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# Vagal nerve stimulation blocks interleukin 6-dependent synaptic hyperexcitability induced by lipopolysaccharide-induced acute stress in the rodent prefrontal cortex

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ABSTRACT

The ratio between synaptic inhibition and excitation (sI/E) is a critical factor in the pathophysiology of neuropsychiatric disease. We recently described a stress-induced interleukin-6 dependent mechanism leading to a decrease in sI/E in the rodent temporal cortex. The aim of the present study was to determine whether a similar mechanism takes place in the prefrontal cortex, and to elaborate strategies to prevent or attenuate it.

We used aseptic inflammation (single acute injections of lipopolysaccharide, LPS, 10 mg/kg) as stress model, and patch-clamp recording on a prefrontal cortical slice preparation from wild-type rat and mice, as well as from transgenic mice in which the inhibitor of IL-6 trans-signaling sgp130Fc was produced in a brain-specific fashion (sgp130Fc mice). The anti-inflammatory reflex was activated either by vagal nerve stimulation or peripheral administration of the nicotinic $\alpha_7$ receptor agonist PHA543613.

We found that the IL-6-dependent reduction in prefrontal cortex synaptic inhibition was blocked in sgp130Fc mice, or – in wild-type animals – upon application sgp130Fc. Similar results were obtained by activating the *"anti-inflammatory reflex"* – a neural circuit regulating peripheral immune response – by stimulation of the vagal nerve or through peripheral administration of the $\alpha_7$ nicotinic receptor agonist PHA543613.

Our results indicate that the prefrontal cortex is an important potential target of IL-6 mediated trans-signaling, and suggest a potential new avenue in the treatment of a large class of hyperexcitable neuropsychiatric conditions, including epilepsy, schizophrenic psychoses, anxiety disorders, autism spectrum disorders, and depression.



## 1. Introduction

A number of neurologic and psychiatric conditions including epilepsy, psychotic schizophrenia, and post-traumatic stress disorder are associated with acute, mostly transient, temporal phases characterized by central hyperexcitability which manifests itself with specific clinical features depending on the peculiar nature of the illness (i.e. seizures in epilepsy, psychotic episodes in schizophrenia, low threshold irritability and propensity to aggressive behavior in post-traumatic stress disorder, etc. Bauer et al., 2014; Centonze et al., 2005; Wondolowski and Dickman, 2013). Stress often triggers acute hyperexcitable episodes and increases the level of endogenous compounds including steroid hormones, monoamines, and several peptides, mainly of hypothalamic origin.

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A number of these peptides, like the pro-inflammatory cytokine interleukin 6, are produced by "psychogenic" stress, like social challenge (Audet et al., 2011) or sustained changes in circadian rhythmicity (Monje et al., 2011), as well as by systemic stress (Burton et al., 2013; Mays et al., 2012; Merlot et al., 2004; Powell et al., 2009). The relationship between IL-6 and the onset of neuropsychiatric disorder has been previously discussed (Atzori et al., 2012a). For all these reasons we and others focused our work on the central effects of IL-6, finding that this molecule decreases the response of inhibitory GABAergic synapses in the spinal cord (Kawasaki et al., 2008) and mediates the stress-induced decrease of the ratio between synaptic inhibition and excitation (sI/E) in the temporal cortex (Garcia-Oscos et al., 2012).

In this context we looked here for effective methods to reduce the effects of LPS on cortical inhibition, using intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) as a model of acute stress to determine whether the medial prefrontal cortex (mPFC) – an area of the brain whose dysfunctions are at the core of hyperexcitable conditions in the psychiatric spectrum-displays IL-6-dependent hyperexcitability similar to the spinal cord and the temporal cortex.

### 1.1. Extracellular mechanism of action of IL-6

IL-6 has been found to activate the intracellular Janus kinase/signal transducer activator of transcription (JAK/STAT) cascade using two distinct extracellular mechanisms, named "classical" and "trans-signaling" pathways. In the "classic" pathway, IL-6 binds a composite membrane receptor containing a core represented by the so-called IL-6 receptor (IL-6R) associated with a dimer of the glycoprotein 130 (gp130). Binding of the complex IL-6/IL-6R/gp130, in turn, leads to activation of JAK kinases and subsequent tyrosine-phosphorylation of the gp130 dimer, which in turn activates the STAT cascade. While this specific process has only been identified in a few cellular types including immune cells and hepatocytes, membrane-bound gp130 appears to be present in all nucleated cells and is the signal-transducer for at least 5 other ligands besides IL-6 (Wang et al., 2009).

A second mechanism through which IL-6 can activate membrane-bound gp130 is by previous binding to extracellular IL-6Rs "shed" by immune cells following the activation of the membrane bound proteolytic enzyme ADAM 17 (Rose-John, 2012). In this pathway, named "trans-signaling", the law of mass action linking IL-6, soluble IL-6Rs and their membrane-bound cognate receptor gp130 determines the efficacy of IL-6 in activating the corresponding intracellular cascade. Soluble versions of gp130 are present systemically, (sgp130) sequestering the complex IL-6/IL-6R, and acting as actual IL-6 antagonist (Wolf et al., 2014). Among the tools of this work we have used genetically modified mice (GFAP-sgp130Fc mice) in which a sequence for the production of a dimerized soluble version of gp130 (sgp130Fc) has been placed under the transcriptional control of the promotor for glial fibrillary acidic protein (GFAP), specifically expressed in brain astrocytes (Rose-John, 2012; Yamamoto and Rose-John, 2012). We have recently shown that this manipulation prevents most of the pathological central effects of IL-6 (Campbell et al., 2014).

### 1.2. The "anti-inflammatory" reflex

We reasoned that since peripheral inflammation contributes to the etiology of psychiatric conditions (Burton and Johnson, 2011; Burton et al., 2013, 2011), attenuation of inflammation may decrease symptoms associated with such spectrum of illnesses. The *anti-inflammatory reflex*, consists in the inhibition of harmful effects of inflammation by inhibiting immune cells through $\alpha_7$ nicotinic receptors (Tracey, 2002). This phenomenon has been widely documented (Andersson and Tracey, 2012a,b), and has been shown to reduce the effects of immune activation caused by a number of stimuli including LPS (Borovikova et al., 2000). The anti-inflammatory reflex can be activated in at least two ways: by direct administration of $\alpha_7$ nicotinic agonists, or by vagal nerve stimulation (VNS). VNS has been successfully tested during the last two decades by tens of thousands of patients worldwide without significant side effects as a treatment for pharmacologically untreatable epilepsy (Marras et al., 2013) and major depression (Wani et al., 2013), and is being currently tested as treatment for other conditions like tinnitus (Engineer et al., 2010).

## 2. Materials and methods

For this study we used 46 wild type mice (C57BL/6J, Charles River), offspring from 3 founding pairs donated by the vivarium of the Instituto de Neurobiologia of the Autonomous University of Mexico (UNAM) in Juriquilla (Queretaro, generous gift from Dr. Raul Paredes), other 20 mice of the same strain, offspring from mice genetically modified in the laboratory of SRJ (for details see this Section 2.4.1), and 112 rats (Sprague Dawley rats, Charles River, Wilmington, MA). Rats were used to routinely perform vagal nerve stimulation (VNS). The use of mice in addition to rats was necessary because of the need to compare wild-type with transgenic animals. Unfortunately we were not successful in performing VNS on mice due to their small size. Otherwise, the general procedures for similar experiments conducted on rat and on mice did not substantially differ between the two species and are reported below.

Given the use of two different species in order to test in order to challenge the generality of our results, we did not pursue a further development of the experimental design with heterozygous mice for the GFAP-sgp130Fc allele, which might have yielded non-conclusive results along with interpretative problem associated with an intermediate concentration of sgp130Fc.

### 2.1. Brain slices

Animals in their early adolescence, close to reproductive age (25–50-day-old, mean = 35-day-old) were chosen because of their high sensitivity to stress (Harrison and Baune, 2014). Experimental animals were anesthetized with isoflurane (Baxter, Round Lake, IL), sacrificed according to the National Institutes of Health Guidelines, and their brains sliced with a vibratome (VT1200, 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 $MgCl_2$, 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) were cut from the prefrontal cortex after removal of the olfactory lobes as in previous work (Atzori et al., 2005; Gonzalez-Burgos and Barrionuevo, 2001). Slices from pretreated animals (see next sections in "Section 2") were subsequently incubated in ACSF at 32 °C before being placed in the recording chamber.

### 2.2. Electrophysiological recording

#### 2.2.1. Patch-clamp recordings

Slices were rapidly transferred to 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). The recording area in the prefrontal cortex was selected in the intermediate area of the medial aspect (Cg3) from coronal slices of the frontal lobes (Gonzalez-Burgos and Barrionuevo, 2001). In a few trials, synaptic currents and the corresponding parameters were monitored and

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