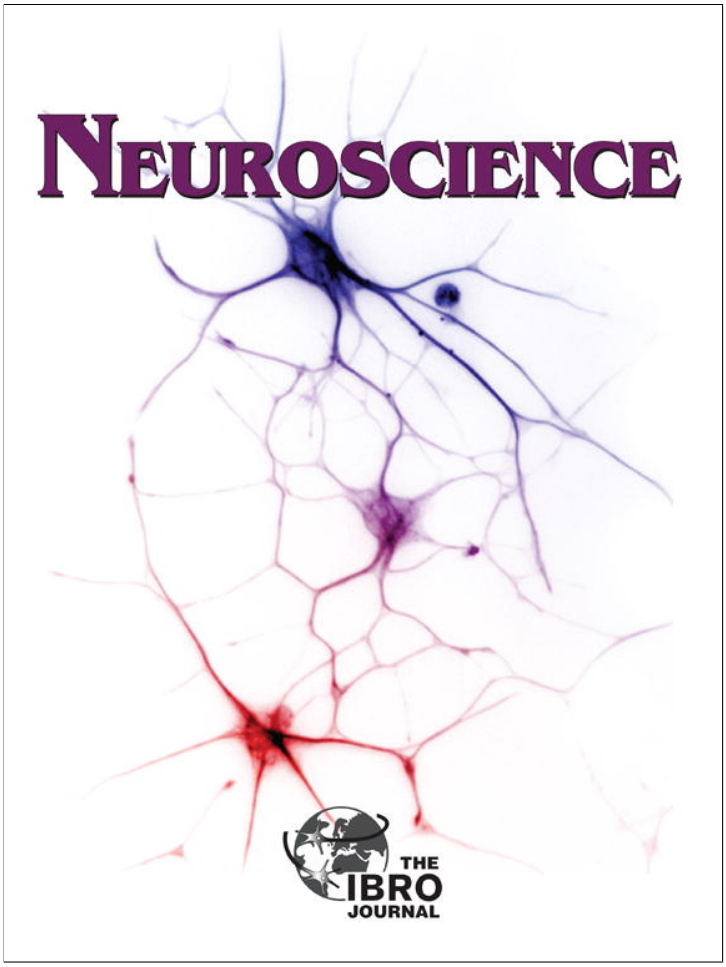

Neuroscience 246 (2013) 94–107

REPEATED RESTRAINT STRESS IMPAIRS AUDITORY ATTENTION AND GABAERGIC SYNAPTIC EFFICACY IN THE RAT AUDITORY CORTEX

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Abstract—Chronic stress induces dendritic atrophy in the rat primary auditory cortex (A1), a key brain area for auditory attention. The aim of this study was to determine whether repeated restraint stress affects auditory attention and synaptic transmission in A1. Male *Sprague–Dawley* rats were trained in a two-alternative choice task (2-ACT), a behavioral paradigm to study auditory attention in rats. Trained animals that reached a performance over 80% of correct trials in the 2-ACT were randomly assigned to control and restraint stress experimental groups. To analyze the effects of restraint stress on the auditory attention, trained rats of both groups were subjected to 50 2-ACT trials one day before and one day after of the stress period. A difference score was determined by subtracting the number of correct trials after from those before the stress protocol. Another set of rats was used to study the synaptic transmission in A1. Restraint stress decreased the number of correct trials by 28% compared to the performance of control animals ($p < 0.001$). Furthermore, stress reduced the frequency of spontaneous inhibitory postsynaptic currents (sIPSC) and miniature IPSC in A1, whereas glutamatergic efficacy was not affected. Our results demonstrate that restraint stress decreased auditory attention and GABAergic synaptic efficacy in A1.

Key words: stress, attention, learning, auditory system, synaptic transmission.

INTRODUCTION

Stress is a complex biological reaction common to all living organisms that allows them to restore homeostasis and adapt to environmental pressure (i.e. stressors) (Selye, 1936; Goldstein and McEwen, 2002). The stress response is mediated strongly by activation of the hypothalamus–pituitary–adrenal (HPA) axis, leading to the secretion of glucocorticoids (corticosterone in rodents and cortisol in humans) from the adrenal gland; which bind to glucocorticoid receptors in the brain and peripheral tissues (Herman et al., 1996, 2003; Smith and Vale, 2006). Stress can be positive (eustress) when the stressors are mild, brief and controllable (Tafet and Bernardini, 2003). Strong and persistent stressors trigger distress or chronic stress (Tafet and Bernardini, 2003). Uncontrollable stressors significantly increase HPA axis activity and plasma corticosterone levels in chronically stressed rats compared to unstressed animals, leading to a maladaptive response (Tafet and Bernardini, 2003; Ferraz et al., 2011).

Limbic structures like the hippocampus, amygdala and medial prefrontal cortex have high concentrations of glucocorticoid receptors (Gray and Bingaman, 1996; Joels, 2001; Wellman, 2001). Chronic glucocorticoid administration and chronic stress alter the dendritic architecture and function of brain areas related to memory, learning and emotional processing (Watanabe et al., 1992; Magariños and McEwen, 1995; Magariños et al., 1998; Wellman, 2001; Vyas et al., 2002; Mitra and Sapolsky, 2008).

Stress effects on the auditory system and learning

The emotional processing of the acoustic information in the brain depends on the intensity of the acoustic stimuli (McDonald, 1998; Wilensky et al., 2006). Acoustic stimuli are processed at the subcortical level through the neuronal pathway formed by the cochlea nucleus and superior olivary complex–lateral lemniscus. The inferior colliculus (IC) receives all projections from these nuclei, which are then sent to the medial geniculate nucleus (MG, auditory thalamus). Part of the auditory information received in the MG is sent directly to the lateral amygdala (McDonald, 1998; Wilensky et al., 2006). Auditory stimuli $\leqslant$80 dB must be associated with an

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Abbreviations: 2-ACT, two-alternative choice task; A1, primary auditory cortex; CE, central amygdaloid nucleus; dB, decibel; DS, difference score; DS-CT, difference score of correct trials; EGTA, ethylene glycol tetraacetic acid; EPSCs, excitatory postsynaptic currents; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPA, hypothalamus–pituitary–adrenal; IC, inferior colliculus; IPSCs, inhibitory postsynaptic currents; ITI, inter-trial interval; kHz, kilohertz; L-CT, latency of correct trials; L-CT/CT, ratio of latency of correct trials/number of correct trials; m, meter; MG, medial geniculate nucleus; mIPSC, miniature inhibitory postsynaptic currents; ms, millisecond; mV, millivolt; PO, posterior thalamic nucleus; PPR, paired-pulse ratio; s, second; sEPSCs, spontaneous excitatory postsynaptic currents; sIPSC, spontaneous inhibitory postsynaptic currents; TTX, tetrodotoxin.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.04.044

aversive unconditioned stimulus, such as footshock, to acquire the ability to elicit conditioned fear responses (Monfils et al., 2009). The acquisition of auditory emotional memories in the amygdala is associated with neuronal plasticity in the basolateral amygdala and MG (Maren et al., 2001; Poremba and Gabriel, 2001). Both brain areas exhibit associative plasticity of spike firing during fear conditioning (Maren et al., 2001). On the other hand, acoustic stimuli equal to or higher than 90 dB are sent from the dorsal nucleus of the lateral lemniscus to the posterior thalamic nucleus (PO), located just medially to the posterior intralaminar nucleus (Kudo et al., 1983; Paré et al., 2004). The PO also receives auditory projections from the nucleus of the brachium of the IC (Kudo et al., 1983). The PO efferents are sent directly to the central amygdaloid nucleus (CE) and to the primary somatic sensory cortex, indicating the possibility that the CE receives auditory input from the thalamus (Paré et al., 2004). Through this neuronal pathway, the CE is activated and fear responses such as freezing are performed independent of the pathway formed by the IC–MG–auditory cortex.

Studies in animal models have shown that chronic stress impairs the major nuclei of the auditory system. For instance, a recent study using micro Positron Emission Tomography supports these findings in that chronic mild stress induces significant deactivation in the IC, the main nucleus of the auditory system (Hu et al., 2010a). In this line, we previously demonstrated that restraint stress induces dendritic atrophy in the rat IC, MG, and neurons from layers II/III and V/VI of the primary auditory cortex (A1) (Dagnino-Subiabre et al., 2005, 2009; Bose et al., 2010). Chronic stress significantly impaired auditory learning in rats subjected to a two-way-signaled active avoidance learning procedure, where animals had been trained in a shuttle box to avoid a footshock signaled by acoustic cues (Dagnino-Subiabre et al., 2005).

Stress effects on synaptic transmission

One factor that has not received sufficient attention yet in this topic is the effect of chronic stress on glutamatergic and GABAergic signaling at the cortical level. The balance of excitation and inhibition in the brain is essential for synaptic plasticity and cognitive functions (Buzsaki and Chrobak, 1995; Cobb et al., 1995). Intracellular recording studies have shown that a synergic effect of excitatory and inhibitory inputs to A1 neurons is key for auditory processing (Wehr and Zador, 2005; Tan and Wehr, 2009). As well interneurons exert a strong control over balance and synchronization of the brain circuit. Thus, modulation of glutamatergic and GABAergic synaptic efficacy is the main regulatory element for auditory learning and complex cognitive processes in both health and disease (Oswald et al., 2006; Levy and Reyes, 2012). Chronic stress and glucocorticoids increase the GABAergic synaptic transmission in the hippocampus (Hu et al., 2010b; Martisova et al., 2012), while in the basolateral amygdala chronic stress decreases GABAergic inhibition (Rodríguez Manzanares et al., 2005; Reznikov et al., 2009; Roozendaal et al., 2009). At the cortical level, stress decreases the inhibition/excitation ratio in the temporal cortex (Garcia-Oscos et al., 2012).

Attention

All animals live in a world of competition, with multiple stimuli from environment that must be resolved in order so that they behave adaptively. Attention is a complex cognitive function that allows them the ability to select from an overabundance of stimuli, responses, and memories, and in doing so, ignore any that are irrelevant (Raz, 2004).

Attention involves a unitary description of three attentional control systems in the brain: ‘‘alerting’’, ‘‘orienting’’ and ‘‘executive’’ (Posner and Petersen, 1990; Raz and Buhle, 2006). ‘‘Alerting,’’ relates to preparedness for an imminent stimulus through maintaining an alert state. Human studies on neuroimaging show that the alert state is associated to activity in the prefrontal and parietal cortices, mainly in the right hemisphere (Coull et al., 1996). ‘‘Orienting’’ is related to the ability to select information from several sensory stimuli; this is associated with activity in both the superior parietal cortex activity and superior colliculus, respectively (Corbetta, 1998; Corbetta et al., 2000). ‘‘Executive attention’’ is the complex monitoring and resolution of conflict between different brain regions.

Attention is studied in rats by the behavioral paradigm two-alternative choice task (2-ACT) (Jaramillo and Zador, 2011). In this paradigm, auditory attention is associated with increases in neural activity in A1, which are required to process attentional information (Hromádka and Zador, 2007; Otazu et al., 2009). Likewise, electrophysiological and behavioral studies have shown a significant positive correlation between the animal’s performance in the 2-ACT and increased neuronal activity in A1 (Jaramillo and Zador, 2011).

These findings raise the question of whether complex cognitive functions such as auditory attention are affected by chronic stress, and if this effect is mediated by an alteration of glutamatergic or GABAergic signaling in A1. The objective of this study was to test whether repeated restraint stress impairs auditory attention and decreases synaptic efficacy in A1. We performed four experiments; the first was to measure corticosterone plasma levels of trained rats in a 2-ACT. The second experiment was to analyze whether restraint stress affects the spatial memory of trained rats. In addition, we studied the stress markers of body weight gain and anxiety in all animals. The third experiment was to determine whether restraint stress affects auditory attention in the 2-ACT. Finally, in the fourth experiment we analyzed the efficacy of the glutamatergic and GABAergic systems in A1 of control and chronically stressed rats. The main results of our research were that restraint stress decreased both auditory attention and inhibitory synaptic efficacy in A1 compared to controls.

EXPERIMENTAL PROCEDURES

Ethics statement

All procedures related to animal maintenance and experimentation were approved by the Institutional Animal Ethics Committee of the Faculty of Sciences. Universidad de Valparaíso (Chile) and were in strict accordance with animal care standards outlined in National Institute of Health (USA) guidelines. Efforts were made to minimize the number of animals used and their suffering.

Animals and restraint stress protocol

Male *Sprague–Dawley* rats were housed in groups of three per cage under a 12/12 light/dark cycle (lights on at 08:00 h.), with *ad libitum* access to food (rat chow, Champion®, Santiago, Chile) and water in a temperature and humidity controlled room (20 ± 1 °C, 55% respectively). Animals were weighed every day on a digital scale (Model WLC2/A1, Radom, Radwag, Poland). Beginning on postnatal day 23, the animals were trained for 3 weeks in a 2-ACT, a behavioral paradigm to analyze auditory attention in rats (Fig. 1A). Trained animals were randomly assigned to two experimental groups: control, $n = 48$ and stress, $n = 48$, for all experiments. Control animals, which were littermates of the stress-treated animals, were housed in separate rooms and cages, and not subjected to any type of experimental stress. Restraint-stressed rats were placed in a plastic rat restrainer (6 cm diameter × 12 cm long and then 6 cm diameter × 20 cm long as the rats grew) in their home cages for 6 h daily, beginning at 10.00–16.00 h for 21 consecutive days. Restraint occurred during the light phase the light/night cycle applied. To monitor the overall effects of the stress protocol the percentage gain in body weight of all animals (net change in weight after experiment × 100/ weight at the beginning of experiment) was measured.

2-ACT

Apparatus and stimuli delivery. To measure auditory attention in rats we used the 2-ACT paradigm. Four modular rat operant chambers and accessories (LE1005, LE10022, LE100575, LE100560, Panlab S.L., Barcelona, Spain) were used in the attention task, each within of a 67 × 67 × 67 cm^3 sound-attenuating cubicle lined with 7.5 cm acoustic foam (Vroka S.A., Santiago, Chile). The operant chamber was illuminated to 200 lux (measured by a digital lux meter, Model # LX-1010B, Weafo Instrument Co., Shanghai, China) and the background noise level was 30 dB. During training, the auditory stimuli were delivered through a speaker calibrated with a precision sound level meter (Model # 1100, Quest Technologies, Oconomowoc, WI, USA) to generate 70 dB in the range of 1–15 kHz at the position of the subject. The duration of the auditory stimuli was 0.1 s. The speaker was mounted in front of the three nose-poke, each connected with a liquid dispenser (Fig. 1A). Operant modules were regulated by the Packwin V1.2 software (Panlab S.L., Barcelona, Spain). All experiments were recorded with an IP camera (VIVOTEK, Sunnyvale, CA, USA) fixed above each operant chamber. Videos were acquired by Nuuo software (Nuuo, Taipei, Taiwan).

Behavioral task. Three days after weaning, male *Sprague–Dawley* rats (23 days old at the start of the experiment) were trained in the behavioral 2-ACT paradigm. Animals were water deprived over night under a protocol approved by the Institutional Animal Ethics Committee of the Faculty of Sciences, Universidad de Valparaíso, Chile. Afterward, the rat initiated a trial by inserting its nose into the center nose-poke of a three-port operant chamber (Fig. 1A), which triggers the computer to present two types of acoustic stimuli at random one a low-frequency tone of 1 kHz and the other a high-frequency 15 kHz tone. The rats were trained to respond with right pokes for low tones and left pokes for high tones. Correct trials were rewarded with water (Fig. 1A). All operant chambers were thoroughly cleaned with a 5% ethanol solution after each trial. The control and chronically stressed groups were evaluated at the same time.

The 2-ACT training has three steps; in each of which it is possible to independently analyze learning, memory consolidation and auditory attention (Fig. 1B). In the first week of training, rats learned to respond with right pokes for low tones and left pokes for high tones (learning period). In the second week, rats were trained in 50 2-ACT trials until reaching 70% correct trials. At the end of this week the rats' memory related to 2-ACT was consolidated (memory consolidation period). Beginning in the third week, the rats recalled the task and improved their auditory attention increasing correct responses to over 80% of correct trials (auditory attention period).

Experimental design. Fig. 1B shows a schematic drawing of the experimental design used in this study. After 3 weeks of training, rats that reached a correct performance of over 80% in 50 trials were included for four experiments, using different sets of animals for each experiment. Experiment 1 analyzed whether repeated restraint stress paradigm affects the stress levels of trained rats (control, $n = 12$, stress, $n = 12$) one day after stress ended. The most conventional method to determine if the animals are stressed is to measure the plasma levels of the stress hormone corticosterone. Animals were subjected to a new stressor (swimming in a water maze) and corticosterone plasma levels were quantified before and after water maze exposure. Experiment 2 consisted of analyzing both 2-ACT training and restraint stress effects on the locomotor activity (open field), anxiety (elevated plus-maze), and memory (Y-maze) (control, $n = 9$, stress, $n = 9$). In experiment 3 we studied the effect of restraint stress on auditory attention. The animals were randomly assigned to two groups: control, $n = 15$, and stress, $n = 15$, and one day before and after the stress protocol, the rats were subjected to 50 trials of 2-ACT

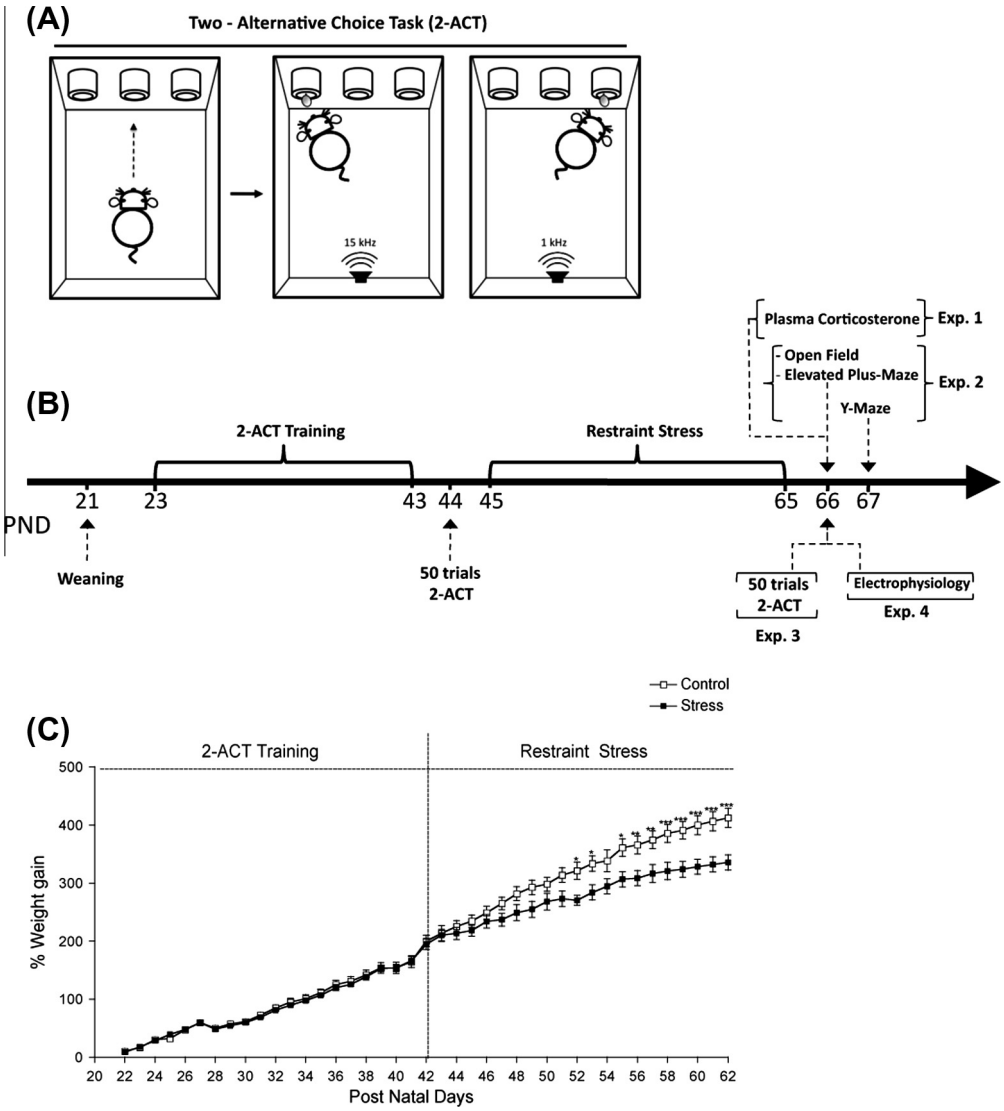

Fig. 1. Structure of the basic two-alternative choice task (2-ACT), schematic drawing of the experimental design, and the influence of restraint stress on the body weight. (A) 2-ACT is an auditory attentional task; the rat initiates a trial when decides to introduce its nose into the center port (*left*). This elicits the computer to randomly present two types of auditory stimuli, a high (15 kHz) or low (1 kHz) frequency tone (*right*). Rats were trained to respond with a left poke for high tones and a right poke for low tones; correct trials were rewarded with water. (B) The arrow represents the postnatal days of the animals (PND). After weaning, rats were trained in the 2-ACT for 20 days. Afterward, animals were randomly assigned to two experimental groups: control, $n = 48$, and stress, $n = 48$. Twenty-four trained rats (control, $n = 12$, stress, $n = 12$) were used in the experiment 1 (Exp. 1) to measure plasma corticosterone levels one day after stress ended. Eighteen trained rats (control, $n = 9$, stress, $n = 9$) were used in the experiment 2 (Exp. 2) to analyze the stress effects on locomotor activity (open field), anxiety (elevated plus-maze), and memory (Y-maze) on days 66 and 67, respectively. In experiment 3 (Exp. 3), to analyze the stress effects on auditory attention, thirty trained rats, (control, $n = 15$, stress, $n = 15$) were subjected to 50 2-ACT trials one day before and after the restraint stress protocol. A difference score was then determined by subtracting the correct trials after from those before chronic stress (DS-CT). Experiment 4 (Exp. 4) analyzed synaptic transmission in A1 (control, $n = 12$, stress, $n = 12$). (C) Training in the auditory task (2-ACT) did not affect the percentage of weight gain. At the beginning of the restraint stress, control animals gained weight gradually throughout the study, however chronically stressed rats failed to gain weight. Data are represented by mean ± SEM.

(Fig. 1B). Experiment 4 analyzed stress effects on synaptic transmission in A1 (control, $n = 12$, stress, $n = 12$).

Experiment 1

Plasma corticosterone measurement. A separate set of animals was used to measure the concentration of corticosterone in plasma, in order to avoid the stressfulness of blood collection on behavioral or electrophysiological experiments. One set of rats (control, $n = 6$, stress, $n = 6$) were given a 60-s probe trial in a water maze at 11:00 h after which the animals were transferred to a heated holding cage for 10 min. Afterward, the animals were transported to a separate room (time used approximately 10 s) and were quickly anesthetized with isoflurane (time used approximately 5 s) and immediately sacrificed via decapitation under deep anesthesia to blood collection. Animals were not

exposed to other decapitated animals before deep anesthesia. Another set of rats (control, $n = 6$, stress, $n = 6$) were not disturbed and sacrificed at 11:11 h under deep anesthesia. The Morris water maze consisted of a blue circular tank (183 cm diameter) in a room that was rich with spatial cues. The tank contained non toxic colored water at 19 °C (black nontoxic tempura paint).

Blood (1 ml) was collected in heparinized microcapillary tubes and centrifuged (Model # MiniSpin Plus; Eppendorf AG, Hamburg, Germany) at 10,000 rpm for 10 min to obtain plasma and then stored at −70 °C. Total corticosterone was determined by an Enzyme Immunoassay kit (Corticosterone BioAssay, Catalog. # C7903-30) purchased from US Biological (Swampscott, MA, USA). Optical density values were measured at 450 nm using a micro-plate reader (Tecan GENios, Tecan Group Ltd., Switzerland). Samples were diluted 1:10 and then processed in duplicate and averaged final values were represented as ng/ml.

Experiment 2

Behavioral testing. The open field and elevated plus-maze tests were conducted 24 h after final completion of the stress protocol, and a day after the Y-maze test was applied (Fig. 1B). All animals were naive to the test situations. Behavioral tests were carried out from 10.00 to 14.00 h. in the test room. The activity of each rat was recorded by IP cameras fixed above the behavioral apparatus and connected to a computer in another room. Videos were acquired by Nuuo software (Nuuo, Taipei, Taiwan) and analyzed using ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA). All mazes were wiped clean thoroughly with 5% ethanol solution after each trial. In all experiments, animals from control and stress experimental groups were evaluated at the same time.

Open field test. Behavioral tests were conducted in a sound-proof and temperature-controlled (21 ± 1 °C) room. Each rat was placed in the center of a black Plexiglass cage (70 × 70 × 40 cm) for 5 min. The background noise level in the open field was 40 dB (Precision sound level meter, Model # 1100, Quest Technologies, Oconomowoc, WI, USA) and the arena was illuminated to 300 lux (measured by digital lux meter, Model # LX-1010B, Weafo Instrument Co., Shanghai, China). Total distance traveled and average speed was analyzed from video recordings and analyzed using ANY-maze video-tracking system (Stoelting Co.).

Elevated plus-maze. Immediately after the analysis of the open field (approximately 10 s) we measured anxiety levels using an elevated plus-maze test. Each rat was individually placed in an elevated plus-maze, consisting of two open arms (60 × 15 cm each), two closed arms (60 × 15 × 20 cm each) and a central platform (15 × 15 cm), arranged so that two arms of each type were opposite to each other. The maze was elevated 100 cm above the floor. The illumination was 300 lux in the open arms and 210 lux in the closed arms. At the beginning of each trial animals were placed at the center of the maze, facing an open arm. During a 5-min test period we recorded the frequency of entries to the open and closed arms, the total number of arm entries, and the amount of time spent in each section of the maze. The number of entries and time spent in the open arms, and the ratio of open to total arm entries (open/total × 100) were used as measures of the anxiety level (Dagnino-Subiabre et al., 2006). Total arm entries were taken as an indicator of general locomotor activity. Entry into an arm was defined as having occurred when the animal placed all four limbs onto the arm.

Y-maze. Spatial memory was tested on the Y-maze 24 h after completing the analysis of the elevated plus-maze. The Y-maze consisted of three equilaterally intersecting black Plexiglas arms (58 cm long × 19 cm wide × 38 cm high) and several extra-maze cues on the surrounding walls. The three arms were assigned as Novel, Start and Other, and were counterbalanced among rats. Control and stressed rats were tested at the same time and in separate Y-mazes. Through training, one arm (Novel) was blocked and the animals were placed on the Start to explore for 15 min both the Start and Other arms. After training, the Novel arm was unblocked and rats were returned to their home cages and room. Four hours later, rats were returned to the same start location used during training, and were allowed to freely explore all arms for 5 min. Rats tend to explore novel environments, consequently an intact spatial memory if the rats showed a preference for the Novel arm. Entry into an arm was defined when the animal placed all limbs onto the arm. Behavior was videotaped and entries were converted into percentages. Entries into all arms were counted (total entries) to determine whether locomotor activity levels were similar between experimental groups. To analyze the stress effect on spatial memory ability, a difference score (DS) was measured subtracting the percentage of entries in the alternate arm from the percentage of entries in the novel arm.

Experiment 3

Trained rats were subjected to 50 trials of 2-ACT one day before and after the stress protocol (Fig. 1B). The following parameters were measured using the Packwin software (Panlab, Barcelona, Spain) in each trial: number of correct trials, ratio of latency of correct trials (L-CT)/number of correct trials (L-CT/CT), and inter-trial interval (ITI). The ratio of L-CT/CT indicates the time used in each correct trial. To analyze the stress effects on the auditory attention, a DS was measured by subtracting the number of correct trials determined before stress (DS-CT) from the number of correct trials obtained on the 50 trials after the stress period. A DS was also calculated for the ratio of L-CT/CT [(DS-(L-CT/CT)] and ITI (DS-ITI).

Experiment 4

Electrophysiology. A new set of trained rats (control, $n = 12$, stress, $n = 12$) was used to study the chronic stress effects on the efficacy of the glutamatergic and GABAergic systems in A1. After completion of the stress protocol each rat was decapitated under deep anesthesia with isoflurane. The brain was removed quickly and submerged in cold (~4 °C) artificial cerebrospinal fluid (in mM: 124.00 NaCl, 2.69 KCl, 1.25 KH_2PO_4, 2.00 $MgSO_4$, 26.00 $NaHCO_3$, 2.00 $CaCl_2$, 10.00 glucose). The pH of the artificial cerebrospinal fluid was stabilized at 7.4 by bubbling carbogen (95% O_2 and 5% CO_2). Coronal brain slices (300–350 μm) were cut with a Vibratome (Campden Instruments, model MA752, England) and incubated in the artificial cerebrospinal fluid (> 1 h, at room temperature; 20–22 °C). Slices were transferred to a 2-ml chamber fixed to an upright microscope stage (NIKON, model Eclipse FN1, Tokyo, Japan) equipped with infrared differential interference contrast (DIC) video microscopy and 40× water immersion objectives. Slices were superfused with carbogen-bubbled artificial cerebrospinal fluid (2 ml/min) and maintained at room temperature (22–24 °C). 2-Amino-5-phosphonopentanoic acid (D-AP5; 50 μM) and 7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile (CNQX; 20 μM) were added to the artificial cerebrospinal fluid as needed.

Recordings were made in neurons located 300–450 mm from the pia surface corresponding to layers II–III of rat auditory neocortex. Whole-cell recordings were made with patch pipettes (4–6 MΩ) filled with an internal solution that contained in mM: 100 Cs-Gluconate, 10 HEPES, 10 EGTA, 4 Na_2-ATP, 10 TEA-Cl and 1 $MgCl_2$–$6H_2O$, buffered to pH 7.2–7.3 with CsOH. Recordings were performed in voltage-clamp modes using an EPC-7 patch-clamp amplifier (HEKA, Instruments). In voltage-clamp experiments the Vh was adjusted to −65 or 0 mV as needed. In the voltage-clamp configuration the series resistance was compensated to ~70% and neurons were accepted only when the seal resistance was > 1 GΩ and the series resistance (7–14 MΩ) did not change > 10% during the experiment. The liquid junction potential was measured (~6 mV) but was not corrected. Voltage-clamp data were low-pass filtered at 3.0 kHz and sampled at rates between 6.0 and 10.0 kHz using an A/D converter (ITC-16, InstruTech) and stored with Pulse FIT software (Heka Instruments). The Pulse Fit program was used to generate stimulus timing signals and transmembrane current pulses. The recording analysis was made off-line with pClamp software (Clamp-fit, Molecular Devices). Inhibitory postsynaptic currents (IPSCs) were evoked with a concentric bipolar electrode (60 μm diameter, tip separation ~100 μm (FHC Inc., ME, Bowdoin), placed at about 100–200 μm lateral from pyramidal neuron somata to stimulate the GABA interneurons or at the base of layer III/IV, in line with the recording electrode to stimulate the thalamocortical axons.

An average of IPSC ($n = 10$) was obtained under voltage clamp by repeated stimulation at 0.3 Hz. Chemicals were purchased from Sigma–Aldrich Chemistry (Santiago, Chile), and Tocris (Bioscience, USA). The paired pulse ratio (PPR) was calculated as 1-(R2/R1) ∗ 100, where R1 and R2 are the peak amplitudes of the first and second IPSCs, respectively.

To determine whether chronic stress could simultaneously affect the glutamatergic and GABAergic pyramidal neuron synapses; we voltage-clamped CA1 PNs at the reversal potential for evoked excitatory or inhibitory synaptic currents (EPSCs or IPSCs, respectively). Values of the reversal potential of EPSCs and IPSCs were estimated from current–voltage relationships of EPSCs (0.3 ± 0.5 mV; $n = 10$) and IPSCs (−64.2 ± 2.3 mV; $n = 10$), respectively. Moreover, in some experiment the excitatory or inhibitory synaptic transmission were isolated after blocking GABAA with picrotoxin (10 μM) or NMDA and AMPA receptors with D-AP5 (50 μM) and CNQX (20 μM).

The spontaneous inhibitory and excitatory postsynaptic currents (sIPSCs or sEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) were analyzed off-line using the Minianalysis software (Minianalysis; Synaptosoft, Decatur, GA, USA), which allowed visual detection of events and selection for analysis of those that exceeded an arbitrary threshold.

Calculation of the multiplicity factor. The multiplicity index was calculated in order to estimate the degree of connectivity between interneurons and pyramidal neurons in A1 (Hsia et al., 1998; Groc et al., 2003). The spontaneous and miniature (action potential-independent) GABAergic postsynaptic currents, sIPSC and mIPSC respectively, were compared in A1 neurons of rats from control and stress groups. To determine the index of multiplicity, first the amplitude and frequency mean values of sIPSC and mIPSC alone were obtained and recorded respectively before and after adding tetrodotoxin (TTX). Multiplicity was calculated as the mean amplitude of action potential-driven events (''a'') divided by mean quantal size (''q'': mean amplitude of mIPSC recorded in TTX). The ''a'' value was determined for each cell, subtracting the contribution of mIPSC to the pool of events collected in the absence of TTX, using the expression for ''a'':

$$a = \frac{f_b b - f_q q}{f_b - f_q}$$

where ''f_b'' and ''f_q'' denote the mean frequency values from events recorded before and after the addition of TTX to the perfusion media, respectively, and ''b'' is the mean amplitude of both sIPSC and mIPSC.

Statistical analysis

Behavioral studies. Locomotor activity, anxiety, and memory studies were analyzed by a Student's unpaired t-test. Body weight gain and DS for correct trials, ratio (L-CT/CT), and ITI were analyzed using a two-way repeated-measure ANOVA [Body weight [groups (control, stress) × post-natal days (22–62)]; DS [groups

(control, stress) × trials (1–50)] followed by a Bonferroni post hoc comparison test.

Plasma corticosterone levels. Results were analyzed by one-way ANOVA to compare groups (control and stress) and swimming conditions (no swimming or swimming on the day of plasma collection) followed by Bonferroni's multiple comparison test.

Electrophysiological studies. Data analysis and statistical evaluations were made with both the pClamp program (Molecular Devices Corporation, Chicago, USA) and Origin 7.0 (Originlab Corporation, Northampton, MA, USA). Results are presented as percentages of control. Statistical analysis was performed using Student's two-tailed *t*-test.

Results are presented as the mean ± SEM, for the electrophysiological studies n = number of cells. A probability level of 0.05 or less was accepted as significant.

RESULTS

Effects of restraint stress on body weight gain

2-ACT training did not affect body weight gain (Fig. 1C). Restraint stress attenuated the percentage of body weight gain compared to controls (Fig. 1C). A 2 × 41 mixed factor ANOVA, with treatment (control, $n = 21$, stress, $n = 21$) as the between-subjects factor and the day (22–62) as the repeated measure showed a significant treatment-day interaction, ($F_{(40,1600)} = 55.65$, $p < 0.0001$), a significant main effect of treatment, ($F_{(1,40)} = 18.51$, $p < 0.0001$), and a significant main effect of day, ($F_{(40,1600)} = 4556$, $p < 0.0001$). At the start of the stress protocol, there were no differences between the body weights of the animals from control and stress groups (Fig. 1C). However, rats that received restraint stress showed less weight gain during the 21 days of the stress protocol (postnatal day 42–62) than to control rats ($p < 0.0001$) (Fig. 1C).

Experiment 1

Effects of restraint stress on plasma corticosterone levels. Stressed animals showed an increase in HPA axis activity and plasma corticosterone levels compared to controls after exposure to an uncontrollable stressor, leading to maladaptive response (Tafet and Bernardini, 2003). In this way, acute swim stress in a water maze increases plasma corticosterone levels of *Sprague–Dawley* rats (McFadden et al., 2011). Therefore, we measured the plasma corticosterone levels of trained animals one day after of the last restraint session, when behavioral and electrophysiological experiments were initially conducted. Fig. 2 shows the level of circulating corticosterone in rats subjected to a 60-s probe trial in the water maze and in animals that were not disturbed. Controls and the rats that were subjected to restraint stress swimming for 60 s in the water maze had higher corticosterone levels than those that were left undisturbed (control group: undisturbed = 130.60 ± 17.06 ng/ml, $n = 6$, acute swimming = 285.10 ± 19.88 ng/ml, $n = 6$, $p < 0.01$; stress group: undisturbed = 89.12 ± 27.78 ng/ml, $n = 6$, acute swimming = 426.00 ± 29.97 ng/ml, $n = 6$, $p < 0.001$). Following acute swimming, animals from the stress group had significantly higher corticosterone levels than control rats (stress group = 426.00 ± 29.97 ng/ml, $n = 6$; control group: 285.10 ± 19.88 ng/ml, $n = 6$; $p < 0.01$).

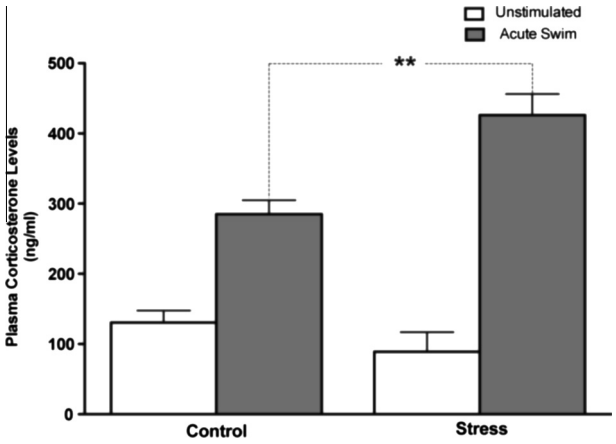

Fig. 2. Stress levels after 21 days of restraint stress paradigm. Acute 60 s swim in a water maze at 19 °C given 10 min before plasma collection caused a significant increases in plasma corticosterone levels in the rats of all experimental groups (gray bars); whereas after acute swim chronically stressed rats had significantly increased serum corticosterone levels compared to control animals. The asterisk (∗) indicates a significant difference relative to control animals. Data are represented by mean ± SEM.

Experiment 2

Effects of restraint stress on locomotor activity, anxiety and memory. Table 1 shows the effects of restraint stress on locomotor activity, anxiety, and memory. Statistical analysis revealed that the restraint stress protocol did not affect locomotor activity in the open field [(total distance traveled, stress: 16.25 ± 1.60 m, $n = 9$;

Table 1. Influence of restraint stress on the locomotor activity, anxiety, and memory of trained rats

	Control	Stress
Locomotor activity (Open Fiel)		
Total distance traveled (m)	14.9 ± 3.2	16.3 ± 1.6
Average speed (m/s)	0.05 ± 0.01	0.05 ± 0.01
Anxiety (EPM)		
Number of open arm entries	4.8 ± 1.3	2.2 ± 0.4*
Number of closed arm entries	12.4 ± 1.2	10.2 ± 0.8
Number of total entries to the arms	8.6 ± 1.5	6.2 ± 1.4
Memory (Y-maze)		
Difference score (% Novel–% Other)	6.7 ± 4.4	0.0 ± 2.9*
Number of total entries to the arms	15.2 ± 2.9	12.8 ± 1.4

The asterisk (∗) indicates significant difference relative to control animals. Data are represented by mean ± SEM.

control: 14.89 ± 3.24 m, $n = 9$; $p = 0.7177$) (average speed, stress: 0.0540 ± 0.0053 m/s, $n = 9$; control: 0.0494 ± 0.011 m/s, $n = 9$; $p = 0.7148$)]. Restraint stress induced a significant reduction in the frequency of the open-arm entries (stress: 2.2 ± 0.4, $n = 9$; control: 4.8 ± 1.2, $n = 9$; $p < 0.05$) on the elevated plus maze (Table 1). There were no treatment differences in the total arm entries, indicating that the stress protocol did not affect locomotor activity (stress: 6.2 ± 1.4, $n = 9$; control: 8.6 ± 1.5, $n = 9$; $p = 0.3055$) (Table 1). These results are indicative of an enhanced anxiety response in the trained stressed animals. Restraint stress impaired the trained rats' performance on the Y-maze. This finding was supported by the 4-h delay version of the Y-maze, restraint stress significantly decreased the %DS between Novel and Other arm entries (stress: 0.0 ± 2.9, $n = 9$; control: 6.7 ± 4.4, $n = 9$; $p < 0.05$). Statistical analysis revealed that restraint stress did not affect the total number of entries made into the arms of the Y-maze (stress: 4.3 ± 0.3, $n = 9$; control: 5.1 ± 0.6, $n = 9$; $p = 0.1515$).

Experiment 3

Auditory attention task. The purpose of this experiment was to analyze the effect of the restraint stress protocol on the auditory attention of rats that were trained in the 2-ACT, a behavioral paradigm to study attention in rats (Fig. 1A, B). Restraint stress did not affect the DS-CT through the first 10 trials of the 2-ACT (Fig. 3A). After of the 10th trial, restraint stress decreased the DS-CT compared to that of control animals (Fig. 3A). Restraint stress did not affect interaction between treatment and trial ($F_{(9,252)} = 1.57$, $p = 0.1250$), while treatment effect was significantly altered ($F_{(1,28)} = 139.3$, $p < 0.0001$). Stress and control group rats had DS-CTs of −9.4 ± 0.6 and 4.6 ± 0.6 correct trials, respectively (Fig. 3B). The majority of control animals had a significantly higher number of correct trials on postnatal day 66 (Fig. 3C). In terms of percentages, the percentages of DS-CT for animals of stress and control groups were −18.8 ± 1.2% and 9.2 ± 1.2% respectively. This is a 28% reduction in the 2-ACT performance of stressed rats compared to that of controls on postnatal day 66 (Fig. 3B). Stressed rats had over 80% of correct trials before restraint stress and 61.2% after the stress period, indicating that restraint stress impaired auditory attention and did not significantly affect memory consolidation related to the 2-ACT after the stress period (Fig. 3C).

Restraint stress decreased the DS-(L-CT/CT) compared to controls (Fig. 3D). Interactions between treatment and trial, and treatment effect decreased significantly [(Interaction: $F_{(9,252)} = 2.51$, $p < 0.001$); (Treatment: $F_{(1,28)} = 5.86$, $p < 0.05$)] (Fig. 3D). Restraint stress significantly decreased the average of total DS-(L-CT/CT) (Fig. 3E). This result demonstrates that control rats take more time for each correct trial than rats from the stress group.

Rats that were subjected to restraint stress showed a significant increase of the DS-ITI relative to control animals (Fig. 3F). There was no effect in the interaction between treatment and trial (Interaction: $F_{(9,252)} = 0.45$, $p = 0.91$), but the treatment effect was significantly affected ($F_{(1,28)} = 16.35$, $p < 0.0001$) (Fig. 3F). The restraint stress protocol significantly increased the average of total DS-(L-CT/CT) (Fig. 3G). This result shows that rats from the stress group used more time compared to controls for decision-making to perform each correct trial in the 2-ACT paradigm.

Experiment 4

Effects of restraint stress on glutamatergic and GABAergic synaptic transmission in A1. To determine whether the restraint stress protocol affects the efficacy of GABAergic synapses on pyramidal neurons of A1, we evoked isolated IPSC by the paired-pulse protocol. Afterward, modifications of the paired-pulse depression (PPR) were quantified by an index (1-(R2/R1) ∗ 100). We observed that in rats from both experimental groups, the PPR was characterized by depression, with the latter (R2) IPSC lower than the former (R1), indicating that the group with stimulated inhibitory synapses had a high release probability. We observed that restraint stress decreased the PPR (24.9 ± 4.9%) compared to controls (42.6 ± 12.2%). These effects suggest that restraint stress protocol induced a depression of GABA release (Fig. 4A, B), which is illustrated by representative neurons in Fig. 4A, top recordings.

According to the presynaptic locus, we observed that restraint stress decreased the frequency of sIPSC and mIPSC. The sIPSC frequency of rats from the stress group was 1.9 ± 0.4 Hz, whereas the frequency reached 2.8 ± 0.4 Hz in control animals ($p < 0.05$; $n = 8$; respectively; Fig. 5A, B). In addition, the mIPSC frequency of rats after restraint stress was 0.4 ± 0.04 Hz and 1.93 ± 0.3 Hz for control rats. The reductions in the sIPSC and mIPSC frequencies in the rats of the stress group were also observed after comparing the cumulative probability plots of the sIPSC frequency relative to controls (Fig. 5B), suggesting that stress-induced reduction of GABA release occurs presynaptically as a result of a decreased probability of release. Moreover, we observed that restraint stress had no effect on postsynaptic GABA efficacy. Thus, the sIPSC and mIPSC amplitudes were 98.1 ± 11.2 and 50.8 ± 4.2 pA in the rats subjected to restraint stress, while in control animals they were 124.3 ± 13.9 and 55.3 ± 1.0 pA ($n = 6$; $p = 0.08$; Fig. 5B). Restraint stress significantly decreased the sIPSC and mIPSC frequencies, whereas the amplitude was not affected than controls (Fig. 5C). In addition, the multiplicity index was examined to estimate synaptic network connectivity (Hsia et al., 1998; Groc et al., 2003). The sIPSC was recorded first and after addition of TTX (500 nM), the mIPSC was recorded ($n = 6$). Afterward, the multiplicity index was calculated for each cell (Groc et al., 2003; Riebe and Hanse, 2012). The multiplicity index was on average 1.8 ± 0.1 for stressed rats and 2.3 ± 0.3 for controls ($n = 4$, respectively; data not shown), indicating

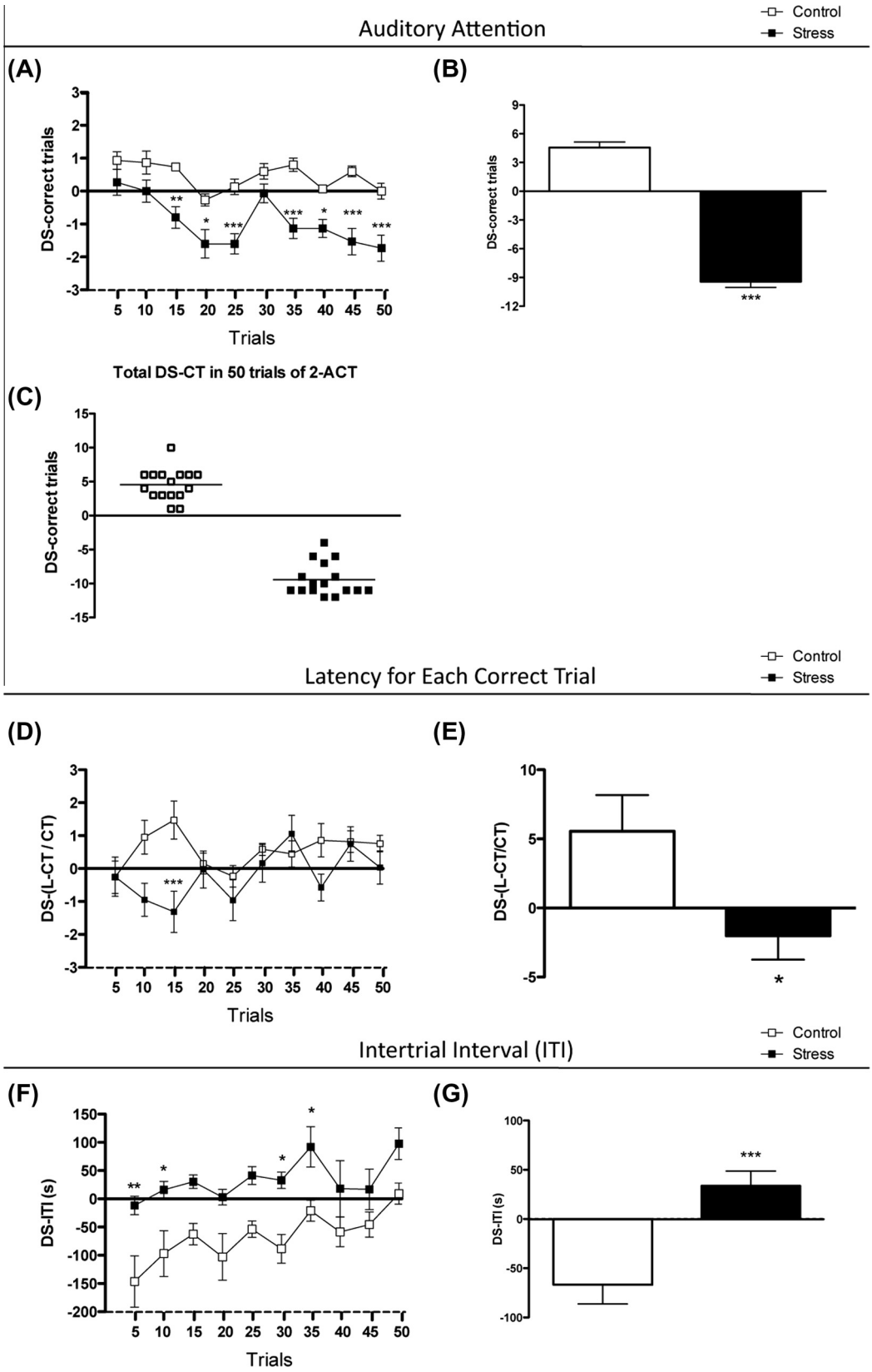

Fig. 3. Influence of restraint stress on the auditory attention, latency of correct trials, and inter-trial interval (ITI). (A) The DS-CT significantly decreased in the stressed rats compared to controls. (B, C) Restraint stress significantly decreased the average of total correct trials in 50 trials of 2-ACT. (D) Ration of DS-(L-CT/CT) was increased in the control rats compared to the stressed rats. (E) Restraint stress significantly decreased the average of total DS-(L-CT/ CT). (F) Restraint stress decreased the DS-ITI. (G) Restraint stress significantly increased the average of total ITI. The asterisk (∗) indicates significant difference relative to control animals. Data are represented by mean ± SEM.

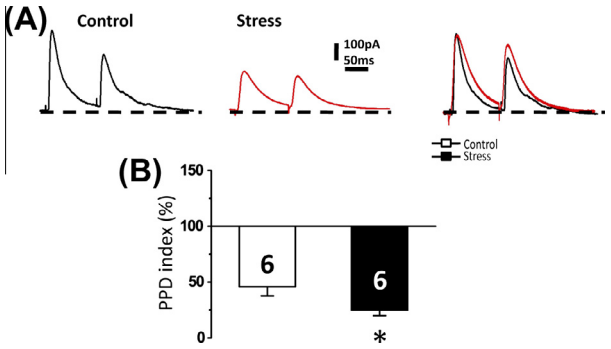

Fig. 4. Restraint stress impaired the probability of GABA release. (A) representative averaged IPSC recorded under control and stress conditions respectively. At the right, superimposed representative averaged IPSC recorded under control (black trace) and stress conditions (red trace). (B) Summary data showing the changes in PPR index between stressed and control rats. The numbers in the bars represent sample size (*n*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that restraint stress did not affect the degree of connectivity between an interneuron and A1 pyramidal neurons.

In a parallel experiment, we analyzed the effect of restraint stress on glutamatergic synaptic transmission. Restraint stress did not affect the sEPSC frequency and amplitude. Fig. 6A shows a representative sEPSC recording from A1 pyramidal neurons in both restraint stress and control condition. The sEPSC frequencies were 0.9 ± 0.3 Hz in the rats of the stress group and 1.1 ± 0.3 Hz in control rats ($p > 0.05$; $n = 6$, respectively), whereas the amplitude reached to 46.6 ± 6.9 pA in the animals that were subjected to restraint stress and 54.8 ± 7.2 pA in controls (Fig. 6B). Furthermore, the cumulative probability plots of the sEPSC frequency and amplitude confirm that restraint stress did not affect the efficacy of A1 excitatory synaptic transmission (Fig. 6C).

DISCUSSION

The present study shows that repeated restraint stress impaired the auditory attention and GABAergic synaptic efficacy in the rat A1. The first step of our investigation was to analyze the stress levels of trained rats one day after the stress protocol ended. Fig. 2 shows that non-stimulated rats of the stress and control groups had similar corticosterone levels suggesting that trained rats would adapt to 21 days of restraint stress. Previous studies have shown that 3 or 6 h per day of restraint stress significantly increases corticosterone plasma levels during the first week, while in the second and third weeks of restraint stress the increases of corticosterone levels were less pronounced (Galea et al., 1997; Cook and Wellman, 2004). Therefore, if the

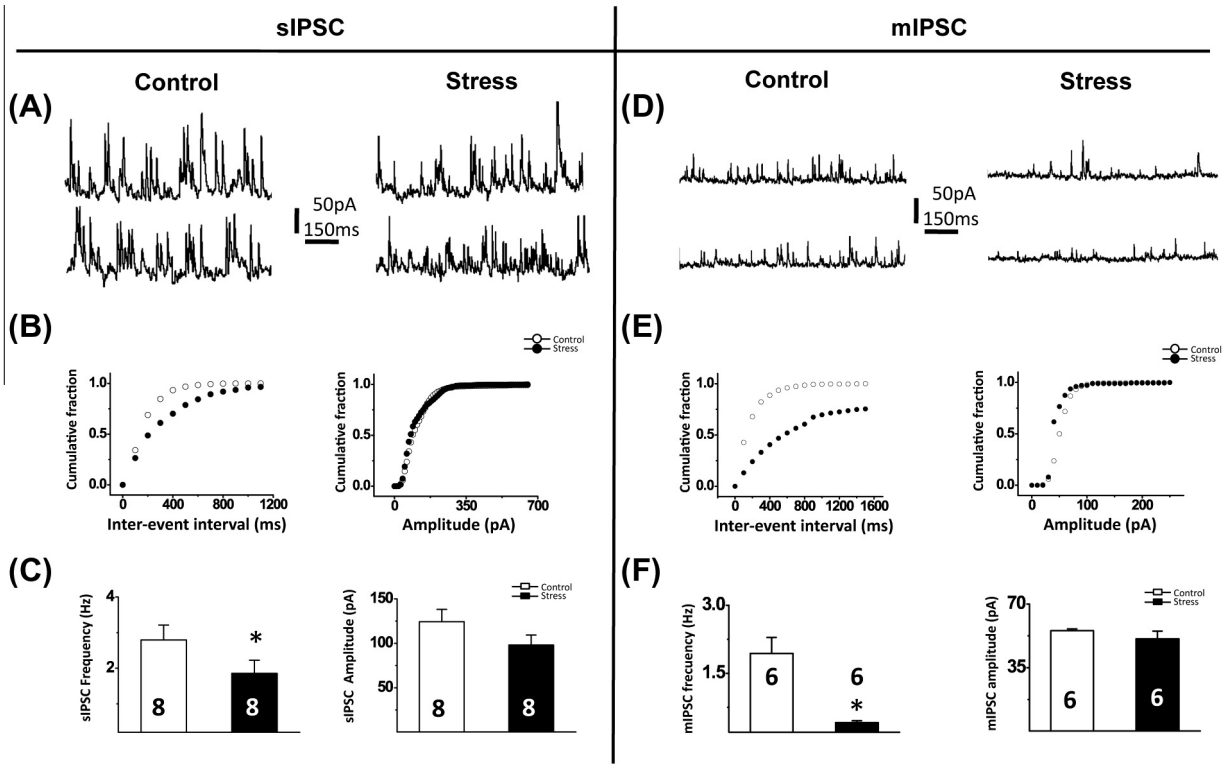

Fig. 5. Restraint stress reduced spontaneous GABAergic activity. (A) Representative sample traces showing sIPSC recorded under control and restraint stress conditions. (B) Cumulative probability histogram of sIPSC frequency and amplitude of control (open circles) and stress (filled circles) conditions. (C) Summary data showing the sIPSC frequency (left) and amplitude (right) under control and stress conditions. The numbers in the bars represent sample size (*n*). (D) Representative traces showing mIPSC recorded for control and stressed rats. (E) Cumulative probability histogram of mIPSC frequency and amplitude of control (open circles) and stress (filled circles) conditions. (F) Summary data showing the mIPSC frequency (left) and amplitude (right) for control and stressed rats. The numbers in the bars represent sample size (*n*).

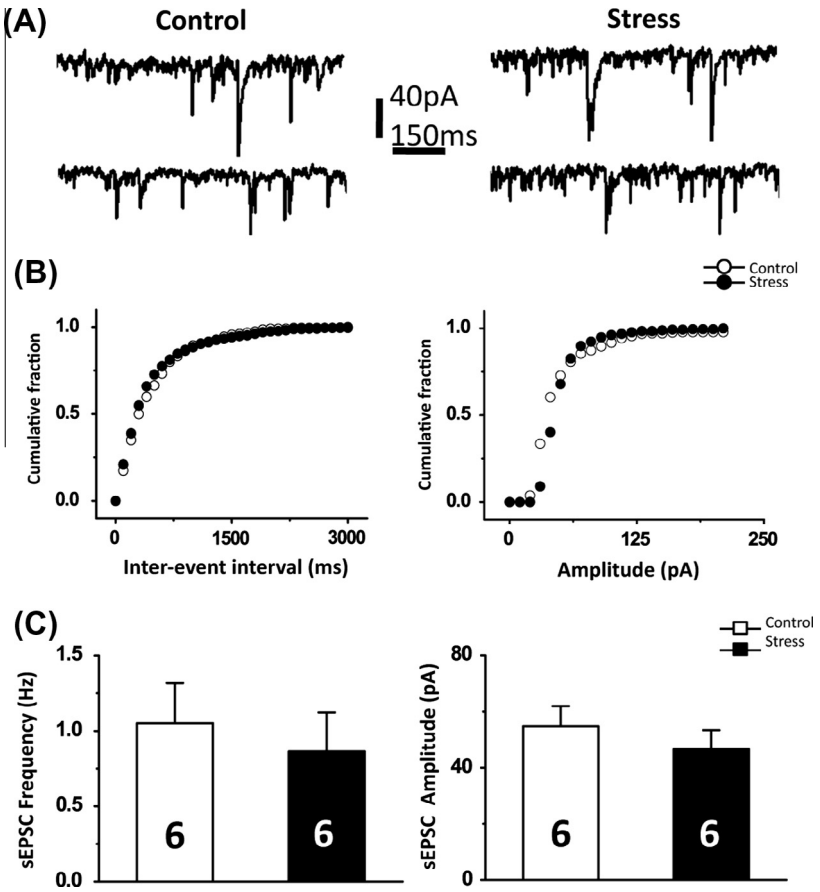

Fig. 6. Restraint stress did not affect Glutamatergic synaptic efficacy. (A) Representative sample traces showing sEPSC recorded under control and stress conditions. (B) Cumulative probability histogram of sEPSC frequency and amplitude of control (open circles) and stress (filled circles) conditions. (C) Summary data showing the sIPSC frequency and amplitude under control and stress conditions. The numbers in the bars represent sample size (*n*).

effects of restraint stress on the HPA axis activity and corticosterone response had been lost during the 21 days of restraint stress, the rats of control and stress groups would have shown comparable plasma corticosterone levels after exposure to a new uncontrollable stressor (acute swimming). However, rats that were subjected to restraint stress had significantly higher plasma corticosterone levels than control rats following one minute of swimming (Fig. 2). The conclusion of this experiment is that one day after restraint stress ended, trained rats of the control and stress groups showed similar HPA axis activity in an environment without stressors. On the other hand, rats of the stress group still showed higher levels of the HPA axis activity than controls animals when were exposed to a new uncontrollable stressor. This neuroendocrine alteration, which induces maladaptive responses to stressors, is characteristic of stressed animals (Tafet and Bernardini, 2003; Ferraz et al., 2011).

After 2-ACT training, chronic stress reduced the percentage of body weight gain compared to that of control animals (Fig. 1C). In addition, restraint stress enhanced anxiety in the elevated plus-maze (Table 1), as reported previously for physiological and behavioral stress markers (Dagnino-Subiabre et al., 2009).

Experiment 2 analyzed whether restraint stress affects the locomotor activity and spatial memory of trained rats. Restraint stress did not affect the distance traveled and average speed in the open field (Table 1). As well stressed- and control rats did not show differences in the number of total arm entries in both the elevated plus-maze and Y-maze (Table 1), indicating that restraint stress did not affect the locomotor activity. Moreover, restraint stress significantly decreased the percentage of DS in the Y-maze. This result demonstrates that restraint stress impaired the spatial memory of trained rats (Table 1). Comparable stress paradigms, such as immobilization and chronic unpredictable stress, show that stress-induced impairment on the spatial recognition memory is correlated with dendritic atrophy of the CA3c pyramidal neurons and decreases of the neurogenesis in the *dentate gyrus* of the hippocampus, a main brain area for the spatial memory (Vyas et al., 2002; McLaughlin et al., 2007).

Effects of restraint stress on the auditory attention

The stress-induced enhancement of anxiety and spatial memory impairment did not affect rat performances in

the 2-ACT during the first trials (Fig. 3A). Positive values for the DS-CT were associated with an increase in correct trials after the stress period compared to correct trials before restraint stress. Stressed rats had a DS-CT of −9.4 ± 0.6 correct trials (Fig. 3B), indicating that restraint stress decreased 2-ACT performance by 18.8%. Therefore, stressed rats had over 80% correct trails before restraint stress and 61.2% after the stress period. This result demonstrates that stressed rats recalled the task because 2-ACT performance over 60% indicates the animals know to respond with right pokes for low tones and left pokes for high tones. In support of this idea, animals of control and stress groups showed similar DS-CT during the first 10 trials of the 2-ACT. If restraint stress impaired the memory related to 2-ACT, which was consolidated before stress period, the stressed rats should have had negative values for the DS-CT during all the first 10 trials of the 2-ACT and significantly fewer than 50% correct trials after the stress period, similar to the performances at the beginning of the second week of training. Interesting, most of stressed animals significantly decreased their 2-ACT performances after the first ten trials (Fig. 3A). This result suggests that auditory attention was significantly impaired in the stressed rats compared to that in control animals (Fig. 3A–C).

After the stress period, the DS-(L-CT/CT) significantly decreased in the rats of the stress group (Fig. 3D, E). Thus, control rats took more time in each 2-ACT trial to achieve a correct response. It was previously reported that neuronal activity in A1 regulated the rat performance in the 2-ACT (Jaramillo and Zador, 2011). In addition, the balance between the excitatory and inhibitory systems is essential for cortical functions such as auditory attention (Buzsaki and Chrobak, 1995; Cobb et al., 1995; Isaacson and Scanziani, 2011). Therefore, we speculate that the stress-induced dendritic atrophy in A1 (Bose et al., 2010) and decreasing of the probability of GABA release (Figs. 4 and 5) may affect the balance between excitation and inhibition in A1; which in turn impairs the sensory representation and perception of the stressed rats through the 2-ACT. Under these conditions, tone frequency discrimination and response accuracy, and auditory attention decrease in rats subjected to restraint stress (Fig. 3A–C).

Rats from the stress group used significantly more time during ITI than the control animals (Fig. 3F, G). It is possible that an increase in DS-CT in controls rats improves the motivation to perform the auditory task. In contrast, the rats of the stress group did not change their motivation to perform the auditory task after restraint stress (Fig. 3F, G).

Effects of restraint stress on synaptic transmission in A1

It has been in this study that repeated restraint stress decreased GABAergic transmission in A1 without affecting glutamatergic transmission (Figs. 4–6). According to the presynaptic locus of expression, we observed that restraint stress decreased the frequency of sIPSC and mIPSC in A1, while the amplitude of sIPSC and mIPSC did not change after restraint stress. However, the cellular mechanisms that underlie this form of stress-dependent GABAergic depression in A1 are currently unknown.

It has been shown that chronic stress affects GABAergic synaptic transmission at the subcortical level (Hu et al., 2010a; Rodríguez Manzanares et al., 2005). Recent evidence suggests that both stress and the glucocorticoids affect GABAergic synaptic efficacy (Hu et al., 2010a), which could be due to direct actions via the neuronal glucocorticoid receptors. Glucocorticoid receptors are expressed widely in the brain; including in layers II–III of the neocortical regions (Sah et al., 2005). Corticosterone binds to cytosolic glucocorticoid receptor, thereby increasing the gene expression of proplastic genes, such as neuronal cell adhesion molecules, NCAM and L1 (De Kloet et al., 1998; Sandi, 2004; Meltser and Canlon, 2011). These molecules are implicated in neurite extension, cell survival and synaptic plasticity (Kiss et al., 2001). Thus, restraint stress may down-regulate the expression of glucocorticoid receptors and proplastic genes in A1 and affect inhibitory parvalbumin-positive neurons and pyramidal neurons, both types of neurons present in A1 (Letzkus et al., 2011). In support of this idea, chronic stress reduces the number of inhibitory parvalbumin-positive neurons in the hippocampus (Hu et al., 2010b) and causes atrophy of the basilar dendrites of pyramidal neurons in layers II and III of A1 (Bose et al., 2010).

Both the stress-dependent GABAergic depression in A1 demonstrated in this study (Fig. 5) and the dendritic atrophy induced by chronic stress in A1 (Bose et al., 2010) can modify the threshold of synaptic plasticity and induce an imbalance between the excitatory and inhibitory systems in A1, which in turn, could have a long-term effect on rat performance on the 2-ACT, specifically by decreasing the auditory attention of the rats that were subjected to restraint stress with respect to control animals in the last forty trials of the 2-ACT, as shown in Fig. 3.

The acoustic environment has a key role in the development of cortical circuits and auditory synaptic plasticity. This process is strongly modulated by the balance between excitation and inhibition that determines the development of the synaptic receptive field in A1 (Sun et al., 2010). In this context, sensory experience induces the refinement of intracortical inhibition, which regulates the organization and functioning of A1 (Dorrn et al. 2010; Sun et al., 2010). Therefore, if balanced excitation and inhibition is necessary to the temporal precision of neuronal activity in A1, restraint stress may disrupt the excitatory-inhibitory balance and change the auditory plasticity affecting complex cognitive functions associated with A1 such as auditory attention.

Agonist for alpha 7 nicotinic receptors increases the probability of GABA releases (Arnaiz-Cot et al., 2008). These receptors are expressed in A1 (Broide et al., 1995) and could increase GABA release in A1 of the stressed rats. Thus, agonist for alpha 7 nicotinic

receptors may improve auditory attention in the stressed rats.

The animal model and the behavioral paradigm to analyze auditory attention used in our research may be useful to study the cellular and behavioral mechanisms associated with attentional deficit in humans with psychosocial stress (Simoens et al., 2007) and patients suffering stress-related disorders. For example, patients with Post-Traumatic Stress Disorders show attentional disturbances (Kimble et al., 2010) and reduction in pre-attentive auditory sensory memory (Kimble et al., 2010). In addition, patients with major depressive disorders show impairment in early auditory processing (Kähkönen et al., 2007).

CONCLUSION

The data presented here demonstrates that repeated restraint stress impairs auditory attention in rats. This result correlates with decreased GABAergic synaptic efficacy in the A1. We propose that stress-induced inhibition of GABA release in A1 could be triggered by the effect of glucocorticoids on synaptic plasticity in A1 during the stress period. This novel molecular mechanism may underlie the effects of restraint stress on auditory attention in rats.

Acknowledgements—This research was supported by FONDECYT 1100413 grant (Dagnino-Subiabre) and FONDECYT 11090059 grant (Fuenzalida), and CID 01/2006 DIPUV. We would like to thank Drs. Marco Atzori (University of Texas at Dallas) and Manuel Roncagliolo (Universidad de Valparaíso) for insightful discussions and critical review of the manuscript.

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(Accepted 21 April 2013)
(Available online 30 April 2013)