, ACSF). Coronal slices (270 μm thickness) 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 temporal cortex (1).

Electrophysiological Recordings

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, Japan) with a 60X objective and an infrared camera system (DAGE-MTI, Michigan City, IN). Whole-cell voltage-clamp recordings from layer II/III pyramidal neurons of the temporal cortex were performed under visual guidance. Neurons were selected by their pyramidal shape and by their pronounced apical dendrite. In most experiments postsynaptic currents (PSCs) were recorded in the whole-cell configuration, in voltage clamp mode, at a holding membrane potential V_h = - 60 mV unless otherwise specified, 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 (BAPTA-K), 1 lidocaine N-ethyl bromide (QX314), 1 $MgCl_2$, 10 N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), 4 glutathione, 1.5 ATPMg, 0.3 $GTPNa_2$, 20 phosphocreatine. In another series of experiments, we measured inhibitory and excitatory currents within a single cell, using a low-Cl^- intracellular solution where CsCl was

lowered to 10 mM, and the remainder 90 mM was substituted with K gluconate, resulting in a theoretical reversal potential for Cl^- of approximately -65 mV. The holding voltage was corrected for the junction potential ($V_{offset} \approx 10$ mV). The intracellular recording solutions were titrated to pH 7.3 and had an osmolarity of approximately 270 mOsm.

Analysis

In our analysis of the postsynaptic current amplitudes, we defined a statistically stable period as a time interval (5-8 min) along which postsynaptic current mean amplitude measured during any two-minute assessment did not vary according to Mann-Whitney U test. All data are expressed as mean ± SEM. Paired-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 (2). The effects of drug application on the PSC amplitude changes were reported as A_{treat}/A_{ctrl}, where A_{treat} and A_{ctrl} are the mean PSC amplitude in treatment and in control respectively. The coefficient of variation was defined as the ratio between standard deviation and mean on samples of 50 evoked responses. Drug effects were assessed by measuring and comparing the different parameters (A_{treat}/A_{ctrl}, inhibitory PSC and excitatory PSC mean amplitudes, or other parameters as indicated) of baseline (control) vs. treatment, with a Mann-Whitney U-test. One way ANOVA with Tukey post hoc test was used for comparisons between different groups of cells. Wilcoxon test was used for comparing between PPRs, and Student *t*-test (paired or unpaired, depending on the experiment) was used for all other comparison.

1. Rutkowski RG, Miasnikov AA, Weinberger NM (2003): Characterisation of multiple physiological fields within the anatomical core of rat auditory cortex. *Hear Res*. 181:116-130.
2. Kim J, Alger BE (2001): Random response fluctuations lead to spurious paired-pulse facilitation. *J Neurosci*. 21:9608-9618.