EFFECT OF CHRONIC STRESS ON PREFRONTAL CORTICAL FUNCTION

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ABSTRACT

The prefrontal cortex (PFC) is a brain region thought to mediate cognitive functions such as working memory. Chronic stress has been shown to reduce working memory. In this thesis study, the effect of chronic stress on PFC functions was assessed in adult rats.

First, contrary to previous evidences, chronic stress induces working memory performance alterations differentially in two populations of rats. One group displayed a decrease of performance only at 30 second delay, while the other had a decrease and increase at 0 and 30 seconds respectively.

Then, the effect of chronic stress on synaptic plasticity induction in the hippocampus-PFC network was investigated. High-frequency tetanic stimulation (HFS) of the dorsal hippocampus that induced long-term potentiation (LTP) in the prelimbic and infralimbic cortex in normal conditions was unable to induce LTP after chronic stress in the infralimbic cortex, whereas long-term depression (LTD) instead of LTP was induced in the prelimbic cortex.
Given that synaptic plasticity has been shown to depend on NMDA receptors in the PFC, NMDA subunit expressions before and after chronic stress was examined. There was a decrease of NR1 subunits expression in the prelimbic, but not infralimbic cortex. In contrast, the NR2A/NR2B ratio was increased in the infralimbic, but not prelimbic cortex. These results suggest that chronic stress disrupts PFC functions through dynamic modulation of distinct neural networks within the PFC.
RÉSUMÉ

Le cortex préfrontal (PFC) est une région du cerveau qui contrôle les fonctions cognitives comme la mémoire de travail. Dans cette thèse, l'effet du stress chronique sur des fonctions du PFC a été analysé chez des rats adultes.

Premièrement, les performances de la mémoire de travail ont été mesurées avant et après exposition au stress chronique. Nous avons constaté que le stress chronique induit des changements de performances de la mémoire de travail différemment selon deux populations de rats. Une des populations a démontré une diminution de performance seulement à 30 secondes de délai. Au contraire, l'autre a démontré une diminution de performance à 0 seconde et une amélioration de performance à 30 secondes.

En plus, nous avons évalué l'effet du stress chronique sur l'induction de la plasticité synaptique dans le réseau reliant l'hippocampe au PFC. Dans les conditions initiales, une stimulation tétanique à haute fréquence (HFS) dans l'hippocampe dorsal provoquait une potentialisation à long terme (LTP) dans le cortex prélimbique et infralimbique. Or après exposition au
stress chronique, une stimulation tétanique à haute fréquence n’a pas entraîné de potentialisation à long terme dans le cortex infralimbique. De plus, une exposition au stress chronique a provoqué l’apparition dans le cortex prélimbique d’une dépression à long terme (LTD) plutôt qu’une potentialisation à long terme.

Étant donné que la plasticité synaptique dépend des récepteurs de NMDA dans le PFC, nous avons examiné l’expression de sous-unité de NMDA avant et après exposition au stress chronique. En accord avec les changements synaptiques distincts de plasticité entre le cortex prélimbique et infralimbique après exposition au stress chronique, nous avons observé que l’expression de la sous-unité NR1 a diminué dans le prélimbique, mais non dans l’infralimbique. En revanche, le ratio de NR2A/NR2B a augmenté dans le cortex infralimbique, mais non dans le prélimbique. Ces résultats suggèrent que le stress chronique perturbe les fonctions du PFC par la modulation dynamique des réseaux distincts neurologiques dans le PFC.
INTRODUCTION

The prefrontal cortex (PFC) is among the most recent addition to our brain in terms of evolution and has evolved into one of the largest brain structure in humans. It is the latest part of the brain to mature as we develop (Fuster 2002). The PFC mediates an assortment of higher cognitive functions (Arnsten 2009) such as working memory, behavioral flexibility, attention, decision making, and future planning.

ANATOMY AND FUNCTION OF THE PREFRONTAL CORTEX

The PFC mediates diverse cognitive and affective functions with its extensive connections to other cortical and subcortical regions. In primates, these connections are arranged in a topographical manner, distinguishing subareas within the PFC, such as the ventromedial PFC regulating emotional processes by interaction with the amygdala (Price and Amaral 1981; Price et al. 1996; Ghashghaei and Barbas 2002). Whereas, the dorsolateral part of the PFC mediates cognitive functions through interactions with the hippocampus, the thalamus, and other cortical areas such as the parietal cortex (Nathaniel-James and Frith 2002). The PFC of rodents is slightly different from that of humans, such that the medial PFC, which refers as the prelimbic and infralimbic regions,
is thought to be analogous to the dorsolateral and the ventromedial PFC of primates, respectively (Vertes 2004; Hoover and Vertes 2007).

While the prelimbic and infralimbic regions are anatomically and functionally overlapping brain areas, there are also significant differences. Precisely, the infralimbic cortex possess stronger synaptic interactions with limbic areas compared to the prelimbic cortex; suggesting that the infralimbic cortex is more strongly involved in affective information processing, whereas the prelimbic cortex primarily mediates cognitive functions (Hoover and Vertes 2007).

The prelimbic cortex demonstrates action-outcome association capability, which is critical for goal-oriented behavior (Dickinson 1994), i.e. the actions taken are meant to achieve a specific goal, as opposed to just habits that are automatic-responses to stimuli. In contrast, the infralimbic cortex plays a role in stimuli-responses associations through driving of the basal ganglia, leading to the formation of habits after extensive training (Dickinson 1995). Another line of evidences suggests that the prelimbic cortex is required for “expression”, whereas the infralimbic cortex is crucial for “inhibition” of learned behaviors including habits (Peters et al. 2009). For example, using animal models of drug addiction, it has been shown that the prelimbic cortex is required for the initial acquisition as
well as reinstatement (relapse after behavior extinction) of drug-seeking behavior, while the infralimbic is required for extinction of drug-seeking behavior (Peters et al. 2008). Similarly, affective behaviors are also differently mediated by the prelimbic and infralimbic cortices, the prelimbic cortex mediating fear expression induced by stress (Baeg et al. 2001), while the infralimbic cortex mediating fear extinction (Morgan and LeDoux 1995).

**DOPAMINE**

Dopamine (DA) is one type of catecholamines, which modulates synaptic transmission. DA neurons are located in the ventral tegmental area (VTA) and substantia nigra pars compacta in the brainstem and projects to brain areas including the PFC, the hippocampus (HPC), and the striatum. The PFC receives DA innervations from the VTA. The PFC in turn sends excitatory projections into DA neurons of the VTA. Together, these connections organize a reciprocal loop regulating DA release in the PFC. This mesocortical DA system has been identified to be critical for PFC functions.

DA release occurs in two different processes; tonic and phasic release. A tonic release determines the low background level of DA concentration
which is thought to be important for cognitive functions (Raiteri et al. 1979; Borland and Michael 2004), whereas a phasic release is an intrasynaptic, transient, and massive release of DA that is evoked by salient environmental stimuli (Grace 1991).

DA binds and activates DA receptors grouped into two main categories: (1) D1-class: D1 and D5, (2) D2-class: D2, D3 and D4. All DA receptors are metabotropics and activate or inhibit intracellular signaling cascades. The D1-class receptors activate, whereas D2-class receptors inhibit intracellular signaling network consisting of adenylate cyclase, cyclic adenosine monophosphate, and protein kinase A (Seeman 1987). The resulting outcome of DA receptors stimulation and intracellular signaling is diverse; however, it generally results in increased cellular activity and plasticity for the D1-like receptors stimulation and vice versa for the D2-like receptors.

**CATECHOL-O-METHYLTRANSFERASE**

Catechol-O-Methyltransferase (COMT) is an enzyme which enables degradation of DA, and is one of many molecular candidates suggested for schizophrenia (Weinberger 1995; Mannisto and Kaakkola 1999; Weinshilboum et al. 1999; Harrison and Weinberger 2005). In both
rodents and humans, COMT is highly expressed in the PFC and HPC, while expressed in lower amount in the striatum; suggesting that DA release in the PFC is primarily removed by COMT, whereas DA release is removed by other mechanisms (i.e. dopamine transporters) in the striatum (Kastner et al. 1994; Matsumoto et al. 2003).

**TYROSINE HYDROXYLASE**

Tyrosine hydroxylase (TH) is a rate limiting enzyme for the biosynthesis of DA and noradrenaline (NA) (Bolte Taylor et al. 1998). Immunohistochemistry staining is commonly used to reveal DA innervation in the PFC. Post-mortem studies of schizophrenia brains have shown reduced TH expression in the PFC (Akil et al. 1999; Venator et al. 1999), suggesting that DA release is decreased in the PFC of schizophrenia patients. Another method using glutaraldehyde-conjugated DA staining to labels DA fibers in the PFC demonstrated that animals reared in impoverished conditions show reduced DA fiber labeling, while animals raised in enriched environment show increased DA fiber staining. As well, these changes correlate with improved working memory performance in delayed alternation task (Winterfeld et al. 1998).
Taken together, COMT and TH play major roles in regulation of DA release in the PFC and alterations of these molecules can affect PFC functions.

**WORKING MEMORY**

Working memory refers to a type of memory with which we are able to retain information only transiently, such as a phone number until we dial it after which the memory vanish rapidly. For example, working memory test such as delayed-alternation task is commonly used to examine working memory function in rodents. In this task, animals have to enter into the left and right arms of the maze alternately to obtain reward, such that the animals have to temporarily remember the arm they entered in the previous trial. Moreover, the task also requires animals to learn the rule of the task to alternately enter into the left and right arms of the T-maze, which is associated with long-term memory. Using this task, it has been shown that short-term retention of information critically depends on the DA tone in the mPFC and more specifically an optimal level of DA D1 receptor stimulation (Vijayraghavan et al. 2007). It turn out, too little or too much DA release impairs performance, while an adequate level of DA provide the best performances, which refer to the inverted-U shape hypothesis (Seamans and Yang 2004; Williams and Castner 2006; Goto et al. 2007).
The glutamatergic transmission, specifically NMDA receptors in the dorsal HPC, was also implicated in spatial working memory (McHugh et al. 2008). Acute stress enhances glutamate transmission through insertion of both AMPA and NMDA receptors at the cell membrane (Yuen et al. 2009). The effect of D1 receptor activation in the PFC also depends on NMDA receptor functionality (Rios Valentim et al. 2009). The NR2B subunit was demonstrated to decrease in the PFC after injection of corticosterone, however a more complete picture with other NMDA receptor subunits has yet to be drawn (Gourley et al. 2009).

SYNAPTIC PLASTICITY

Synaptic plasticity is the cellular mechanism underling dynamic changes in the strength of synapses. Molecular and cellular mechanisms of long-term potentiation (LTP), long-term depression (LTD), persisting facilitation, and attenuation of synaptic transmission induced by electrical stimulation of synapses have been extensively studied as models of physiologically-induced synaptic plasticity.

DA plays a major role in modulation of synaptic plasticity induction in the PFC, striatum, and limbic structures. In the PFC, it has been shown that LTD is preferentially induced with high-frequency tetanic stimulation in the
in vitro slice preparation, which is mediated by activation of either D1 or D2 receptor. However, recent studies reveals that such preferred LTD induction with DA application is due to a decreased background DA tone in brain slice, and that supplying exogenous DA into the slice recover LTP induction (Otani et al. 2003; Matsuda et al. 2006), similar to that reported in the in vivo brain (Gurden et al. 1999). It was further demonstrated that this mechanism is mediated by co-activation of D1 and D2 receptors and subsequent activation of post-synaptic extracellular signal-regulated kinases (ERKs) (Kolomiets et al. 2009). In the in vivo condition, D1 antagonist pre-treatment blocked LTP induction, while D1 agonist accentuated LTP. In contrast, D2-like antagonist does not affect LTP induction (Gurden et al. 2000; Ishikawa et al. 2005), whereas D2 agonist attenuates LTP (Goto & Grace 2006). In support of D1 modulation of LTP induction, D1 receptors activation, through a PKA-dependent mechanism, lead to surface expression of GluR1-containing AMPA receptors at PFC extra-synaptic compartment, which can further be trafficked into synapses when NMDA receptors are activated (Sun et al. 2005); thereby facilitating excitatory synaptic drives of PFC neurons.

Synaptic plasticity induction in the PFC also requires activation of N-methyl-d-asparate (NMDA) receptors. Although, non-NMDA receptors mediated synaptic plasticity specifically exist for LTD (Matsuda et al.
NMDA receptors are one of glutamate’s receptors linked to ion channels permeating calcium into neurons upon activation. NMDA receptors are composed of four subunits, two of which have to be NR1, while the other two subunits can be any of the following: NR2A, NR2B, NR2C, NR2D, and NR3 (Cull-Candy and Leszkiewicz 2004). The key element about the function of NMDA receptors is the presence of a magnesium ion that blocks the flow of calcium unless the post-synaptic neuron is already depolarized prior to stimulation of NMDA receptors by glutamate release.

The NR2A and NR2B subunits are the most prevalent in the PFC according to the Allen Brain Atlas website. The expression of NR2A subunits increases throughout development of the brain and has been implicated in glutamate transmission in mature synapses (Barria and Malinow 2002). Meanwhile the NR2B subunits are highly expressed early in neurodevelopment and decrease in mature synapses, which has been implicated in synaptogenesis of silent synapses (Bellone and Nicoll 2007). Silent synapses contain all the required cellular machinery to function, yet they lack AMPA receptors such that synaptic transmission does not occur, but they can quickly become fully functional by insertion of AMPA receptors (Isaac et al. 1995). Furthermore, blocking the NR2A subunit was shown to block LTP but not LTD induction, while the opposite was true for
NR2B in which LTD is inhibited but not LTP (Liu et al. 2004), although this finding is challenged by many controversial studies, and therefore selective involvements of NR2A and NR2B on LTP and LTD is still inconclusive. The effect of tetanic stimulation of dorsal HPC afferent toward the medial PFC demonstrated a decrease of LTP amplitude after chronic stress (Cerqueira et al. 2007); however, a more careful examination of the prelimbic and infralimbic cortex within the medial PFC could be interesting given their different functional role.

**STRESS**

Stress was defined as “an animal’s state of threatened homeostasis... The disturbing forces or threats to homeostasis [are] called stressors and the counteracting re-establishing forces [. . .] adaptive responses “ (Chrousos 1998). When stress is unavoidable, the hypothalamic-pituitary-adrenal (HPA) axis is activated in order to counter-act the stressor (Engelmann et al. 2004). The activated HPA axis results among many other things in the release of cortisol in human and corticosterone in rodents.

However, if the stress is avoidable by fight or flight responses, the response to stress mainly activates another circuit: the sympatho-adrenergic system (SAS). The end results of this SAS activation is the
release of NA and epinephrine in the blood stream. In both case, it is believed that the goal of the SAS and HPA activation is to enhance the rate of learning and neural circuit plasticity in order to remember better the events that caused this elevated stress, such that we can prevent or cope with it better if similar events occur in the future (Engelmann et al. 2004). While single exposure of moderate stress, which we refer to acute stress, can be beneficial, prolonged chronic stress is believed to cause maladaptive responses.

Acute psychological stress activates the amygdala and stress-response pathways in the hypothalamus and brainstem, by which both NA and DA releases are evoked. Acute stress has been shown to increase DA release in the PFC (Morrow et al. 2000) peaking between 15 and 20 minutes and return to the baseline level thereafter even while animals are still under stress exposure. DA neurons also exhibit increased burst spike firing up to 24 hours after acute stress induction (Anstrom and Woodward 2005). The phenomenon is partly due to the surface expression and increased mobility of AMPA receptors in the cell membrane, which was shown to occur after corticosterone treatment of HPC cultures (Martin et al. 2009).
Further, lesion studies of the central nucleus of the amygdala disrupt the usual DA release evoked by stress in the PFC. (Davis et al. 1994). The increase of DA release in the PFC after moderate amount of controllable stress appears to improve cognitive functions, and therefore, this could be the mechanism of avoidance of the stressful stimuli (Giorgi et al. 2003). However, uncontrollable stress with excessive DA release disrupts cognitive functions. In monkeys, stress induces a deficit of PFC function due to over-stimulation of D1 receptors and NA alpha-1 receptors, due to abnormal increase of DA and NA release (Arnsten 2000). Not only is NA and DA release increased by stress, DA release is also increased by rewards, which link it to goal-oriented behaviors (Taber and Fibiger 1997). Thus, DA release is not only associated with reward, but also linked with aversive stimuli, which suggest that DA facilitate plasticity for enhanced learning of goal-directed behavior to obtain rewards as well as to avoid aversive events.

The PFC is a major brain area regulating the stress response, but differently between the prelimbic and infralimbic cortices. Prelimbic lesions results in increased levels of c-fos expression and corticotrophin-releasing factor (CRF), a biological marker that is known to be increased with emotional stress, in the paraventricular nucleus of the hypothalamus during acute restraint-induced stress. Whereas infralimbic lesion do not
results in increased c-fos or CRF expression in the same region, suggesting that the prelimbic cortex is important for deactivating the stress response (Herman et al. 2005; Radley et al. 2006).

Two to three weeks of chronic repeated stress exposures induces a decrease of DA tone in the mPFC (Gresch 1994; Gresch et al. 1994; Mizoguchi et al. 2000). Furthermore, a large number of DA neurons in the VTA had lower spontaneous tonic spike firing following seventeen days of chronic cold stress (Moore et al. 2001), consistent with decreased tonic DA tone in the PFC after chronic stress. Three weeks of chronic stress restraint (6 hours/day) exposure induces dendritic spines atrophy in PFC (prelimbic) neurons (Radley et al. 2008). In contrast, brief uncontrollable stress exposure (1 to 3 day swim stress) has been shown to induce retraction of terminal branches of apical but not basilar dendrites in the infralimbic area, while no discernable changes are observed in the prelimbic cortex (Izquierdo et al. 2006), suggesting distinct stress vulnerability of PFC neurons morphology between prelimbic and infralimbic cortex.

Reports show that corticosterone treatment can increase NMDAR subunits expression in the hippocampus (Weiland et al. 1997). However, it is
unclear how the various NMDAR subunits are altered during chronic stress in the prelimbic and infralimbic region of the PFC (Gourley et al. 2009).

**Schizophrenia**

Schizophrenia, one of many mental diseases affected by stress, displays deficits in cognitive functions, such as working memory (Lenzenweger et al. 1991; Goldman-Rakic and Selemon 1997). It has been hypothesized that schizophrenia involves hypofrontality associated with mesocortical DA activity reduction, which in turn increases DA release in the striatum, causing imbalance of DA release between the PFC and striatum (Grace 2003b; Boyce and Finlay 2005). More importantly, the PFC is very susceptible to the effect of stress and cause abnormality in cognitive functions (Arnsten 1998). Emotional stress plays a significant role in triggering the symptoms of schizophrenia (Rabkin 1980). It appears that vulnerability to stress can be used as a potential marker for schizophrenia (Docherty et al. 1996), because several studies demonstrated overactive stress responses in schizophrenia patients (Gruzelier 1981; Gruzelier et al. 1981; Dawson 1992; Dawson et al. 1992).
Thus, understanding the effect of stress on PFC cognitive functions could lead to new methods for treating psychiatric disorders such as schizophrenia.

HYPOTHESIS

The general hypothesis is that chronic stress induces alterations in the neuronal circuits of the PFC which impairs working memory. The neuronal circuits’ alterations may be due in part through modifications of glutamatergic neurotransmission such as an imbalance of NR2A/NR2B ratio composing NMDA receptors responsible for initiating synaptic plasticity.

Aim 1: To examine how chronic stress affects working memory performance in a delayed-alternation task.

Hypothesis: Chronic stress should induce more severe working memory impairments at longer delays of 30 seconds, while having smaller effects at 0 and 10 seconds.

Previous experiments demonstrated working memory impairments at 0 second delays, but it should be expected that as the task become more difficult with longer delays, the impairments should become more
significant. However, it is also known that other brain structures such as the hippocampus are more critical than the PFC at five minutes delays. It is unclear at which time point, the switch occur, and if the effect of chronic stress plays on this phenomenon. For these reasons, the results of this first aim will provide the ground work for the planning of further experiments.

AIM 2: TO EXAMINE HOW CHRONIC STRESS AFFECTS SYNAPTIC PLASTICITY INDUCTION (LTP) IN THE mPFC.

Hypothesis: The prelimbic cortex should have impaired synaptic plasticity capacity after chronic stress, while the infralimbic cortex should be much less impaired.

Since the prelimbic cortex is involved in goal-oriented learning, which is less essential during stressful events, synaptic plasticity of this region should be impaired and even suffer from neuronal circuit weakening during chronic stress. On the other hand, the infralimbic cortex is involved in habits learning, which is critical during stressful events. Thus, during chronic stress, the infralimbic cortex should maintain a normal degree of synaptic plasticity. Previous studies demonstrating the reduction of NMDA receptors expression in the PFC might be misleading, as such this aim will provide more precise data regarding each subregions of the mPFC. This
ground work is also necessary in order to perform pharmacological treatment to pinpoint the exact molecular entity critical.

**AIM 3: TO EXAMINE HOW CHRONIC STRESS AFFECTS NMDAR SUBUNITS EXPRESSIONS IN THE mPFC.**

Hypothesis: The NR1 subunit should be decreased in the prelimbic cortex more so than in the infralimbic cortex. As well the ratio of NR2A/NR2B should be altered for both regions.

Given previous studies demonstrating NR2B reduction in the PFC after corticosterone treatment, it is of interest to assess all major NMDA receptors subunits for both subregions of the mPFC. However, considering the controversies regarding the role of NR2A versus NR2B for synaptic plasticity, it is unclear how to make accurate predictions. Meanwhile, it is of critical importance to obtain data regarding this topic in order to eventually clear up this controversy. Once pharmacological treatment experiments targeting NMDA receptors, will be conducted following aim 2, it should provide strong evidence for the actual role of NR2A and NR2B for synaptic plasticity in relation with chronic stress.
METHODOLOGY

Figure M1. Behavior Experimental Design.

ANIMALS

All experiments were conducted using male Sprague-Dawley rats in strict accordance with the Canadian Council on Animal Care (CCAC) guidelines, and were approved by the McGill University Animal Care and Use Committee.

Animals started the experiments between 275-300 grams. Food was restrained during behavioral training and testing to maintain above 85% of body weight. Water was available ad libitum, except when rats were restrained, they were unable to move to drink.
Handling was first performed for 20 minutes per day for three consecutive days to habituate the animals to the experimenters.

**STRESS INDUCTION**

Chronic stress was induced by immobilization stress by placing the animals in clear plexiglass restrainers for six hours a day for 21 days.

**WORKING MEMORY TEST**

After animal handling, three days of maze apparatus (8 arms radial maze, Model 89001B, Lafayette Instrument) habituation was performed, such that animals were allowed to explore the maze freely in order to recover fruit loops for 20 minutes.

Training was then initiated until animals were able to eat 24 fruit loops within 30 minutes without returning to the central segment of the maze to eat. This training period varied between one to two weeks depending on the animals, after which the testing phase was ready to start. Each day animal received one training session composed of 24 trials with a delay of 0 seconds between each trial. The initial trial of each training session was
randomly selected for bait to start in either left or right, after which each trial would alternate which arm is baited. Baited arm was opened; while unbaited arm was close to teach the animals to alternate.

A delayed alternation task in a T-maze was used to examine the effect of stress on PFC-dependent working memory. Animals were first trained to achieve above 85% success rate in the 0 second delay condition, which was then considered day 1 of testing. The food reward was not put in the opposite arm unless the animal succeeded the trial, thus animals could perform more than 24 trials, but would never eat more than 24 rewards. Delays between trials varied between 0 (no delay – Day 1), 10 (Day 2), and 30 seconds (Day 3). Animals were placed in their home cage during the delay. Animals were tested again following chronic stress for each delay for 3 days. Acute stress performance was collected, but the results are not presented in this chronic stress oriented thesis. Animals were sacrificed after the last day of training.

**Western Blot**

Six control and six chronic stressed animals were deeply anesthetized with pentobarbital (100mg/kg, I.P.) and decapitated. Brain tissue was frozen in dry ice less than five days before homogenizing the sample with lysis
buffer modified from a RIPA buffer (50 mM Tris-HCl PH 7.4, 1% Triton X-100, 0.2% Na-deoxycholate, 0.2% SDS, 1mM Na-ethylenediaminetetraacetate). Proteinase inhibitor (Fisher, PI78415, 1:100) was used directly before performing the lysis step. Cells were then ground mechanically and further sonicated for ten minutes with a Fisher sonicator FS20 in ice cold water. Supernatant was collected after centrifugation at 4°C and processed with Bradford reagent for protein concentration measurement. Samples were adjusted to even concentration 2 ug/ul. We used an ECL Plex Rainbow molecular marker (CA95040-080L Ge Healthcare) and Invitrogen NuPage Western blot equipment with NuPage 4-12% BisTris gels.

Antibodies were mixed with 2% MPBS and 0.05-0.1% Tween. NR2B Millipore 06-600 were diluted at 1:500; NR2A Millipore 07-632 at 1:2000, tubulin SIGMA at 1:40000 MAB5566, and finally NR1 SIGMA at 1:5000. Incubation lasted overnight at 4°C before secondary antibody treatment. Dilution for secondary antibody was 1:3000 for goat antirabbit HRP Abcam ab6721 and 1:2000 for rabbit antimouse HRP Abcam ab6728. Membranes were developed with ECLPlus and visualized on a Typhoon Trio Plus. The medial part of the PFC (prelimbic and infralimbic cortex) was processed for NMDAR subunit, NR1, NR2A, NR2B, expression. NMDAR subunits were quantified as the relative optical density (R.O.D.) to α-tubulin expression.
IN VIVO ELECTROPHYSIOLOGY IN ANESTHETIZED ANIMALS

The effect of stress on synaptic plasticity was investigated in the hippocampal-PFC pathway of seven prelimbic cortex and seven infralimbic cortex control animals, as well as five prelimbic cortex and seven infralimbic cortex chronic stressed animals. All animals were male Sprague-Dawley described above. Animals were anesthetized with Urethane (1g/kg, I.P.) and placed in a stereotaxic frame. Rectal temperature was maintained at 37°C by a homeothermic warming blanket. The experimental procedures for implantation and recording of extracellular local field potentials (LFP) has been described before (Rocher et al. 2004). Briefly, LFP’s were recorded with a glass electrode implanted in the mPFC (5.0 mm below pial surface for infralimbic and 3.0 mm for prelimbic; both 0.5-0.6 mm lateral; 2.7-3.0 mm anterior to bregma) evoked by electrical stimulation (Master-8, A.M.P.I, Israel), using 200µsec test pulse every 30 seconds for a 50-60% of maximal intensity, of the ipsilateral posterior dorsal hippocampus (6.4 mm posterior to bregma; 5.5 mm lateral, 3.0-6.0 mm below pial surface), by concentric bipolar stimulation electrode, according to the atlas of Paxinos and Watson fifth edition. LFP recording was amplified a 1000 times (Differential Extracellular Amplifier, Model ER-1, Cygnus Technology, Inc.) with a high
pass filter (0.1 Hz) and a low pass filter (0.1 kHz). To induce synaptic plasticity, two session of high frequency stimulation (10 trains of 100 pulses of 250Hz at maximal intensity ~1mA repeated a second time after 6 minutes recovery) was administered to the hippocampus which was shown to induce long-term potentiation (LTP) on hippocampal afferents projecting into the mPFC. The data was converted by an A/D converter (Digidata 1440A, Molecular Devices) and analyzed with Axoscope (Molecular Devices) and Matlab (The Mathworks, Inc.).
RESULTS

Aim 1 – Working Memory Performance.

Table 1.1. Working memory performance on a T-maze task at 0, 10 and 30 second delay for control and chronic stress group.

<table>
<thead>
<tr>
<th>RatID</th>
<th>Baseline Score (%)</th>
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Mean | 93.8 | 85.4 | 80.5 | 83.8 | 82.5 | 80.0 |
S.E.M. | 1.0 | 3.1 | 3.8 | 2.6 | 3.2 | 3.8 |

Mean | 95.3 | 87.7 | 75.2 | 91.8 | 84.0 | 79.3 |
S.E.M. | 1.2 | 3.1 | 6.0 | 1.9 | 1.4 | 4.8 |
RESULTS

Figure 1.1. Working memory performance on a T-maze task at 0, 10 and 30 sec delay for Chronic Stress group. (n=13)

Figure 1.1. Working memory performance on a T-maze delayed-alternation task with 0, 10 and 30 second delay between baseline and chronic stress. Performance at 0 second delay did decrease significantly from baseline 93.8±1.0% to 83.8±2.6% after chronic stress, p=0.01. Performance at 10 second delay did not decrease significantly from baseline 85.4±3.1% to 82.5±3.2% after chronic stress, p=0.46. Similarly, performance at 30 second delay also did not decrease significantly from baseline 80.5±3.8% to 80.0±3.8% after chronic stress, p=0.9. Baseline performance demonstrate delay-dependent decrease from 93.8±1.0% at 0 second delay to 80.5±3.8% at 30 second delay, p=0.001. Chronic stress performance on the other hand become non-delay dependent with 83.8±2.6% at 0 second delay to 80.0±3.8% at 30 second delay, p=0.34.]
Based on the data in figure 1.1 and 1.2, we can observe that chronic stress induces a significant decrease at 0 second delay from 93.8±1.0% baseline down to 83.8±2.6% after chronic stress, p=0.01 (all p-values in the figures’ caption represent the p-values of the Fisher’s LSD post-hoc test obtained using the following method). The statistical analysis was performed by an ANOVA design of repetitive measures, such that we
compared pre and post chronic stress for 0, 10 and 30 seconds delay. There was a main effect for the delay (n=13, df=24, \(F=6.13, p=0.007\) for the chronic stress group and n=5, df=8, \(F=11.657, p=0.004\) for the control group). Post-hoc analysis was performed with Fisher’s least significant difference (LSD). These results contradict our initial hypothesis, where longer delays should have been the most affected by chronic stress. Considering that maybe not all rats are created equal, such that the effect of chronic stress may be masked by an opposite trend in different group of animals. Thus, clustering animals into two groups was performed. The criteria used was based on the difference of performance between 0 and 30 seconds delay for both baseline and after chronic stress, data is shown in table 1.1, columns five and nine and plotted in figure 1.3. In order to quantify which animals belong in which groups, a K-means clustering algorithm (Statistica, Statsoft Inc.) was used in which two clusters was specified for analysis. The clustering results were also compared with visual inspection of the scatterplot in figure 1.3. It became clear that the difference in performance before stress is not a good indicator for clustering, meanwhile the difference between 0 and 30 second delay after chronic stress correspond well to the results obtained with the K-means algorithm.
Figure 1.3. Scatterplot showing the difference between 0 and 30 second delay for baseline and after chronic stress. For example, performance at 30 seconds was subtracted from performance at 0 seconds both after chronic stress for each rat to plot the data along the x axis, while before chronic stress data was used for the Y axis. Clusters can be separated along the x-axis around the origin (x=0); however the K-means clustering algorithm classified the value at 0 belonging to the group 2.]
Figure 1.4. Working memory performance on a T-maze task at 0, 10 and 30 second delay for the pattern 1 group. (n=6)

[Figure 1.4. Working memory performance on a T-maze delayed-alternation task with 0, 10 and 30 second delay between baseline and chronic stress for the pattern 1. Performance at 0 second delay did significantly decrease from baseline 94.7±1.7% to 78.3±3.7% after chronic stress (p=0.003). Performance did not change at 10 second delay from baseline 80.0±5.2% to 80.5±2.9% after chronic stress (p=1.0). Surprisingly, performance at 30 second delay did significantly increase from baseline 73.7±6.2% to 89.8±1.5% after chronic stress (p=0.007). Baseline performance demonstrate delay-dependent decrease from 94.7±1.7% at 0 second delay to 73.7±6.2% at 30 second delay (p=0.001). Chronic stress performance remained delay dependent, with 78.3±3.7% at 0 second delay to 89.8±1.5% at 30 second delay (p=0.03).]
RESULTS

Figure 1.5. Working memory performance on a T-maze task at 0, 10 and 30 sec delay for the pattern 2 group. (n=7)

[Figure 1.5. Working memory performance on a T-maze delayed-alternation task with 0, 10 and 30 second delay between baseline and chronic stress for the pattern 2. Performance at 0 sec delay did not significantly decrease from baseline 93.1±1.1% to 88.4±2.5% after chronic stress (p=0.25). Performance did not significantly decrease at 10 second delay from baseline 90.0±2.6% to 84.1±5.2% after chronic stress (p=0.25). However, performance at 30 second delay did significantly decrease from baseline 86.3±3.0% to 71.6±4.6% after chronic stress (p=0.002). Baseline performance demonstrate non-delay dependent change from 93.1±1.1% at 0 second delay to 86.3±3.0% at 30 second delay (p=0.06). Chronic stress performance did significantly decrease from 88.4±2.5% at 0 second delay to 71.6±4.6% at 30 second delay (p=0.0003).]

Statistical analysis of the data in figures 1.3 and 1.4 was performed with an ANOVA design of repetitive measures comparing performance at 0, 10 and 30 seconds delay pre and post chronic stress for both pattern 1 and 2. There was a significant main effect for the interaction term between pre
RESULTS

and post stress, delay and group cluster (n=6 for pattern 1, n=7 for pattern 2, df=22, F=8.895, p=0.001). Post hoc analysis with a Fisher’s LSD was performed on the interaction term to obtain the p-value described for figures 1.4 and 1.5. Overall, there is a significant decrease of performance at 0 second delay from 94.7±1.7% to 78.3±3.7% after chronic stress (p=0.003) for the pattern 1. Surprisingly, there is a significant increase at 30 second delay from 73.7±6.2% to 89.8±1.5% after chronic stress (p=0.007) for the pattern 1. Meanwhile, for the pattern 2, there is only a significant decrease of performance at 30 second delay from 86.3±3.0% to 71.6±4.6% after chronic stress (p=0.002).

AIM 2: LONG-TERM POTENTIATION INDUCTION IN THE mPFC.

[Figure 2.1. LTP induction in the prelimbic cortex for the control group. Normalized amplitude indicates a significant LFP amplitude increase after tetanic stimulation that persisted for more than 90 minutes (p=0.001 for late-LTP).]
RESULTS

Figure 2.2 - LTP induction in the prelimbic cortex: chronic stress group (n=5)

[Figure 2.2. LTP induction in the prelimbic cortex for the chronic stress group. Normalized amplitude indicates a significant decrease after tetanic stimulation that persisted for more than 70 minutes (p=0.003 for early and medium-LTP, p=0.1 for late-LTP).]

Figure 2.3 - LTP induction in the infralimbic cortex: control group (n=7)

[Figure 2.3. LTP induction in the infralimbic cortex for the control group. Normalized amplitude indicates a significant increase after tetanic stimulation that persisted for more than 90 minutes (p=0.008 for late-LTP).]
High-frequency stimulation (HFS) of the dorsal HPC resulted in a significant normalized extracellular LFP amplitude increase ($p=0.001$ for late-LTP) in the prelimbic cortex during the control condition as shown in figure 2.1. The same HFS failed to induce LTP after chronic stress and became an LTD instead ($p=0.02$, $p=0.003$, $p=0.03$ for early, mid and late-LTD respectively). Meanwhile, the infralimbic cortex recordings, demonstrated a strong LTP induction ($p=0.004$ for late-LTP), which was completely attenuated after chronic stress ($p=0.95$ for late-LTP). P-values were obtained by a Fisher’s LSD post-hoc analysis on the statistical results of an ANOVA design of repetitive measures comparing the mean of values of each five minute bins grouped into four 20 minutes blocks representing
RESULTS

baseline, early-LTP, mid-LTP and late-LTP. Significant main effects were seen for all groups (e.g. Prelimbic Control: df=18, F=10.696, p=0.0002) except the infralimbic cortex after chronic stress, suggesting synaptic plasticity is non-existent under those conditions (df=15, F=0.111, p=0.95).

AIM 3: NMDAR SUBUNITS EXPRESSION IN THE MPFC.

Figure 3.1. Infalirilic NMDAR subunits expression by western blot for the infralimbic cortex before and after chronic stress. All p-values were obtained by independent student T-test. NR1 expression did reduce non-significantly after chronic stress (1.13±0.05 for control versus 1.01±0.18 for stress, df=10, t-value=1.54, p=0.16). NR2A expression did reduce non-significantly with chronic stress (0.79±0.07 for control versus 0.71±0.06 for stress, df=10, t-value=2.05, p=0.07). NR2B expression did reduce significantly after chronic stress (0.84±0.08 for control versus 0.65±0.05 for stress, df=10, t-value=4.93, p=0.001).
RESULTS

Figure 3.2. Prelimbic NMDAR subunits expression before and after chronic stress (n=6)

[Figure 3.2. NMDAR subunits expression by western blot for the prelimbic cortex before and after chronic stress. NR1 expression did reduce significantly after chronic stress (1.52±0.14 for control versus 1.23±0.16 for stress, p=0.005). NR2A expression did reduce non-significantly with chronic stress (1.06±0.22 for control versus 0.86±0.22 for stress, p=0.07). NR2B expression did reduce significantly after chronic stress (0.87±0.12 for control versus 0.75±0.08 for stress, p=0.048). Further, NR1 expression was significantly higher in the prelimbic (1.52±0.14 R.O.D.) then the infralimbic (1.13±0.05) (p=0.00008)]

Figure 3.3. NR2A/NR2B expression ratio before and after chronic stress (n=6)

[Figure 3.3. NR2A/NR2B expression ratio by western blot for the infralimbic and prelimbic cortices before and after chronic stress.
stress. For the infralimbic cortex, the NR2A/NR2B ratio did increase significantly from 0.95±0.14 to 1.09±0.07 after chronic stress, p=0.04. For the prelimbic cortex, the NR2A/NR2B ratio did not reduce significantly from 1.22±0.14 to 1.16±0.10 after chronic stress, p=0.40. The NR2A/NR2B ratio is significantly lower in the infralimbic (0.95±0.14) versus the prelimbic (1.22±0.14), p=0.0007).

Western blot for NMDA receptor subunits in the infralimbic cortex revealed a significant decrease of NR2B from 0.84±0.08 in control to 0.65±0.05 after chronic stress (p<0.001), which lead to a significant NR2A/NR2B ratio increase from 0.95±0.14 baseline to 1.09±0.07 after chronic stress (p=0.04). However, there is no significant differences for NR1 subunits 1.13±0.05 for control versus 1.01±0.18 for chronic stress (p=0.16). Meanwhile, the prelimbic cortex displayed a significant reduction of NR1 subunits from 1.52±0.14 for control versus 1.23±0.16 for chronic stress (p=0.005), while the NR2A/NR2B ratio had no significant differences from
1.22±0.14 for baseline to 1.16±0.1 after chronic stress (p=0.4). All p-values were obtained by independent student t-test.
DISCUSSION

The study has shown that chronic stress induces distinct alterations between the dorsal (prelimbic cortex) and ventral (infralimbic cortex) PFC. The behavioral experiments demonstrated that chronic stress effect on working memory performance can be segregated into two groups of rats based on the differences of performance between 0 and 30 seconds delay after chronic stress on the delayed-alternation task. In pattern 1, as shown in figure 1.4, there is a significant performance decrease at 0 second delay, but a significant performance increase at 30 second delay between control and chronic stress. Whereas, the pattern 2, shown in figure 1.5, demonstrate a significant performance decrease at 30 seconds delay between control and chronic stress.

Furthermore, in the control group, LTP induced by HFS in the dorsal HPC that was shown to results in LTP in both the infralimbic and prelimbic cortices was distinctly altered as shown in figure 2.1 and 2.3 respectively. The HFS in the HPC resulted in an attenuated LTP induction in the infralimbic cortex, whereas the prelimbic cortex displayed a conversion from LTP into LTD after chronic stress, as shown in figure 2.2 and 2.3 respectively.
Finally, NMDA receptor subunits expressions, quantified by western blot, were also distinctly altered by chronic stress between prelimbic and infralimbic cortex. With a significant decrease of NR2B and an increase of NR2A/NR2B ratio in the infralimbic cortex as shown in figure 3.1 and 3.3 respectively. Whereas, in the prelimbic cortex, there was a significant decrease of NR2B which was too modest to alter a ratio of NR2A/NR2B, as shown in figure 3.2, and 3.3 respectively. In addition, there was a significant decrease of NR1 subunit in the prelimbic, but not infralimbic cortex, as shown in figure 3.2.

**WORKING MEMORY PERFORMANCE IS ALTERED BY CHRONIC STRESS.**

Based on our data in figure 1.1 and 1.2, we demonstrated that increasing the inter-trial delay from 0 to 10 and 30 seconds decrease performance gradually from 93.8±1.0% to 85.4±3.1% and finally to 80.5±3.8% respectively (p=0.001 between 0 and 30 second delay, n=13). While, there was no differences before and after chronic stress for 10 and 30 seconds, there was a significant decrease at 0 second from 93.8±1.0% in baseline to 83.8±2.6% after chronic stress (p=0.01). The performance decrease at 0 second delay observed is not believed to be due to having three weeks of T-maze navigational inactivity, since performance for the control group in figure 1.2 was not decreased at all. However, these
findings are very surprising, since it does not corroborate past experiments in the field, and it would be expected that increasing the delay should make the task more difficult especially after chronic stress. As such, careful examination of the raw data by computing the difference of performance between 0 and 30 seconds for both baseline and after chronic stress, shown in table 1.1 was performed. It was then possible to display the resulting values in a scatterplot to identify possible clusters of data, which could represent different rodent populations, as shown in figure 1.3. Through visual inspection, it was possible to identify post chronic stress difference to be a better indicator for the clusters then performance difference before stress. However, to corroborate our visual inspection and grouping criteria, a K-means clustering algorithm was used in which two clusters were specified, and the optimal classification grouped our results according to the patterns shown in figure 1.3.

Following the K-means clusters classification of our data, statistical analysis through a mixed ANOVA design composed of a cluster factor with two factors of repetitive measures for pre/post chronic stress and inter-trial delays was performed. As shown in figure 1.4, the first group of rats displayed a significant performance decrease at 0 second delay from 94.7±1.7% baseline to 78.3±3.7% after chronic stress (p=0.003, n=6) combined with a significant performance increase at 30 second delay from
DISCUSSION

73.7±6.2% baseline to 89.8±1.5% after chronic stress (p=0.007). Hypothetically, the performance decrease at 0 second is could be due to a lower DA tone, but the exact mechanism of the increase at 30 seconds is unknown; it is possible that a separate brain regions may take control over the task.

Studies have shown that short-term memory could be mediated by the mPFC and the dorsal HPC in parallel, but at different timescale ranging in the order of ten seconds and five minutes for both regions respectively (Lee and Kesner 2003). While the exact time scale where the switch occurs between the two regions is unknown, these two brain areas are involved in temporal storage of information depending on duration of the retention (Yoon et al. 2008). These studies and our own data could speculatively suggest that animals classified in group one have such a low DA tone that their performance is decreased at 0 seconds after chronic stress because the neuronal circuits in the PFC become too weak to mediate the working memory task. Although speculative as well, at 30 second delay, performance is increased possibly because the competition between the HPC and PFC becomes weaker due to such low activity in the PFC enabling the HPC to take full control over the task. Further experimentations would be required for more insights on this issue. On the other hand, also hypothetically if we consider that animals classified in
the group two expresses a higher DA tone than animals in the group one, it could explain why performance is decreased only at 30 second delay. A possible explanation would be that the DA tone after chronic stress would still be sufficient for appropriate neuronal activity in the PFC at 0 seconds, but would be slightly too weak at 30 seconds leading to competitions between brain regions at that later delay.

Alternatively, it is possible that the DA tone before stress exposure is not different in both populations, but reactivity to stress is, such that animals classified in the pattern two have lower reactivity to stress leading to a smaller decrease of DA tone; however, since performance at baseline for 30 second delay is 73.7±6.2% versus 86.3±3.0% for the pattern 1 and 2 respectively, we believe the reactivity to stress is an unlikely explanation, since performance is already different for both groups before stress.

SYNAPTIC PLASTICITY INDUCTION IS ALTERED DIFFERENTIALLY BETWEEN THE PRELIMBIC AND INFRALIMBIC PFC REGION BY CHRONIC STRESS.

HFS in the dorsal HPC demonstrated an induction of LTP in the prelimbic cortex (p=0.001 for late-LTP, n=7). Statistical analysis was performed with an ANOVA with a repetitive measures design used for each group
DISCUSSION

separately, such that the mean of values for five minutes bins in blocks of 20 minutes (20 minutes blocks: baseline, E-LTP, M-LTP and L-LTP) was used for comparisons. Early-LTP represents the first 20 minutes, whereas mid-LTP represent 35 to 55 minutes, and late-LTP in 70 to 90 minutes after HFS. P-values were obtained from a Fisher’s LSD post-hoc analysis.

As shown in figure 2.2, the HFS used to induces LTP in the prelimbic cortex resulted in LTD (p=0.003 for both early and mid-LTP, while late-LTP had a p-value of 0.1, n=5), suggesting a possible attenuation of neuronal activity in this region after chronic stress. In contrast, the infralimbic cortex demonstrated a strong LTP induction in control animals (p=0.008 for late-LTP, n=7), but LTP induction was impaired after chronic stress (p=0.8, n=7), which suggests infralimbic network is incapable of dynamic network changes after chronic stress. Given the role of the prelimbic area in goal-oriented behavior or expression of learned behavior (Peters et al. 2009), and habits-formation or inhibition of learned behavior with the infralimbic cortex, we can appreciate that conversion of LTP into LTD attenuates expression of learned behavior for goal-directed actions, whereas a loss of synaptic plasticity could provide behavioral inflexibility in order to maintain habits.

**NMDAR subunits are altered differentially between the prelimbic and infralimbic PFC regions by chronic stress.**
NR1 expression was significantly higher in the prelimbic region compared to the infralimbic cortex (1.52±0.14 and 1.13±0.05 R.O.D., respectively, p=0.0001, t-value=-6.38, df=10), as well as the NR2A/NR2B ratio for infralimbic versus prelimbic cortices (0.95±0.14 and 1.22±0.14 R.O.D., respectively, p=0.007, t-value=-3.35, df=10) for baseline condition. Statistical analysis was performed by independent t-test comparing each subunits between control and chronic stress. The prelimbic area shows a significant decrease of NMDAR receptors (1.52±0.14 versus 1.23±0.16 for control versus chronic stress respectively, p=0.005), with a non significant decrease of NR2A subunits (1.06±0.22 versus 0.86±0.13 for control and chronic stress respectively, p=0.07). NR2B did significantly decrease, although this decrease is modest, such that the ratio of NR2A/NR2B is not significantly altered before and after chronic stress (1.22±0.14 versus 1.16±0.1 for control and chronic stress respectively, p=0.4).

On the other hand, chronic stress did not change infralimbic NR1 subunits (1.13±0.05 baseline versus 1.01±0.18 chronic stress, p=0.16), however NR2B is largely down-regulated (0.84±0.08 versus 0.65±0.05 for control and chronic stress respectively, p=0.0006), which significantly increase the NR2A/NR2B ratio (0.95±0.14 versus 1.09±0.07 for control and chronic stress respectively, p=0.04). There is a trend that NR2A subunits
is also down-regulated (0.79±0.07 versus 0.71±0.06 for control and chronic stress respectively, p=0.07).

Given that NR1 is an essential subunit for functional NMDA receptors, higher expression of NR1 suggests that NMDA receptors are more abundant in the prelimbic cortex than in the infralimbic cortex. The abundance of NMDA receptors may be therefore reflected as a higher magnitude of LTP induction in the infralimbic cortex than in prelimbic cortex; however the electrophysiology data is not available to corroborate this difference.

Considering previous research implicating NR2B with LTD and NR2A with LTP, it was expected that the prelimbic cortex would have a decrease in NR2A/NR2B ratio, explaining the conversion from LTP to LTD shown in our electrophysiological experiments. However, this was not corroborated in the PFC with chronic stress exposure in terms of NMDA receptors subunits. While some studies have shown that NR2A was shown to be linked with LTP induction and NR2B to LTD, there are many controversial studies as well (Barria and Malinow 2002; Berberich et al. 2005; Toyoda et al. 2005; Zhao et al. 2005; Morishita et al. 2007; Miwa et al. 2008; Yashiro and Philpot 2008; Muller et al. 2009). Thus, the interrelation
between NMDA receptors subunits and synaptic plasticity changes remain unclear.

**FUTURE DIRECTION OF THE STUDY**

Microdialysis sampling of cerebrospinal fluid DA and NE in the PFC with HPLC assay could be used to quantify the DA/NE tone at rest and during working memory task before and after chronic stress in correlation with the performance of working memory task between the two distinct groups of rats observed.

Considering the role of the prelimbic cortex in goal-oriented behavior and the infralimbic cortex in habits formation, chronic stress may impair goal-oriented behavior through abnormal induction of LTD in the prelimbic cortex while disrupting plasticity change of the neural circuit for habits formation in the infralimbic cortex. However the mechanisms behind these differences are still unknown. Thus, it would be critical to conduct electrophysiology experiments with selective DA/NE receptors agonists/antagonists and NMDA receptors agonists/antagonists in order to assess if DA, NE, and/or NMDA receptors are the critical molecules responsible for the distinct alterations of synaptic plasticity induction between prelimbic and infralimbic cortices. Furthermore, in vivo electrophysiological recordings in behaving animals could shed light on
possible neural network activity differences between baseline and chronic stress animals.
CONCLUSION

In conclusion, the behavior data suggests that chronic stress effects on working memory performance differs between rats. After all rats may not all be created equals in terms of genetics and early life experiences. In addition, the electrophysiology data demonstrates different synaptic plasticity alterations between the prelimbic and infralimbic cortices, such that chronic stress render the infralimbic cortex incapable to increase or reduce synaptic strength, while the prelimbic cortex is weakened through abnormal induction of LTD. Western blot assays revealed that such distinct synaptic plasticity alterations may be associated with NMDA receptors subunits changes. Specifically a decrease of NR1 and NR2B subunits in the prelimbic cortex, whereas only a decrease of NR2B in the infralimbic cortex.

Stress induces maladaptive changes in the brain, causing impairments of brain functions. This study provides one aspect of such maladaptive changes of the brain systems, with which cognitive functions such as working memory is disrupted through alterations of molecular expressions in the glutamate system and network plasticity in the PFC. Stress has been shown to play a major role on schizophrenia, contributing onset and exacerbation of symptoms. Since disruptions of the PFC is thought to be
one of the central pathophysiological mechanisms underlying this disorder, maladaptive responses to chronic stress in the PFC that have been reported in this study could contribute onset and exacerbation of symptoms by interacting with pathological conditions in schizophrenia brains. Therefore, molecular and network changes observed in the PFC with chronic stress may be a promising pharmacotherapeutic targets of schizophrenia.
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