PRIORITY COMMUNICATION

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

Francisco Garcia-Oscos, Humberto Salgado, Shawn Hall, Feba Thomas, George E. Farmer, Jorge Bermeo, Luis Charles Galindo, Ruben D. Ramirez, Santosh D'Mello, Stefan Rose-John, and Marco Atzori

Background: Although it is known that stress elevates the levels of pro-inflammatory cytokines and promotes hyper-excitable central conditions, a causal relationship between these two factors has not yet been identified. Recent studies suggest that increases in interleukin 6 (IL-6) levels are specifically associated with stress. We hypothesized that IL-6 acutely and directly induces cortical hyper-excitability by altering the balance between synaptic excitation and inhibition.

Methods: We used patch-clamp to determine the effects of exogenous or endogenous IL-6 on electrically evoked postsynaptic currents on a cortical rat slice preparation. We used control subjects or animals systemically injected with lipopolysaccharide or subjected to electrical foot-shock as rat models of stress.

Results: In control animals, IL-6 did not affect excitatory postsynaptic currents but selectively and reversibly reduced the amplitude of inhibitory postsynaptic currents with a postsynaptic effect. The IL-6-induced inhibitory postsynaptic currents decrease was inhibited by drugs interfering with receptor trafficking and/or internalization, including wortmannin, Brefeldin A, 2-Br-hexadecanoic acid, or dynamin peptide inhibitor. In both animal models, stress-induced decrease in synaptic inhibition/excitation ratio was prevented by prior intraventricular injection of an analog of the endogenous IL-6 trans-signaling blocker gp130.

Conclusions: Our results suggest that stress-induced IL-6 shifts the balance between synaptic inhibition and excitation in favor of the latter, possibly by decreasing the density of functional γ-aminobutyric acid A receptors, accelerating their removal and/or decreasing their insertion rate from/to the plasma membrane. We speculate that this mechanism could contribute to stress-induced detrimental long-term increases in central excitability present in a variety of neurological and psychiatric conditions.

Key Words: 2-Br-hexadecanoic acid, postsynaptic, Brefeldin A, dynamin inhibitory peptide, foot-shock, γ-aminobutyric acid (GABA), interleukin-6 (IL-6), lipopolysaccharide (LPS), PI3K/AKT, patch-clamp, stress, gp130, rat, temporal cortex, trans-signaling, wortmannin

Temporary stress-induced increase in excitability is a symptom common to many psychiatric conditions, such as schizophrenic psychoses (1,2), posttraumatic stress disorder (3), anxiety (4), depression (1), and autistic spectrum disorders (ASD) (5–7), but also to neurological conditions, including epilepsy (8,9) and tinnitus (10). Such increase in excitability can lead to impaired behavior (abnormal startle response, irritability, aggression), abnormal perception (hyperacusis, hypersensitivity to touch, sensory-induced seizures), and/or altered emotion (paranoia, delusions, emotional arousal).

A plethora of factors (adenosine triphosphate depletion, infection, trauma, chronic fatigue, acute stress) potentially triggering hyper-excitable neurological or psychiatric conditions are also known to elevate the synthesis and release of cytokines (11–14). Pro-inflammatory cytokines—including interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α)—are known to affect the brain at the behavioral, morphological, and functional level, inducing for example, sickness behavior (15), neurogenesis (16), and synaptic plasticity (17).

Recent studies have indicated a specific role of IL-6 in stress-related pathophysiology. For example, messenger RNA levels of IL-6, synthesized in pyramidal neurons of the cortex (18) and of the hippocampus (19), are greatly increased by psychological stress (20), whereas abnormal increases in systemic levels of IL-6 follow the administration of a standardized social stress test in nonpsychiatric patients with a history of childhood maltreatment but not in control subjects (21). Furthermore, noninflammatory stressors selectively activate IL-6-producing vasopressin-positive neurons of the paraventricular and supraoptic nuclei of the hypothalamus, which in turn release systemic IL-6 (22).

Although neuronal membranes do not seem to display IL-6 receptors (23), they possess gp130 receptors—which upon stimulation by the complex formed by IL-6 and its soluble receptor "shed" by non-neuronal brain cells initiate the Janus kinase/signal transducer and activator of transcription/extracellular signal-regulated kinase/phosphatidyl inositol 3 kinase (PI_3K) signal transduction, a process referred to as "trans-signaling pathway" (24,25). Importantly, an abnormally high level of IL-6 has the potential to induce *status epilepticus*, probably by decreasing the expression of the β_{2-3} and γ_2 subunits of γ-aminobutyric acid-A receptors ($GABA_A$Rs) in several brain regions, including the temporal cortex (26). A temporary or long-term hyper-excitability of the temporal cortex is hy-

From the School of Behavioral and Brain Sciences (FG-O, FT, GEF, MA); School of Natural Sciences (SH, RDR, SDM); Eric Jonsson School of Engineering (JB), University of Texas at Dallas, Richardson Texas; Centro de Investigaciones Regionales Hideyo Noguchi (HS), Merida, Yucatan; Universidad Nacional Autonoma de Mexico (LCG), Mexico City, Mexico; and the Institute of Biochemistry (SR-J), Christian Albrechts University, Kiel Germany.

Address correspondence to Marco Atzori, Ph.D., Laboratory of Cellular and Synaptic Physiology, School for Behavioral and Brain Sciences, University of Texas at Dallas, GR41, 800 West Campbell Road, Richardson, TX 75080; E-mail: marco.atzori@utdallas.edu.

Received Jul 11, 2011; revised Oct 26, 2011; accepted Nov 11, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.11.018

pothesized to underlie the onset of tinnitus and hyperacusia, positively correlated with increased levels of IL-6 (27). An exquisite vulnerability of the temporal lobe to damage by diverse stressors might contribute to its relevance to schizophrenic psychoses, autism, and epilepsy (28).

The causal relationship linking the increase in IL-6 levels and central hyper-excitability is yet poorly understood. We considered the possibility that IL-6 increases neuronal excitability by a direct action at the synaptic level. To test this hypothesis we determined the effects of the exogenous application of IL-6 as well as the IL-6-dependence of the balance between inhibitory and excitatory synaptic transmission on two types of stress, on a rat temporal cortex preparation. We found that acute administration of IL-6, lipopolysaccharide (LPS) systemic injection, or foot-shock (FS) shifts the balance between excitation and inhibition in favor of the former. The pharmacological sensitivity of the IL-6 modulation of GABAergic responses is consistent with an increased, possibly ligand-dependent, internalization of $GABA_A$Rs from the neuronal membrane.

Materials and Methods

Preparation

We used a temporal cortex slice preparation similar to a previously described one (Supplement 1) (29). 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (10 μmol/L), kynurenate (2 mmol/L) were used in a series of experiments for blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor- and *N*-methyl-D-aspartate receptor-mediated currents. Gabazine (20 μmol/L) was used to block $GABA_A$R-mediated currents.

Drugs and Solutions

Recombinant rat IL-6 (R&D Systems, Minneapolis, Minnesota) was activated with phosphate-buffered saline at a concentration of .5 μg/1 mL and was used at 10 ng/mL, in the range of central (up to 2 ng/mL) and systemic (up to 15 ng/mL) concentrations measured after immune challenge (30,31). The blocker of the IL-6 trans-pathway (32), soluble glycoprotein 130Fc (sgp130Fc), was produced in the laboratory of S.R.-J. at the Department of Biochemistry at the Christian Albrecht Universitat (Kiel, Germany). Lipopolysaccharide (serotype 0127:B8) was purchased from Sigma (St. Louis, Missouri). All other drugs were purchased from Sigma or Tocris (Ellisville, Missouri). 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 in Statistical Analysis). For slice incubation with wortmannin (200 nmol/L), the drug was dissolved in ethanol (final dilution 1/2000). Brefeldin A (400 nmol/L), similar to 2-Br-hexadecanoic acid (20 μmol/L) was also dissolved in ethanol (final dilution 1/500). The dynamin inhibitory peptide (P4) (20 μmol/L) was dissolved as described previously (33).

Electrophysiology

Electrophysiological methods are discussed in detail in Supplement 1. Electrically evoked postsynaptic currents were measured by delivering one or two electric stimuli (90–180 μsec, 10–50 μA) 100 msec apart, every 10 sec, with an isolation unit, through a glass stimulation monopolar electrode filled with artificial cerebrospinal fluid at approximately 100–200 μm from the recorded neuron. Synaptic responses were monitored at different stimulation intensities before baseline recording. Detection threshold was set at approximately 150% of the SD of the noise (typical noise approximately 4–5 pA, threshold approximately 7–8 pA). A −2 mV 100-msec-long voltage pulse was applied at the beginning of every episode 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. Reversal potential for postsynaptic currents were evaluated, determining current-voltage (I-V) relationships for the evoked postsynaptic current (peak amplitude of 10 events at each holding potential V_h in the range from $V_h = -90$ mV up to $V_h = +60$ mV). Evoked inhibitory postsynaptic currents (IPSCs) reversed polarity close to the theoretical reversal potential of −65 mV (−64 ± 2 mV, $n = 3$), whereas evoked excitatory postsynaptic currents (EPSCs) reversed at $V_{exc} = 10.5 \pm 3$ mV ($n = 3$). All experiments were performed at room temperature (22°C).

Surgery

Rats were anesthetized with isoflurane, and body temperature and respiration were maintained at physiological levels. The surgical site was shaved and sterilized, and subjects were positioned in a stereotaxic (David Kopf Instruments, Tujunga, California) instrument so that the frontal and parietal bones of the skull were parallel to the surgical platform. Rats received stereotaxic implantation with bilateral guide cannulae in the lateral ventricles under aseptic conditions, as follows. Skull screw anchors were fixed to the skull, and cannulae (23-gauge stainless steel) were implanted (coordinates: from bregma P-1.0 mm, L-1.3 mm; from cortical surface −3.5 mm) and secured in place with dental acrylic. A dummy cannula was inserted in the guide cannulae to prevent occlusion and infection. Rats received intraperitoneal (IP) injection with aspirin (150.0 mg/kg) after surgery and then again 7–8 hours later to aid with postoperative discomfort and were allowed 72 hours to recover. Accurate placement of the cannulae was confirmed by allowing 2 μL of sterile saline to flow via gravity into the lateral ventricles. If cannulae placement could not be confirmed, the animal was excluded from the study. All procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Animal Studies

LPS Injections. On test day, rats were injected (CMA/100 microinjector, 10 μL Hamilton syringe) intracerebro-ventricularly (ICV) with sterile saline containing .1% bovine serum albumin (BSA) (vehicle) or 100 ng sgp130Fc dissolved in 2 μL vehicle. One hour later, rats received IP injection, with sterile saline (.3 mL) or a dose of LPS (.33 mg/kg body weight) eliciting a pro-inflammatory cytokine response in the brain (34) and were decapitated by a guillotine 8 hours after treatment for obtaining brain slices (29).

Footshock. Rats were placed in an FS chamber (30 cm × 10 cm). A 1.6-mA FS lasting 5 sec was administered every 4 min for a total session duration of 64 min. Immediately after treatment, rats were sacrificed, and brains slices were obtained. One hour before being subjected to the FS procedure, animals received injection with the microinjector through the cannulae with either .1% BSA (vehicle) or 100 ng sgp130Fc dissolved in 2 μL vehicle.

Statistical Analysis

Statistical methods are described in the online Analysis section in Supplement 1. Data comparisons were reported as different only if $p < .05$. Single, double, and triple asterisks (*, **, and ***) indicate $p < .05$, $p < .02$, or $p < .01$, respectively, unless otherwise indicated.

Results

IL-6 Selectively Decreases GABAergic Postsynaptic Currents

IL-6 application (10 ng/mL or 45 nmol/L) did not change the mean evoked excitatory postsynaptic currents (eEPSC) amplitude (time course in Figure 1A) (Figure 1B: mean eEPSC amplitude in control or after IL-6). Neither paired pulse ratio (PPR) (Figure 1C) nor

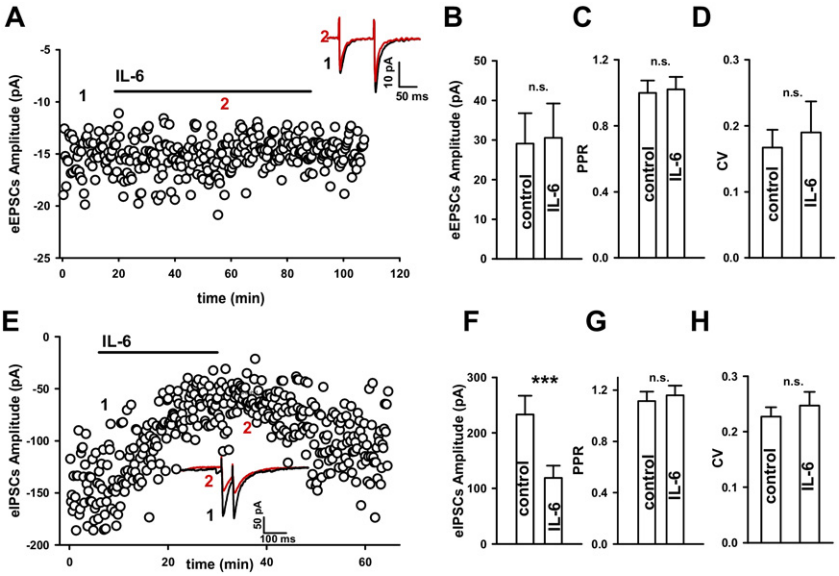

Figure 1. Interleukin (IL)-6 modulation of synaptic activity. **(A)** Representative example of temporal course of the effect of IL-6 (10 ng/mL) on the amplitude of excitatory postsynaptic currents (EPSCs) recorded from cortical layer II/III neurons. The insert displays the average of 10 traces corresponding to the points indicated by the numbers. **(B)** The mean amplitudes of evoked excitatory postsynaptic currents (eEPSCs) do not differ before and after IL-6 application (p = n.s., n = 10). **(C)** The ratio between second and first response amplitude (paired pulse ratio [PPR] ≡ A_2/A_1) in a pair pulse protocol (interpulse interval = 100 msec) was not changed by application of IL-6 (p = n.s., same sample). **(D)** The EPSC coefficient of variation (CV) (calculated across 50 responses) is independent of the presence of IL-6 (p = n.s., same sample). **(E)** Representative example of temporal course of the effect of IL-6 on the amplitude of inhibitory postsynaptic currents (IPSCs) (13.7 ± 1.4 min from the start of IL-6 application to 50% maximal effect, n = 20). **(F)** The mean amplitudes of evoked inhibitory postsynaptic currents (eIPSCs) are significantly smaller after IL-6 application (***$p < .0001$, n = 20). **(G)** The PPR is still unchanged after the application of IL-6 (p = n.s., same sample). **(H)** The IPSC CV is independent of IL-6 (nonsignificantly different [n.s.]).

the coefficient of variation (CV) (Figure 1D) changed after application of IL-6, suggesting that on average excitatory synaptic transmission is not affected by IL-6. On the contrary, bath-application of IL-6 reliably resulted in the decrease of approximately 50% in the amplitude of gabazine-sensitive evoked inhibitory postsynaptic currents (eIPSC) (example of time course in Figure 1E, average in Figure 1F). The change in eIPSC amplitude was not accompanied by changes in mean PPR (Figure 1G) or CV (Figure 1H), suggesting a postsynaptic action of IL-6.

IL-6 Shifts the Balance Between Inhibition and Excitation

To test the single-cell balance between inhibitory and excitatory synaptic input we used an intracellular solution allowing separate measurement of EPSCs and IPSCs within a single-cell recording (see Materials and Methods). We determined the resting potential for inhibitory synaptic currents in the presence of DNQX and kynurenic acid (Figure 2A; insert shows a sample of traces at increasing holding potentials), as the intersection of the I-V curve with the voltage axis ($V_{inh} = -64 \pm 2$ mV, $n = 3$). The value obtained experimentally was close to the theoretical reversal potential for Cl^- ($E_{Cl}^- = -65$ mV). Synaptic currents recorded at $V_h = +10$ mV were completely blocked by application of gabazine, demonstrating their GABAergic nature (example of time course in Figure 2B). The inward synaptic currents obtained at a holding potential (V_h) of −65 mV were completely blocked by DNQX (example of time course in Figure 2B), confirming that they were mediated by $GABA_A$Rs. Similarly, we evaluated an I-V relationship in the presence of gabazine (Figure 2C), which displayed the profile of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated glutamatergic currents with an inward rectification probably due to residual polyamine internal block (35).

We determined the excitatory and inhibitory components of the synaptic responses in control as well as after bath-application of IL-6 within the same cell, by identifying a stimulation site and intensity that produced a solid signal at $V_h = -65$ mV as well as at $V_h = +10$ mV, to activate both GABAergic and glutamatergic axons (example traces and time course in Figure 2E and 2F, respectively). This experiment confirmed that IL-6 decreases the cellular synaptic inhibition/excitation ratio (SIER) (same sample, Figure 2G) without changing eEPSC amplitude (n = 9, Figure 2H) while decreasing eIPSC amplitude (same sample, Figure 2I).

IL-6 Decreases Muscimol-Evoked Currents

The stability of PPR and CV in the GABAergic response is suggestive of a postsynaptic effect of IL-6. To determine a postsynaptic component of the modulation more directly, we tested the effect of bath-application of IL-6 on the response to the pressure-applied $GABA_A$R agonist muscimol (100 μmol/L). Brief (3–12 msec) applications of muscimol every 90 sec produced gabazine-sensitive inward currents that greatly outlasted the duration of the muscimol applications themselves (duration of the responses was approximately 10 sec; see insert in Figure 3A). As shown in the example in the time course of Figure 3A, application of IL-6 decreased the maximum amplitude of muscimol-induced responses to an extent similar to the IL-6-induced reduction in SIER (mean IL-6-induced reduction in Figure 3B, n = 9), confirming that IL-6 affected $GABA_A$R-mediated responses mainly or completely by a postsynaptic mechanism.

Pharmacologic Properties of the IL-6-Induced Decrease of GABAergic Responses

We considered the possibility that IL-6 decreased $GABA_A$R-mediated signal by modifying the equilibrium between membrane expression and internalization of $GABA_A$Rs, similar to the action of other modulators (36). To test this hypothesis we used a series of pharmacological agents known to interfere with receptor trafficking and/or internalization.

The process of receptor internalization requires the presence of functional PI_3K/protein kinase B (AKT) (37). Slice pre-incubation during 2 hours or more with the PI_3K/AKT blocker wortmannin (200 nmol/L) completely prevented the IL-6-induced decrease in eIPSC amplitude (representative time course and traces in Figure 4A, left and center, respectively), suggesting that the process is PI_3K-dependent. The normalized effect of IL-6 after incubation in wortmannin is compared with vehicle (Figure 4A, right panel, n = 8, p = nonsignificant [ns], Student t test).

A similar effect was obtained by adding to the pipette solution the ADP-ribosylation factor-protein inhibitor Brefeldin A (38), which blocks small intracellular guanosine triphosphate (GTP)ases, affecting the displacement of endosomes from the cytosol to the membrane and vice versa. Representative time course and traces illustrating the absence of the IL-6 effect with Brefeldin A in the intracellular solution are shown in Figure 4B, left and center, respectively. The normalized effect of Brefeldin A is compared with the

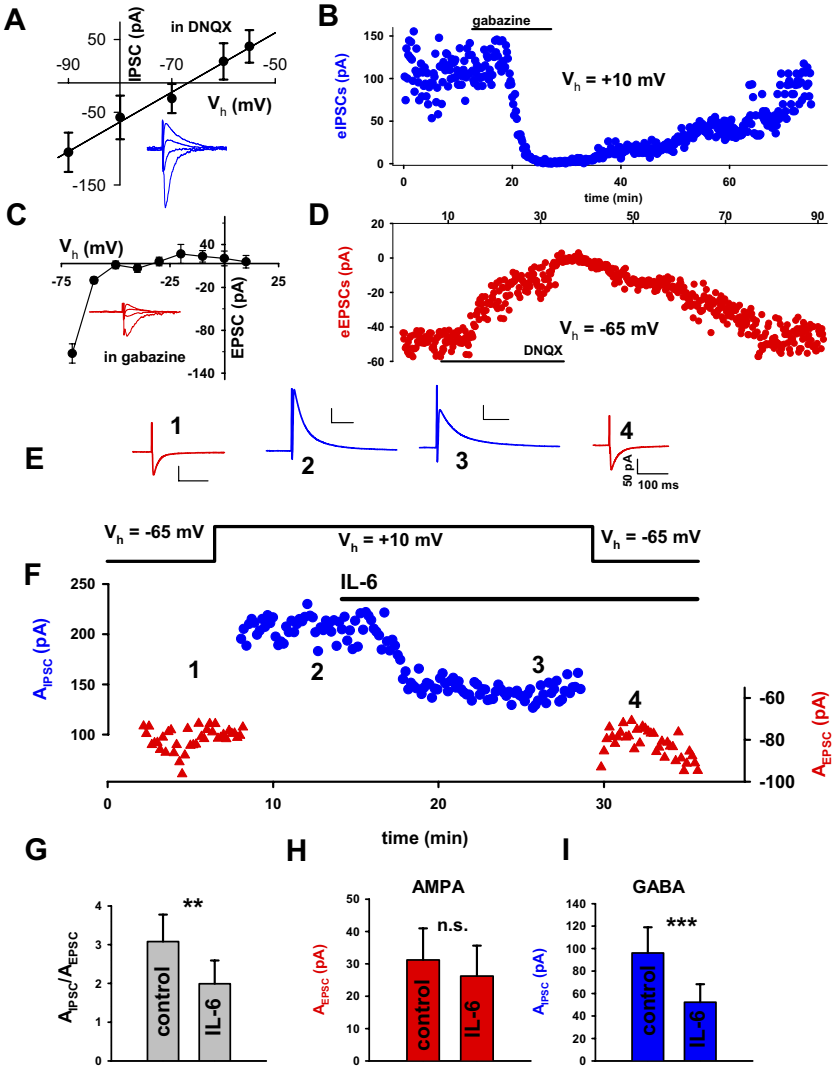

Figure 2. Interleukin-6 decreases synaptic balance between inhibition and excitation. **(A)** Synaptic current-voltage (I-V) relationship obtained mediating 10 eIPSCs in the presence of the glutamate receptor blockers 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (10 μmol/L) and kynurenate (KYN) (2 mmol/L). The reversal potential for γ-aminobutyric acid-A receptor ($GABA_AR$)-mediated currents ($V_{GABA} = -64 \pm 2$ mV, $n = 3$) is close to the Cl- Nernst potential calculated theoretically ($V_{Cl-} = -65$ mV). The insert displays the inversion of inhibitory synaptic currents. **(B)** Effect of SR-95531 (gabazine) application on the amplitude of the signal recorded at $V_h = +10$ mV, confirming the GABAergic nature of the synaptic response. **(C)** The I-V relationship in the presence of the $GABA_AR$ and *N*-methyl-D-aspartate receptor ($NMDA_R$) blockers gabazine and kynurenic acid. Synaptic currents display rectification probably due to the presence of unchelated polyamines ($n = 3$). The insert displays some of the traces. **(D)** The DNQX blocks synaptic currents at $V_h = -65$ mV, confirming the glutamatergic nature of the synaptic response. **(E)** Representative traces (above) and sketch of the holding potential V_h corresponding to the experiment in **(F)**. Scale bars are calibrated as in the rightmost trace. **(F)** After identification of the proper stimulation site and intensity, producing a reliable eEPSC and eIPSC, a baseline of eEPSC was recorded at $V_h = -65$ mV for 15 or more min (red triangles on the left). After depolarization to $V_h = +10$ mV, a baseline of synaptic responses (eIPSCs, blue circles) was recorded before application of IL-6 (10 ng/mL), which lasted for the whole remainder of the experiment. After stabilization of the eIPSC, amplitude was shifted back to $V_h = -65$ mV to monitor possible changes in eEPSC (red triangles on the right). Numbers 1–4 in **(E)** correspond to traces in 1–4 in **(F)**. Traces were obtained by averaging 10 consecutive events. **(G)** The IL-6 decreases the ratio between inhibitory and excitatory synaptic amplitudes (synaptic inhibition/excitation ratio ≡ A_{IPSC}/A_{EPSC}) without changing glutamatergic responses (**$p < .02$) **(H)** but only affecting inhibition **(I)** ($n = 7$; ***$p < .002$). AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; other abbreviations as in Figure 1.

respective data with control intracellular solution in the rightmost panel of Figure 4B ($n = 11$, $p =$ ns, Student *t* test).

Palmitoylation is a reversible covalent modification necessary to anchor and modify membrane protein distribution to the plasma membrane, which is blocked by the competitive blocker of palmitoyl acyl transferase 2-Br-hexadecanoic acid by substituting palmitate on the catalytic site of palmitoylating enzymes (39). The presence of 2-Br-hexadecanoic acid in the pipette solution prevented, on average, the IL-6-induced decrease in eIPSC amplitude (representative time course and traces in Figure 4C, left and center, respectively). The normalized effect of 2-Br-hexadecanoic acid is compared with the respective data with control intracellular solution in the rightmost panel of Figure 4C.

Dynamin inhibitory peptide specifically blocks the dynamin-dependent internalization of $GABA_AR$s after activation of insulin-like and other receptors (33). The presence of P4 in the pipette solution completely blocked the IL-6 depression of the eIPSC amplitude (representative time course and traces in Figure 4D, left and center, respectively). The normalized effect of P4 is compared with the respective data with control intracellular solution in the rightmost panel of Figure 4D.

Together, these results strongly suggest that IL-6 is reducing the amplitude of $GABA_AR$-mediated currents by altering the density of $GABA_AR$s in the plasma membrane.

Effect of Stress on the Synaptic Inhibition/Excitation Ratio

Although the results of our in vitro experiments demonstrate the effect of exogenously applied IL-6 on the SIER, it is yet not clear whether endogenous IL-6 released by actual stressors have the same effect. We tested this hypothesis by using two types of stressors known to increase endogenous levels of IL-6: single-shot systemic administration of LPS (31), and electrical FS (30,40).

LPS Decreases SIER in an IL-6-Dependent Manner. Lipopolysaccharide 8 hours before the preparation of the brain slices significantly reduced SIER in patch-clamp recordings performed up to 3.5 hours after animal sacrifice but not at a later time ($n = 13$ at <3.5 hours, $p < .05$; $n = 7$ at >3.5 hours, ns) (Figure 5A, bars 1 and 2, respectively), compared with control subjects. To establish the IL-6 dependence of the LPS-induced decrease in SIER we repeated similar experiments but accessing the lateral ventricles with cannulae. The IP injection of vehicle and ICV BSA did not change SIER ($n = 8$) (Figure 5A, $p =$ ns; bar 3). The IP injection of LPS and ICV BSA produced the same SIER as LPS injections without any ICV treatment (Figure 5A, bar 4, $n = 7$, $p < .05$). The IP injection of LPS 1 hour after ICV injection of 100 ng of the IL-6 trans-signaling pathway blocker sgp130Fc did not produce the decrease in SIER observed in the absence of sgp130Fc (Figure 5A, bar 5; $n = 13$, $p =$ ns), showing that LPS decreased SIER in an IL-6-trans-signaling-dependent manner.

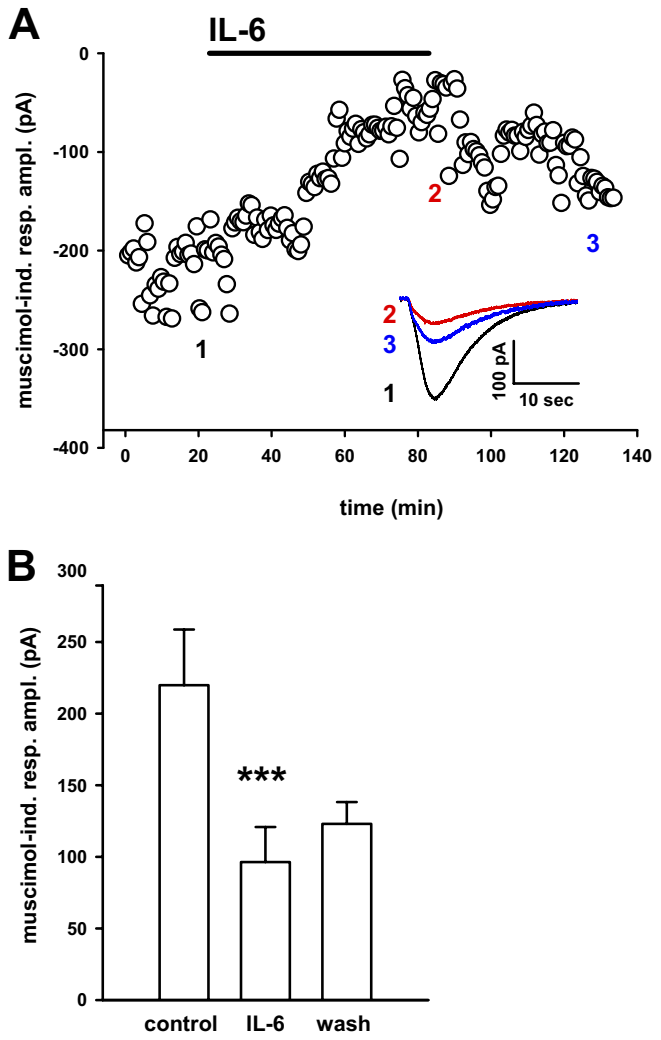

Figure 3. Interleukin-6 decreases muscimol-evoked IPSCs (cIPSCs). **(A)** Temporal course of points of the effect of IL-6 (10 ng/mL) on cIPSC amplitude. The insert shows the signal induced by 10-msec-long delivery of the $GABA_A$ agonist muscimol (100 μmol/L), applied every 90 sec at approximately 100 μmol/L from the recording area produced. **(B)** A summary chart showing that IL-6 significantly decreases cIPSC amplitude (***$p < .001$, $n = 9$). ind. resp. ampl., induced response amplitude; other abbreviations as in Figure 1.

FS Decreases SIER in an IL-6-Dependent Manner. Footshock is known to increase the levels of both peripheral and central IL-6 (30, 40). We stressed experimental animals with electrical FS (1.5 mA, 5 sec every 4 min) during 64 min (see Material and Methods). The FS treatment decreased SIER ($n = 13$, $p < .05$) (Figure 5B, bar 1). In these experiments, previous ICV injection of BSA alone did not change the FS-induced SIER depression ($n = 7$, $p < .05$; Figure 5B, bar 2), whereas ICV administration of BSA with sgp130Fc produced an SIER similar to baseline ($n = 12$, $p =$ ns) (Figure 5B, bar 3). Results are shown together with SIER in control and after IL-6 in the in vitro experiment for comparison. These results suggest that the SIER is subject to acute and reversible modulation by stressors through an increase in IL-6.

Discussion

Our results demonstrate for the first time that acute administration of the pro-inflammatory cytokine IL-6 selectively decreases GABAergic inhibition without changing glutamatergic excitation in the temporal cortex of the rat. As a result, the balance between synaptic inhibitory and excitatory input to cortical neurons shifted toward excitation. In general, cytokines might affect pre- and/or postsynaptic function. In our experiments, the failure of IL-6 to change eIPSC PPR or the CV suggests a postsynaptic nature of the effect, which was further corroborated by the decrease in the response to postsynaptic application of the $GABA_A$R agonist muscimol after IL-6 application.

Several earlier studies suggest that pro-inflammatory cytokines can alter synaptic function in different brain regions in a highly variable and area-dependent manner. For example, somnogenic and motor-depressant effects of IL-1β have been ascribed to an augmentation of $GABA_A$R-mediated muscimol-evoked signal in cortical cultures (41), whereas TNF-α alters excitatory and inhibitory synaptic responses in the visual system and in the hippocampus (42,43), shifting the balance between excitation and inhibition in favor of the former. A similar contribution of IL-6 to synaptic excitability is present in the spinal cord, where IL-6 increases postsynaptic currents induced by applications of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or *N*-methyl-D-aspartate and decreases spontaneous IPSC frequency as well as currents induced by GABA and glycine (44). A postsynaptic IL-6-induced decrease in GABAergic signal as we found in the temporal cortex might similarly shift the balance of excitation/inhibition in favor of the former, further enhancing the presynaptic downregulation of parvalbumin-positive GABAergic neurons caused by nicotinamide adenosine dinucleotide phosphate-oxidase activation, as reported in numerous previous studies (45–47).

Mechanisms of Action of IL-6

Two mechanisms could account for changes in postsynaptic inhibition: direct biophysical modulation of $GABA_A$R function, or $GABA_A$R altered trafficking or internalization. $GABA_A$Rs are known to undergo internalization after binding of clathrin-mediated adaptor protein (AP2) to specific binding motifs of the β_{1-3} or γ_2 $GABA_A$R subunits (33,36,48). Our pharmacological data suggest that IL-6 impairs the translocation of $GABA_A$Rs between plasma membrane and cytosolic compartment in either direction. The Phosphatidyl inositol 3 kinase/AKT is involved in the regulation of synaptic function (37,49) and is ubiquitously involved in the regulation of membrane internalization in many different preparations (50). Phosphatidyl inositol 3 kinase/AKT is also required for $GABA_A$R internalization in the amygdala and in the hippocampus (51). In our experiments, the IL-6-induced decrease of IPSC amplitude was blocked by the PI_3K/AKT inhibitor wortmannin, indicating the involvement of PI_3K/AKT in the IL-6-synaptic shift favoring excitation, resembling the effect of TNF-α—which decreases SIER by inhibiting the expression of $GABA_A$Rs while increasing the expression of glutamate receptor-1 subunits (43).

Brefeldin A impairs intracellular vesicle trafficking (52,53) as well as membrane protein translocation/internalization (53–55) by inhibiting small GTP-ases like ADP-ribosylation factor- or RAB-related proteins, responsible for or otherwise involved in endocytosis (38,56,57). Specifically, Brefeldin A induces $GABA_A$R channel redistribution by inhibiting Brefeldin-A-GDP/GTP exchange factor 2 (BIG2, a postsynaptic mouse protein bound to β_2- and β_3-$GABA_A$R subunits, with 95% homology with its human homolog) and has a critical role in vesicular trafficking of $\beta_{2,3}$-containing $GABA_A$R subunits from/to endoplasmic reticulum and Golgi apparatus (58–61). The presence of Brefeldin A in the recording pipette prevented the IL-6-induced decrease in $GABA_A$R-mediated current. Our results are

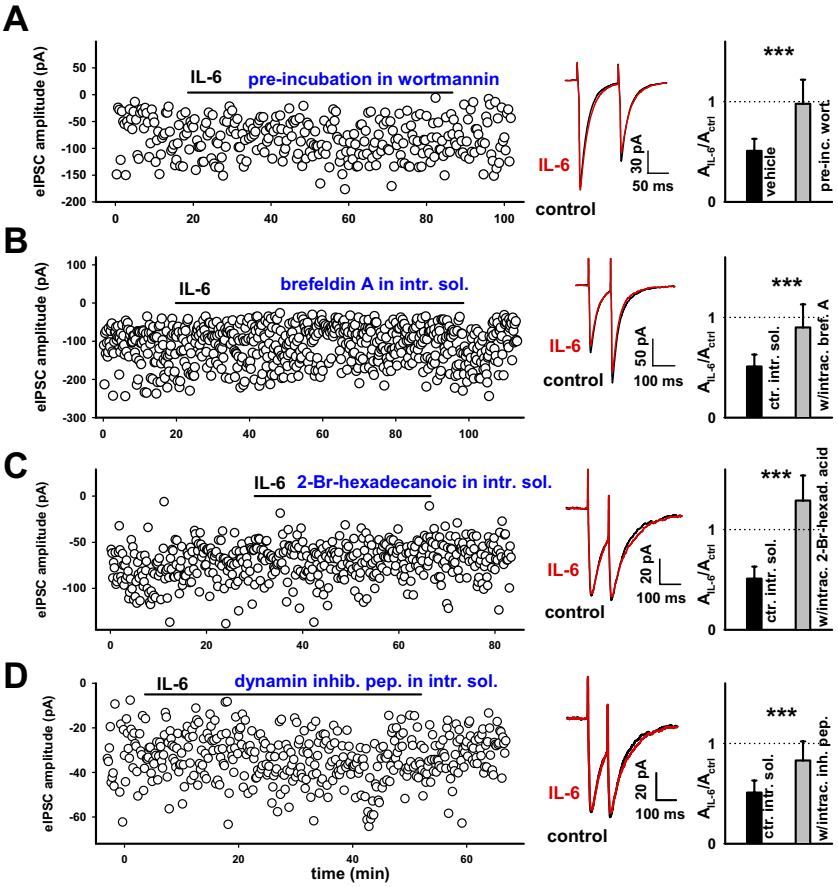

Figure 4. Pharmacology of the IL-6-induced eIPSC modulation. **(A)** The panel on the left displays a representative time course of eIPSCs after slice pre-incubation (>2 hours) with wortmannin (400 nmol/L). Wortmannin prevented the IL-6 induced-decrease in eIPSC ($n = 8$, p = n.s., paired Student t test between eIPSC amplitude before or after IL-6 application), indicating the involvement of phosphatidyl inositol 3 kinase (PI3K)/protein kinase B (AKT). Middle panel in **(A)** shows the corresponding traces in control or after bath-application of IL-6. The rightmost graph compares the normalized effect of IL-6 on eIPSC ($A_{IL\text{-}6}/A_{control}$) in the absence (left bar) or in the presence of wortmannin in the incubation solution. (**B–D)** As in the preceding, leftmost, middle, and rightmost panels illustrate the action of drugs dissolved in the pipette solution on a representative time course (left), corresponding traces (middle), and comparison with the effect of IL-6 in control (right). (**B)** Brefeldin A (400 nmol/L) completely prevents the IL-6-induced depression of eIPSC amplitude ($n = 11$). (**C)** 2-Br-hexadecanoic acid (2-Br-hexad. acid; 20 μmol/L) also blocks the effect of IL-6 on eIPSC amplitude ($n = 8$). (**D)** The presence of the dynamin inhibitor peptide (dynamin inhib. pep.; 20 μmol/L) in the intrapipette solution, similar to the previous drugs, blocks the IL-6-induced eIPSC decrease ($n = 8$). ***Significance level of the statistics comparing eIPSC normalized amplitudes in the presence or in the absence of the drugs (all $p < .01$, unpaired t Student test, $n = 28$, $n = 31$, $n = 28$, and $n = 28$ for **A**, **B**, **C**, and **D**, respectively). ctr. intr. sol., control intracellular solution; other abbreviations as in Figure 1.

qualitatively and quantitatively similar to those obtained in HEK-293T cells (44% decrease in the density of $GABA_AR$ β subunits) (62).

Trafficking and internalization of membrane receptors depends on protein palmitoylation (63). It is known, for instance, that the internalization of the γ_2 subunit of the $GABA_AR$ is palmitoylation-dependent (39,64,65). We found that intracellular postsynaptic block of palmitoylation inhibits the IL-6-induced decrease of the IPSCs amplitude, suggesting that the effect depends on a functional anchoring processing of $GABA_ARs$ to a lipid bilayer.

The trafficking of membrane proteins including ionotropic receptors from and to the intracellular compartments depends on the accessibility of specific intracellular moieties to molecular motors including the GTP-ase dynamin. The peptide P4 selectively targets dynamin, blocking vesicular displacement from the membrane to the cytosolic compartment (66). $GABA_AR$ trafficking is specifically impaired in the presence of P4 in both heterologous systems (33,67) as well as in acute slice preparations (68). In our study, P4 in the recording electrode abolished the IL-6-induced depression of eIPSC, suggesting that IL-6 decreases the GABAergic signal through a dynamin-dependent process. Altogether, our results suggest that IL-6 decreases the availability of $GABA_ARs$ to the plasma membrane by altering the cycle of insertion/internalization of $GABA_ARs$.

Trafficking and internalization of $GABA_ARs$ is a highly regulated process (61,69) whose short- and long-term dysregulation is a potential cause of numerous pathologic conditions like epilepsy, particularly in the temporal lobe (26,70,71). Brain infiltration by leukocytes that produce pro-inflammatory cytokines as well as alterations in immune cell transcription were identified as contributors to the pathophysiology of temporal lobe epilepsy (72). Numerous other studies also link the increase in IL-6 with epilepsy and seizures as cause or precipitating factor (73,74). The temporal cortex might be exquisitely sensitive to stress, possibly resulting in dysfunctional sensory and limbic circuits, an important factor in the etiology of schizophrenic psychosis (75,76).

The IL-6-dependent decrease in SIER after systemic LPS or FS delivery reinforces the hypothesis of a critical role played by IL-6 in the stress-induced control of the balance between excitation and inhibition. Our finding of a connection between immune challenge and synaptic inhibition parallels the results of a study showing that perinatal LPS injections increase the levels of pro-inflammatory cytokines, impairing dopaminergic modulation of cortical interneurons in the schizophrenia model of ventral hippocampus lesion (77). Interestingly, a hypothesis on the immune origin of the diseases of the autistic spectrum, which is often comorbid with hyperactivity and epilepsy, is gaining increasing support (78,79). An extrapolation of our results to humans would suggest that prolonged stress could give rise to abnormal plasticity and development of the temporal cortex in genetically vulnerable individuals, leading in turn to a hyperexcitable neocortex, as hypothesized in autism spectrum disorder (6,80).

We speculate that an enhanced excitability caused by a decrease in $GABA_AR$ density might be an adaptive response to temporary stressors, whereas the prolonged presence of stressors might result in unpredictable long-term plasticity and a potentially detrimental maladaptive response. An imbalance between synaptic inhibition and excitation caused by increased synthesis, release, and/or sensitivity to IL-6 might be a shared factor in the etiology of a large and heterogeneous group of neurological and psychiatric conditions associated with behavioral and central hyperexcitability. Our finding supplies an additional contribution to understand-

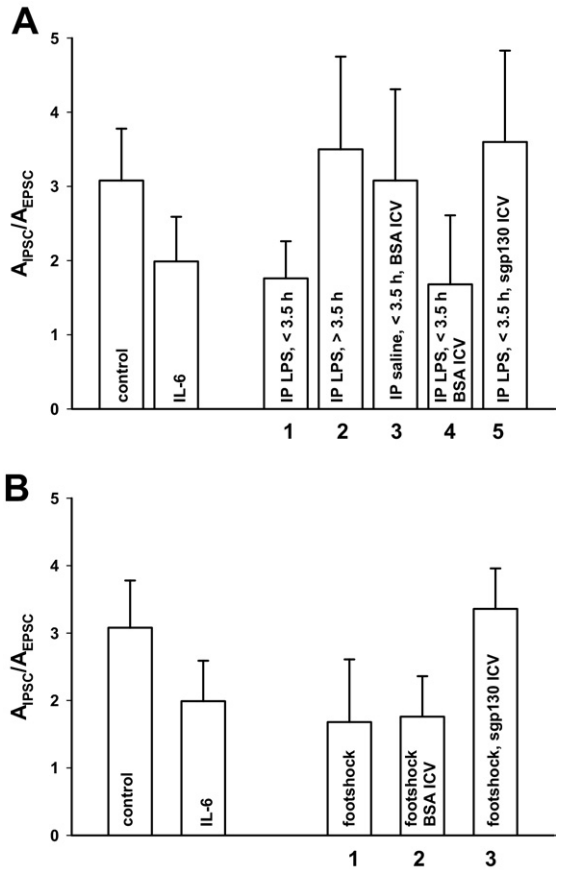

Figure 5. Effect of stress on synaptic inhibition/excitation ratio (SIER). **(A)** Bar graph displaying SIER after IP injections of lipopolysaccharide (LPS) or saline: the first two bars, shown for comparison, represent SIER in control and after bath-application of IL-6. Bars 1 and 2 represent the effect of IP injection of LPS <3.5 hours after injection or >3.5 hours after the injection, respectively. Bar 3 shows the effect of IP saline and bovine serum albumin (BSA) intracerebro-ventricularly (ICV) (<3.5 hours after injection). Bar 4 shows the effect of IP LPS and BSA ICV (<3.5 hours). Bar 5 represents the effect of IP LPS and soluble sgp130Fc ICV (<3.5 hours). (**B**) First two bars, as in preceding. Bar 1 represents the effect of FS on SIER. Bar 2 and 3 represent the effect of FS preceded by BSA or soluble sgp130Fc ICV, respectively. Abbreviations as in Figure 1.

ing why drugs that restore the balance between inhibition and excitation, like $GABA_AR$ enhancers such as benzodiazepines and barbiturates and/or antiglutamatergics such as lamotrigine (81), are so effective in the early treatment of patients suffering from stress-related conditions—who often are prescribed condition-specific long-term treatments only at a later stage.

The study has been funded with the University of Texas at Dallas Behavioral and Brain Sciences research funds 2010 (MA). We would like to thank Drs. J. Burr and R. Draper, of the Department of Biology of the School of Natural Sciences, and Mrs. A. Banerjee and Dr. L. Dinh from the School of Behavioral and Brain Sciences at the University of Texas at Dallas for insightful discussions and critical review of the manuscript. Dr. Rose-John is funded by grants from the Deutsche Forschungsgemeinschaft, Bonn, Germany (SFB654, project C5; SFB841, project C1; SFB877, project A1) and by the Cluster of Excellence "Inflammation at Interfaces."

Dr. Rose-John is an inventor on the patent describing the function of sgp130Fc. He is also a shareholder of the CONARIS Research Institute (Kiel, Germany), which is commercially developing sgp130Fc as a therapy for inflammatory diseases. All other authors reported no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

1. Chambers RA, Bremner JD, Moghaddam B, Southwick SM, Charney DS, Krystal JH (1999): Glutamate and post-traumatic stress disorder: Toward a psychobiology of dissociation. *Semin Clin Neuropsychiatry* 4:274–281.
2. Paz RD, Tardito S, Atzori M, Tseng KY (2008): Glutamatergic dysfunction in schizophrenia: From basic neuroscience to clinical psychopharmacology. *Eur Neuropsychopharmacol* 18:773–786.
3. Rossi S, De Capua A, Tavanti M, Calossi S, Polizzotto NR, Mantovani A, *et al.* (2009): Dysfunctions of cortical excitability in drug-naive posttraumatic stress disorder patients. *Biol Psychiatry* 66:54–61.
4. Rosenkranz JA, Venheim ER, Padival M (2010): Chronic stress causes amygdala hyperexcitability in rodents. *Biol Psychiatry* 67:1128–1136.
5. Gomes E, Pedroso FS, Wagner MB (2008): Auditory hypersensitivity in the autistic spectrum disorder. *Pro Fono* 20:279–284.
6. Markram H, Rinaldi T, Markram K (2007): The intense world syndrome—an alternative hypothesis for autism. *Front Neurosci* 1:77–96.
7. Oblak A, Gibbs TT, Blatt GJ (2009): Decreased GABAA receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. *Autism Res* 2:205–219.
8. Garcia-Cairasco N (2009): Puzzling challenges in contemporary neuroscience: Insights from complexity and emergence in epileptogenic circuits. *Epilepsy Behav* 14 Suppl 1:54–63.
9. Nowak M, Bauer S, Haag A, Cepok S, Todorova-Rudolph A, Tackenberg B, *et al.* (2011): Interictal alterations of cytokines and leukocytes in patients with active epilepsy. *Brain Behav Immun* 25:423–428.
10. Al-Mana D, Ceranic B, Djahanbakhch O, Luxon LM (2008): Hormones and the auditory system: A review of physiology and pathophysiology. *Neuroscience* 153:881–900.
11. Skilbeck KJ, Johnston GA, Hinton T (2010): Stress and GABA receptors. *J Neurochem* 112:1115–1130.
12. Lange T, Dimitrov S, Born J (2010): Effects of sleep and circadian rhythm on the human immune system. *Ann N Y Acad Sci* 1193:48–59.
13. Gaab J, Rohleder N, Heitz V, Engert V, Schad T, Schurmeyer TH, *et al.* (2005): Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. *Psychoneuroendocrinology* 30:188–198.
14. Lambert C, Ase AR, Seguela P, Antel JP (2010): Distinct migratory and cytokine responses of human microglia and macrophages to ATP. *Brain Behav Immun* 24:1241–1248.
15. Hanff TC, Furst SJ, Minor TR (2010): Biochemical and anatomical substrates of depression and sickness behavior. *Isr J Psychiatry Relat Sci* 47:64–71.
16. Song C, Wang H (2011): Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 35:760–768.
17. Boulanger LM (2009): Immune proteins in brain development and synaptic plasticity. *Neuron* 64:93–109.
18. Johnson EA, Kan RK (2010): The acute phase response and soman-induced status epilepticus: Temporal, regional and cellular changes in rat brain cytokine concentrations. *J Neuroinflammation* 7:40.
19. Gadient RA, Otten U (1994): Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. *Brain Res* 637:10–14.
20. Huang CJ, Stewart JK, Franco RL, Evans RK, Lee ZP, Cruz TD, *et al.* (2011): LPS-stimulated tumor necrosis factor-alpha and interleukin-6 mRNA and cytokine responses following acute psychological stress. *Psychoneuroendocrinology* 36:1553–1561.
21. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH (2010): Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* 35: 02617–02623.
22. Jankord R, Zhang R, Flak JN, Solomon MB, Albertz J, Herman JP (2010): Stress activation of IL-6 neurons in the hypothalamus. *Am J Physiol Regul Integr Comp Physiol* 299:R343–R351.
23. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S (2011): The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 1813:878–888.

24. Jones SA, Scheller J, Rose-John S (2011): Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J Clin Invest* 121:3375–3383.
25. Garbers C, Thaiss W, Jones GW, Waetzig GH, Lorenzen I, Guilhot F, *et al.* (2011): Inhibition of classic signaling is a novel function of soluble GP130 which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor. *J Biol Chem* 286:42959–42970.
26. Naylor DE, Liu H, Wasterlain CG (2005): Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci* 25:7724–7733.
27. Weber C, Arck P, Mazurek B, Klapp BF (2002): Impact of a relaxation training on psychometric and immunologic parameters in tinnitus sufferers. *J Psychosom Res* 52:29–33.
28. Umeoka EH, Garcia SB, Antunes-Rodrigues J, Elias LL, Garcia-Cairasco N (2011): Functional characterization of the hypothalamic-pituitary-adrenal axis of the Wistar Audiogenic Rat (WAR) strain. *Brain Res* 1381:141–147.
29. Atzori M, Lei S, Evans DI, Kanold PO, Phillips-Tansey E, McIntyre O, *et al.* (2001): Differential synaptic processing separates stationary from transient inputs to the auditory cortex. *NatNeurosci* 4:1230–1237.
30. Girotti M, Donegan JJ, Morilak DA (2011): Chronic intermittent cold stress sensitizes neuro-immune reactivity in the rat brain. *Psychoneuroendocrinology* 36:1164–1174.
31. Burton MD, Sparkman NL, Johnson RW (2011): Inhibition of interleukin-6 trans-signaling in the brain facilitates recovery from lipopolysaccharide-induced sickness behavior. *J Neuroinflammation* 8:54.
32. Jostock T, Mullberg J, Ozbek S, Atreya R, Blinn G, Voltz N, *et al.* (2001): Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur J Biochem* 268:160–167.
33. Kittler JT, Chen G, Honing S, Bogdanov Y, McAinsh K, Arancibia-Carcamo IL, *et al.* (2005): Phospho-dependent binding of the clathrin AP2 adaptor complex to GABAA receptors regulates the efficacy of inhibitory synaptic transmission. *Proc Natl Acad Sci U S A* 102:14871–14876.
34. Berg BM, Godbout JP, Kelley KW, Johnson RW (2004): Alpha-tocopherol attenuates lipopolysaccharide-induced sickness behavior in mice. *Brain Behav Immun* 18:149–157.
35. Bowie D, Mayer ML (1995): Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. *Neuron* 15:453–462.
36. Kittler JT, Moss SJ (2003): Modulation of GABAA receptor activity by phosphorylation and receptor trafficking: Implications for the efficacy of synaptic inhibition. *Curr Opin Neurobiol* 13:341–347.
37. Man HY, Wang Q, Lu WY, Ju W, Ahmadian G, Liu L, *et al.* (2003): Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. *Neuron* 38:611–624.
38. Donaldson JG, Radhakrishna H, Peters PJ (1995): The ARF GTPases: Defining roles in membrane traffic and organelle structure. *Cold Spring Harb Symp Quant Biol* 60:229–234.
39. Rathenberg J, Kittler JT, Moss SJ (2004): Palmitoylation regulates the clustering and cell surface stability of GABAA receptors. *Mol Cell Neurosci* 26:251–257.
40. Cao L, Hudson CA, Moynihan JA (2007): Chronic foot shock induces hyperactive behaviors and accompanying pro- and anti-inflammatory responses in mice. *J Neuroimmunol* 186:63–74.
41. Miller LG, Galpern WR, Dunlap K, Dinarello CA, Turner TJ (1991): Interleukin-1 augments gamma-aminobutyric acidA receptor function in brain. *Mol Pharmacol* 39:105–108.
42. Lee RH, Mills EA, Schwartz N, Bell MR, Deeg KE, Ruthazer ES, *et al.* (2010): Neurodevelopmental effects of chronic exposure to elevated levels of pro-inflammatory cytokines in a developing visual system. *Neural Dev* 5:2.
43. Stellwagen D, Beattie EC, Seo JY, Malenka RC (2005): Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. *J Neurosci* 25:3219–3228.
44. Kawasaki Y, Zhang L, Cheng JK, Ji RR (2008): Cytokine mechanisms of central sensitization: Distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. *J Neurosci* 28:5189–5194.
45. Behrens MM, Ali SS, Dugan LL (2008): Interleukin-6 mediates the increase in NADPH-oxidase in the ketamine model of schizophrenia. *J Neurosci* 28:13957–13966.
46. Behrens MM, Sejnowski TJ (2009): Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? *Neuropharmacology* 57:193–200.
47. Dugan LL, Ali SS, Shekhtman G, Roberts AJ, Lucero J, Quick KL, *et al.* (2009): IL-6 mediated degeneration of forebrain GABAergic interneurons and cognitive impairment in aged mice through activation of neuronal NADPH oxidase. *PLoS ONE* 4:e5518.
48. Kittler JT, Chen G, Kukhtina V, Vahedi-Faridi A, Gu Z, Tretter V, *et al.* (2008): Regulation of synaptic inhibition by phospho-dependent binding of the AP2 complex to a YECL motif in the GABAA receptor gamma2 subunit. *Proc Natl Acad Sci U S A* 105:3616–3621.
49. Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, *et al.* (2003): Control of synaptic strength, a novel function of Akt. *Neuron* 38:915–928.
50. Manning BD, Cantley LC (2007): AKT/PKB signaling: Navigating downstream. *Cell* 129:1261–1274.
51. Mou L, Heldt SA, Ressler KJ (2011): Rapid brain-derived neurotrophic factor-dependent sequestration of amygdala and hippocampal GABA(A) receptors via different tyrosine receptor kinase B-mediated phosphorylation pathways. *Neuroscience* 176:72–85.
52. Chardin P, McCormick F (1999): Brefeldin A: The advantage of being uncompetitive. *Cell* 97:153–155.
53. Damke H, Klumperman J, von Figura K, Braulke T (1991): Effects of brefeldin A on the endocytic route. Redistribution of mannose 6-phosphate/insulin-like growth factor II receptors to the cell surface. *J Biol Chem* 266:24829–24833.
54. Shome K, Xu XQ, Romero G (1995): Brefeldin A inhibits insulin-dependent receptor redistribution in HIRcB cells. *FEBS Lett* 357:109–114.
55. Wood SA, Park JE, Brown WJ (1991): Brefeldin A causes a microtubule-mediated fusion of the trans-Golgi network and early endosomes. *Cell* 67:591–600.
56. Qualmann B, Kessels MM (2002): Endocytosis and the cytoskeleton. *Int Rev Cytol* 220:93–144.
57. Radhakrishna H, Donaldson JG (1997): ADP-ribosylation factor 6 regulates a novel plasma membrane recycling pathway. *J Cell Biol* 139:49–61.
58. Gilbert SL, Zhang L, Forster ML, Anderson JR, Iwase T, Soliven B, *et al.* (2006): Trak1 mutation disrupts GABA(A) receptor homeostasis in hypertonic mice. *Nat Genet* 38:245–250.
59. Charych EI, Yu W, Miralles CP, Serwanski DR, Li X, Rubio M, *et al.* (2004): The brefeldin A-inhibited GDP/GTP exchange factor 2, a protein involved in vesicular trafficking, interacts with the beta subunits of the GABA receptors. *J Neurochem* 90:173–189.
60. Eshaq RS, Stahl LD, Stone R 2nd, Smith SS, Robinson LC, Leidenheimer NJ (2010): GABA acts as a ligand chaperone in the early secretory pathway to promote cell surface expression of GABAA receptors. *Brain Res* 1346:1–13.
61. Chen ZW, Olsen RW (2007): GABAA receptor associated proteins: A key factor regulating GABAA receptor function. *J Neurochem* 100:279–294.
62. Lo WY, Lagrange AH, Hernandez CC, Harrison R, Dell A, Haslam SM, *et al.* (2010): Glycosylation of {beta}2 subunits regulates GABAA receptor biogenesis and channel gating. *J Biol Chem* 285:31348–31361.
63. Huang K, El-Husseini A (2005): Modulation of neuronal protein trafficking and function by palmitoylation. *Curr Opin Neurobiol* 15:527–535.
64. Fang C, Deng L, Keller CA, Fukata M, Fukata Y, Chen G, *et al.* (2006): GODZ-mediated palmitoylation of GABA(A) receptors is required for normal assembly and function of GABAergic inhibitory synapses. *J Neurosci* 26:12758–12768.
65. Keller CA, Yuan X, Panzanelli P, Martin ML, Alldred M, Sassoe-Pognetto M, *et al.* (2004): The gamma2 subunit of GABA(A) receptors is a substrate for palmitoylation by GODZ. *J Neurosci* 24:5881–5891.
66. Robinson PJ, Sontag JM, Liu JP, Fykse EM, Slaughter C, McMahon H, *et al.* (1993): Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals. *Nature* 365:163–166.
67. Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, Moss SJ (2000): Constitutive endocytosis of GABAA receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *J Neurosci* 20:7972–7977.
68. Swant J, Stramiello M, Wagner JJ (2008): Postsynaptic dopamine D3 receptor modulation of evoked IPSCs via GABA(A) receptor endocytosis in rat hippocampus. *Hippocampus* 18:492–502.
69. Luscher B, Keller CA (2004): Regulation of GABAA receptor trafficking, channel activity, and functional plasticity of inhibitory synapses. *Pharmacol Ther* 102:195–221.

70. Goodkin HP, Sun C, Yeh JL, Mangan PS, Kapur J (2007): GABA(A) receptor internalization during seizures. *Epilepsia* 48 Suppl 5:109–113.
71. Bouilleret V, Loup F, Kiener T, Marescaux C, Fritschy JM (2000): Early loss of interneurons and delayed subunit-specific changes in GABA(A)-receptor expression in a mouse model of mesial temporal lobe epilepsy. *Hippocampus* 10:305–324.
72. Zattoni M, Mura ML, Deprez F, Schwendener RA, Engelhardt B, Frei K, *et al.* (2011): Brain infiltration of leukocytes contributes to the pathophysiology of temporal lobe epilepsy. *J Neurosci* 31:4037–4050.
73. Kalueff AV, Lehtimaki KA, Ylinen A, Honkaniemi J, Peltola J (2004): Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. *Neurosci Lett* 365:106–110.
74. Sallmann S, Juttler E, Prinz S, Petersen N, Knopf U, Weiser T, *et al.* (2000): Induction of interleukin-6 by depolarization of neurons. *J Neurosci* 20: 8637–8642.
75. Jalbrzikowski M, Bearden CE (2011): Clinical and genetic high-risk paradigms: Converging paths to psychosis meet in the temporal lobes. *Biol Psychiatry* 69:910–911.
76. Kates WR, Antshel KM, Faraone SV, Fremont WP, Higgins AM, Shprintzen RJ, *et al.* (2011): Neuroanatomic predictors to prodromal psychosis in velocardiofacial syndrome (22q11.2 deletion syndrome): A longitudinal study. *Biol Psychiatry* 69:945–952.
77. Feleder C, Tseng KY, Calhoon GG, O'Donnell P (2010): Neonatal intrahippocampal immune challenge alters dopamine modulation of prefrontal cortical interneurons in adult rats. *Biol Psychiatry* 67:386–392.
78. Patterson PH (2011): Maternal infection and immune involvement in autism. *Trends Mol Med* 17:389–394.
79. Buehler MR (2011): A proposed mechanism for autism: An aberrant neuroimmune response manifested as a psychiatric disorder. *Med Hypotheses* 76:863–870.
80. Rinaldi T, Silberberg G, Markram H (2008): Hyperconnectivity of local neocortical microcircuitry induced by prenatal exposure to valproic acid. *Cereb Cortex* 18:763–770.
81. Ketter TA, Post RM, Theodore WH (1999): Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders. *Neurology* 53:S53–S67.