Prenatal Stress Produces Persistence of Remote Memory and Disrupts Functional Connectivity in the Hippocampal–Prefrontal Cortex Axis

Ignacio Negrón-Oyarzo[1], David Neira[1], Nelson Espinosa[1], Pablo Fuentealba[1,2] and Francisco Aboitiz[1]

[1]Departamento de Psiquiatría, Facultad de Medicina, Centro Interdisciplinario de Neurociencia, Pontificia Universidad Católica de Chile, Santiago, Chile and [2]Fundación San Juan de Dios, Barcelona, Spain

Address correspondence to Francisco Aboitiz or Pablo Fuentealba, Centro de Investigaciones Médicas, Pontificia Universidad Catolica de Chile Ave. Marcoleta #391, Santiago, Chile. Email: faboitiz@puc.cl (F.A.); pfuentealba@uc.cl (P.F.)

Prenatal stress is a risk factor for the development of neuropsychiatric disorders, many of which are commonly characterized by an increased persistence of aversive remote memory. Here, we addressed the effect of prenatal stress on both memory consolidation and functional connectivity in the hippocampal–prefrontal cortex axis, a dynamical interplay that is critical for mnemonic processing. Pregnant mice of the C57BL6 strain were subjected to restraint stressed during the last week of pregnancy, and male offspring were behaviorally tested at adulthood for recent and remote spatial memory performance in the Barnes Maze test under an aversive context. Prenatal stress did not affect the acquisition or recall of recent memory. In contrast, it produced the persistence of remote spatial memory. Memory persistence was not associated with alterations in major network rhythms, such as hippocampal sharp-wave ripples (SWRs) or neocortical spindles. Instead, it was associated with a large decrease in the basal discharge activity of identified principal neurons in the medial prefrontal cortex (mPFC) as measured in urethane anesthetized mice. Furthermore, functional connectivity was disrupted, as the temporal coupling between neuronal discharge in the mPFC and hippocampal SWRs was decreased by prenatal stress. These results could be relevant to understand the biological basis of the persistence of aversive remote memories in stress-related disorders.

Keywords: medial prefrontal cortex, memory, prenatal stress

Introduction

Stress is a physiological and behavioral response triggered by threats that allow adaptation to challenging environments (McEwen 1998). When this response becomes chronic, it turns into a risk factor for the development of affective disorders, including depression (Hammen 2005) and post-traumatic stress disorder (Pitman et al. 2012). Interestingly, not all individuals who experience chronic stress develop such disorders. Thus, other factors, such as prenatal stress, may confer vulnerability to chronic stress in adulthood (Huizink et al. 2004; Koenen et al. 2007; Green et al. 2011).

Affective disorders are characterized by an exacerbated and maladaptive retention of long-term aversive memories (American Psychiatric Association 2013), a concept also known as memory persistence (White and Wood 2013). In rodent models, prenatal stress induces persistence of aversive-related memories (Markham et al. 2010; Mueller and Bale 2007). This effect could be mediated by morphologic alterations on hippocampus and medial prefrontal cortex (mPFC) (Jia et al. 2010; Mychasiuk et al. 2012; Muhammad et al. 2012). However, little is known about the neurophysiological mechanisms underlying the persistence of aversive memories induced by prenatal stress.

Remote memories are gradually established from a relatively labile form to a more permanent state, in a process known as consolidation (Squire and Alvarez 1995). Currently, the most accepted model for memory consolidation is the 2-stage model (Buzsáki 1989), which proposes that new information is first acquired during the waking state, and then consolidated during sleep (Diekelmann and Born 2010). In this model, the hippocampus acts as a transient storage structure for recently acquired information, since hippocampal lesions impaired the ability to form new memories, but did not affect the recall of remote memories (Squire et al. 1975; Jarrard 1995).

On the other hand, the permanent storage of memories depends on neocortical networks (Buzsáki 1989). The mPFC receives direct synaptic inputs from the hippocampus (Jay and Witter 1991) and plays a critical role in the recall of consolidated memories (see reviews of Wiltgen et al. 2004; Frankland and Bontempi 2005; Nieuwenhuis and Takashima 2011). Indeed, the mPFC is gradually activated as memory becomes permanent (Bontempi et al. 1999; Takehara et al. 2003; Frankland et al. 2004; Takashima et al. 2006). Furthermore, inactivation of the mPFC impairs the recall of remote memories, but not of recent memories (Takehara et al. 2003; Maviel et al. 2004). Considering that stimulation of CA1 induces synaptic plasticity in the mPFC (Laroche et al. 1990), it is believed that the hippocampus and mPFC are functionally connected during memory consolidation (Frankland and Bontempi 2005; Girardeau and Zugaro 2011).

Another important issue is that synchrony between the activities of connected cerebral structures is relevant for memory consolidation (Axmacher et al. 2006; Fell and Axmacher 2011). The hippocampus and mPFC synchronize at theta (4–8 Hz) and gamma (30–80 Hz) frequencies (Siapas et al. 2005; Benchenane et al. 2010; Sirota et al. 2008), which is related to the acquisition and temporal storage of information (Jones and Wilson 2005). However, memory consolidation may preferentially occur during slow-wave sleep (SWS) (Diekelmann and Born 2010), when delta activity (1–4 Hz) is most prominent (Sirota et al. 2003). During sleep the CA1 area of the hippocampus displays high-frequency (100–300 Hz) synchronized field oscillations known as sharp-wave ripples (SWRs) (Buzsáki 1986). SWRs are synchronized with both mPFC spindles (1–2 s oscillations of 7–12 Hz) and mPFC neuronal spikes (Siapas and Wilson 1998; Wierzynski et al. 2009) during SWS. This suggests that SWRs could represent the off-line transference of information from the hippocampus to cortical networks, a process that seems to be critical for memory consolidation (Girardeau and Zugaro 2011).

Prenatal stress produces hippocampal- and mPFC-dependent behavioral alterations both in rodent models

([Mueller and Bale 2007](); [Green et al. 2011]()) and humans ([Mennes et al. 2006](); [Entringer et al. 2009](); [Buss et al. 2011](); [Schwabe et al. 2012]()). However, there is little information regarding neurophysiological substrates in the hippocampus and mPFC underlying the behavioral consequences of prenatal stress. Thus, in the present study we evaluated the effect of prenatal stress on recent and remote memory in adult mice, and its relationship with neuronal activity and synchrony in the hippocampus and the mPFC, using in vivo local field potential (LFP) recordings in urethane anesthetized mice, a model of SWS ([Clement et al. 2008]()). We found that prenatal stress induced persistence of remote memory, together with a decrease in the firing rate of mPFC neurons, and an altered synchrony between mPFC neuronal spikes and hippocampal SWRs.

Materials and Methods

Animals and Prenatal Stress

Mice of the strain C57/BL6 were housed in a temperature and humidity controlled room ($22 \pm 1°C$) with food and water *ad libitum*. All experimental procedures related with animal experimentation were approved by Institutional Animal Ethics Committee of the Pontificia Universidad Católica de Chile (protocol code: CEBA-12-040). Efforts were made to minimize the number of animals used and their suffering.

Prenatal Stress and Stress Markers

Pregnant female mice (confirmed by the presence of vaginal plug, which was considered gestation Day 0) were randomly assigned to control or prenatal stress groups. Pregnant mice of the prenatal stressed group (PNS) were placed in cylindrical acrylic restrainers (3 cm wide × 10 cm long) for 45 min 3 times per day in their home cages every day from gestational Day 14–21. Control pregnant mothers were not subjected to any stress. Chronic stress induces physiological and behavioral alterations related to the generation of the stress response ([McEwen 2007]()). To evaluate whether repeated restraint effectively stressed pregnant mice, we measured their body weight gain, a well-documented marker of stress ([Scherer et al. 2011]()); thus, the percentage of gain in body weight (net change in weight after experiment × 100/ weight at the beginning of experiment) of pregnant mice was determined. After birth, pups born from Control and PNS groups were left undisturbed with their mother. Given that male, but not female, rodents exposed to prenatal stress develop postpubertal deficits in cognitive behaviors specifically supported by the mPFC ([Markham et al. 2010]()), only male offspring were selected at weaning (postnatal Day 21; PND21), and were randomly distributed at 4 animals per cage according to their prenatal treatment.

Behavioral Analysis

The experimental design is displayed in Supplementary Figure 1*A*. Briefly, once the animals of both experimental groups reached PND60, anxiety-like behavior was tested for all animals using the elevated-plus maze test. Next day, recent and remote spatial memory was evaluated in the Barnes Maze test (Control, $n = 22$; PNS, $n = 25$). Twenty-four hours after the end of acquisition, recent memory was evaluated, and the next day, one set of these animals were subjected to electrophysiological recordings (Control, $n = 7$; PNS, $n = 10$). For the rest of the animals (Control, $n = 10$; PNS, $n = 10$), remote memory was evaluated 10 days after the end of acquisition, and, at the next day, they were subjected to electrophysiological recordings.

All behavioral tests were carried out during the dark phase of the light cycle (from 9:00 to 15:00 h) in a sound attenuated room (<40 dB; measured with a digital sound level meter Sonometer JTS1357, China), at $22 \pm 1°C$. The activity of each mouse was recorded with a video camera (ImageLab, model CB3200) fixed above the behavioral apparatus connected to a computer. Videos were acquired by Lab View software and analyzed off-line using ANY-maze video tracking system (Stoelting Co., IL, USA). All mazes were wiped clean thoroughly with 5% ethanol solution after each trial.

Elevated Plus Maze

It has been shown that chronic stress produces behavioral changes, such as elevated anxiety-like behavior and altered locomotor activity ([Vyas and Chattarji 2004]()). To evaluate whether prenatal stress produced these alterations in the adulthood, we tested a different set of adult animals (PND60) in the elevated-plus maze paradigm ([Vyas and Chattarji 2004]()). The maze consisted of 2 open arms (30 × 5 cm), 2 closed arms (30 × 5 cm), and a central platform (5 × 5 cm) arranged so that 2 arms of each type were opposite to each other. The closed arms were surrounded by a wall (25-cm high). The maze was elevated at 70 cm from the floor. The illumination was 300 lux in the open arms and 210 lux in the closed arms measured with a digital lux meter (Tondaj, model LX1010B). 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 amount of time spent in each section of the maze, number of entries to both arms, and the total number of arm entries. The time spent in the open arms and the ratio of open to total arm entries (open/total) were used as measures of anxiety-like behavior. Total arm entries were taken as an indicator of general locomotor activity. Entry into an arm was defined as when the animal placed all 4 limbs onto a particular arm floor.

Barnes Maze

In this test the animal must learn and remember the location of an escape box from an anxiogenic, elevated, and illuminated, circular open field ([Barnes 1979]()). This context is considered aversive because it activates the hypothalamic–pituitary–adrenal-axis ([Harrison et al. 2009]()), the physiological mediator of the stress response ([McEwen 2007]()). The maze consisted of a white circular platform of 70-cm diameter elevated at 70 cm from the floor with 20 equally spaced holes along the perimeter (7-cm diameter each) located at 2 cm from the edge of the platform. Visual cues were located on the walls of the room. Under one of the holes was located a black plexiglass escape box (17 × 13 × 7 cm). The location of the escape box was consistent for a given mouse, but randomized across mice. The maze was rotated daily, with the spatial location of the target unchanged with respect to the distal visual room cues to prevent a bias based on olfactory or the proximal cues within the maze. The maze was illuminated with 2 incandescent lights to yield a light level of ∼600 lux impinging on the circular platform.

For habituation, the mice were placed in the start box for 1 min, then guided to the escape box with the room lights turned off. After 1 min, the mouse was removed from the escape box and returned to the start box for 3 additional training enters into the escape box. For acquisition, each mouse was given 4 trials per day with an intertrial interval (ITI) of 15 min during 4 consecutive days. For every training trial, the mouse was placed in the start box for 10 s with the room lights turned off. After time had elapsed, the chamber was lifted and the mouse was free to explore the maze. The session ended when the mouse entered the escape box or after 3 min elapsed. When the mouse entered the escape box, the lights were turned off and the mouse was allowed to remain in the dark for 1 min before the next trial began. If the mouse did not find the escape box within 3 min, the experimenter guided the mouse to the escape. We measured the latency to enter the escape box (time and distance needed to find and enter the escape box, respectively), and the number of errors (defined as nose-poke in nonescape holes).

To evaluate recent memory, a probe trial (without the escape box) of 90 s was conducted 24 h after the last training session. To assess remote memory, a second probe trial was applied 10 days after the last training trial, without any training trial between recent and remote memory testing. We measured latency time to find the escape box, and the number of pretarget errors. To assess locomotor activity we measured maximum speed in all trials.

Electrophysiological Recordings

To examine simultaneous neuronal activity in the mPFC and hippocampus 1 day after recent or remote memory testing, animals were anesthetized with urethane (0.8 g/kg dissolved in saline, i.p.) and a

mix of ketamine/xylazine (40 mg/kg ketamine; 4 mg/kg xylazine dissolved in saline, i.p.). Anesthesia was maintained with a ketamine/xylazine cocktail (20 mg/kg ketamine; 2 mg/kg xylazine dissolved in saline, i.p.) when required. During the entire experiment, body temperature was maintained at $36 \pm 1°C$ using a heating pad (Harvard Apparatus, MA, USA) and monitored with a rectal probe connected to a temperature controller (Harvard Apparatus, MA, USA). The animals were placed in a stereotaxic device (Stoelting Co.). To simultaneously record neuronal activity of the mPFC (cingulate and prelimbic cortex) and the CA1 area of the dorsal hippocampus, small craniotomies (1 mm) were drilled on the skull (right hemisphere) over the recording sites. The stereotaxic coordinates, indicated by the stereotaxic atlas (Franklin and Paxinos 2007), were (relative to bregma): mPFC, anteroposterior, +1.54 mm; mediolateral, +0.3 mm; and CA1 hippocampus, anteroposterior, −1.94 mm; mediolateral, −0.5 mm. Single borosilicate electrodes (10–25 MΩ) were pulled with a vertical puller (PE-2; Narishige; Tokyo, Japan), filled with 1.5% neurobiotin in 0.5 M NaCl, and slowly lowered via an motorized microdrive (Siskiyou, Grants Pass, OR, USA) to the recording positions. To record in the mPFC, electrodes were positioned at ~1.0–2.0 mm dorsoventrally, and to record in the CA1 of the hippocampus electrodes were positioned at ~1.2 mm dorsoventrally using the firing of CA1 pyramidal cells and the appearance of SWR as hallmark for functional localization.

During recording, signals from the electrodes were amplified ×10 with the headstage (Dagan Instruments, MN, USA) connected to an amplifier (model BVC-700A, Dagan Instruments) for the signal from the mPFC, and another amplifier (model Axoclamp 2A, Axon Instruments, Sunnyvale, CA, USA) for the signal from hippocampus. Signals were sent and amplified with a 4-channel amplifier (model EX-4 400, Dagan Instruments). Signals from mPFC were simultaneously bandpass filtered for LFP (0.3 Hz–2 kHz) and for single unit (300–5 kHz) recording. The signal from hippocampus was bandpass filtered for LFP (0.3 Hz–2 kHz) and for SWR detection (100–300 Hz). Signals from mPFC single unit recording were visualized on line on an oscilloscope (Tenma Test Equipment, CA, USA) and an audio monitor (Dagan Instruments). The amplified and bandpass filtered outputs were digitized (10 kHz) through an analogue–digital converter interface (National Instruments, TX, USA), monitored with a computer using Igor Pro software and stored on a hard disk for off-line analysis.

The electrode in the area CA1 of the hippocampus stayed in the same position during the entire experiment, while the electrode in the mPFC was moved along the dorsoventral axis within the mPFC. Once a spiking neuron was detected, the simultaneous mPFC and hippocampal recording started, and lasted for 10 min. Single units from mPFC were recorded if they met the criteria of signal-to-noise ratio >3:1.

Histology

At the end of the electrophysiological recording, recorded neurons were juxtacellularly labeled as described by Pinault (1996). Briefly, positive current at increasing amplitude (1–15 nA) was applied at 5 Hz pulses (GW Instek 8216A; Good Will Co., Melrose, MA) until the neuron spiking was modulated. Mice were immediately perfused with 20 mL of saline solution followed by 50 mL of 4% paraformaldehyde in phosphate buffered saline (PBS, pH = 7.4). The brain was removed, incubated overnight in 4% paraformaldehyde in PBS buffer and then stored in PBS buffer containing 0.2% sodium azide. Coronal mPFC brain slices (60 μm) were prepared from paraformaldehyde-fixed brains with vibratome (World Precision Instruments, Sarasota, USA) in ice-cold PBS buffer. For visualization of neurobiotin-labeled mPFC neurons, slices were washed 3 times in 0.2% Triton X-100 in PBS buffer at room temperature, and then streptavidin-Alexa Fluor 488 conjugate (0.5 μg/mL; Invitrogen) was added for overnight at 4°C. At the next day, sections were washed 3 times and placed on slides using mounting medium (Dako). Images were acquired with an epifluorescence microscope (Nikon).

Data Analysis

Down-sampling

For data treatment, field potential activity both in mPFC and hippocampus were down-sampled at 1000 Hz. Neuronal spikes were extracted from mPFC recordings using Neuromatic software package

implemented in Igor Pro software. Unless otherwise specified, data analysis was performed using custom software written in Matlab (MathWorks, Natick, MA).

Field Potential Analysis

Power spectral density (PSD) and coherence of field potential were computed using the multitaper Fourier analysis (Mitra and Pesaran 1999) and the Chronux toolbox (http://www.chronux.org). Field potentials were divided into 5000 ms segments with 500 ms overlap, and a time-bandwidth product (TW) of 3 and 5 tapers.

Spike-field Coherence

Spike-field coherence was computed using the multitaper Fourier analysis (Mitra and Pesaran 1999) and the Chronux toolbox (http://www.chronux.org). We used 500 data points at 1000 Hz, a time-bandwidth product (TW) of 3 and 5 tapers, resulting in a half width of 0.6 Hz.

SWRs Detection and Time–Frequency Analysis

We used a recently described method (Logothetis et al. 2012) with some variation. Briefly, the hippocampus LFP was band-pass filtered (80–200 Hz) using a zero phase shift noncausal finite impulse response filter with 0.5 Hz roll-off. Next, the signal was rectified and low-pass filtered at 20 Hz with a 4th order Butterworth filter. This procedure yields a smooth envelope of the filtered signal, which was then z-score normalized using the mean and standard deviation (SD) of the whole signal. Epochs during which the normalized signal exceeded a 3.5 SD threshold were considered as events. The first point before threshold that reached 1 SD was considered the onset and the first one after threshold to reach 1 SD as the end of events. The difference between onset and end of events was used to estimate the SWR duration. We introduced a 50-ms-refractory window to prevent double detections. In order to precisely determine the mean frequency, amplitude, and duration of each event, we performed a spectral analysis using Morlet complex wavelets of 7 cycles. The Matlab toolbox used is available online as LAN-toolbox (http://lantoolbox.wikispaces.com/), which is largely based on Chronux (www.chronux.org) and FieldTrip (http://fieldtrip.fcdonders.nl/) software toolboxes.

Cortical Spindles Detection

For spindles detection, we used the same method as for SWR detection, with the following modifications: the mPFC LFP was down-sampled at 100 Hz, and band-pass filtered between 7 and 15 Hz. Spindles were defined as normalized signals that exceeded the threshold of 2.0 SD, with a minimum duration of 400 ms.

Estimation of phase-locking. Phase-locking analysis was computed using the Matlab toolbox CircStats (http://philippberens.wordpress.com/code/circstats/). Briefly, LFP traces were bandpass filtered at delta range (1–4 Hz, zero phase shift noncausal finite impulse response filter with 0.5 Hz roll-off). Phase locking was quantified as the circular concentration of the resulting phase distribution, which was defined as mean resultant length (MRL = $(nZ)^{0.5}$). The statistical significance of phase-locking was assessed using the Rayleigh test for circular uniformity. To avoid bias we only considered neurons with >50 recorded spikes.

Cross-correlation analysis. Activity of mPFC neurons and hippocampal SWR was cross-correlated by applying the "sliding-sweeps" algorithm (Abeles and Gerstein 1988). A time window of ±1 s was defined with the 0 point assigned to the start time of a SWR. The timestamps of the mPFC neuronal spikes within the time window were considered as template and represented by a vector of spikes number relatives to $t = 0$ s, with a time bin of 50 ms and normalized to the total number of spikes. Thus, the central bin of the vector contained the ratio between the number of mPFC neuronal spikes elicited between±25 ms and the total number of spikes within the template. Next, the window was shifted to successive SWR throughout the recording session, and an array of recurrences of templates was obtained.

Both mPFC neuronal spikes timestamps and start times of SWR where shuffled by randomized exchange of the original interevent

intervals (Nádasdy et al. 1999) and the cross-correlation procedure was performed on the pseudo-random sequence. The statistical significance of the observed repetition of spike sequences was assessed by comparing, bin to bin, the original sequence with the shuffled sequence. An original correlation sequence that presented a statistical distribution different from 100 simulated shuffling was considered as statistically significant, with $P < 0.01$ probability, instead of chance occurrence.

Statistical Analysis

Unpaired t-tests were used to compare mean values between Control and PNS groups. To compare Control and PNS groups across multiple comparisons we used 2-way repeated-measures analysis of variance (ANOVA) followed by Bonferroni post hoc comparison when data were distributed normally, or Mann–Whitney U test when the distribution was not-normal. For significant differences between groups across different testing day (recent and remote memory testing) we used 2-way ANOVA followed by Bonferroni post hoc comparison. Cumulative distributions between groups were compared using Kolmogorov–Smirnov test. Statistical significant difference was considered when P-value reached 0.05 or less. Data are presented as means ± standard error of mean (SEM).

Results

Prenatal Stress Produces Persistence of Remote Spatial Memory

The main objective of the present work was to investigate the effect of the prenatal stress on the acquisition and recall of spatial memory. To this end, Control and PNS animals (PND60; Control, $n = 22$; PNS, $n = 25$) were trained to perform the Barnes Maze test. We first evaluated the effect of prenatal stress on the acquisition of spatial memory. Figure 1A show representative tracking plots during acquisition for both Control and PNS conditions. Figure 1B shows that as training trials progressed, animals from both groups significantly reduced the latency time to find the escape box (effect of trials: $F_{15,448} = 10.43$; $P < 0.0001$; 2-way repeated-measures ANOVA). We found no differences between groups in the latency time to find the escape box across training trials (effect of prenatal stress: $F_{1,448} = 1.96$; $P = 0.1615$; 2-way repeated-measures ANOVA; Fig. 1B). As a measure of locomotor activity in the maze, we quantified the maximum speed and found no significant differences between groups (effect of prenatal stress: $F_{1,448} = 0.565$, $P = 0.4525$; 2-way repeated-measures ANOVA; Supplementary Fig. 4A). Overall, these data suggest that prenatal stress did not affect the acquisition of spatial memory in an aversive context or locomotor activity.

We then assessed the effect of prenatal stress on the consolidation of spatial memory. To this aim, we evaluated the recall of spatial memory, either one (recent; Control, $n = 22$; PNS, $n = 25$) or 10 (remote; Control, $n = 15$; PNS, $n = 13$) days after the last acquisition trial (schematic diagram in Supplementary Fig. 1). We found a significant effect of the type of memory testing ($F_{1,71} = 10.79$; $P = 0.0017$, 2-way ANOVA), prenatal stress ($F_{1,71} = 5.823$; $P = 0.0184$), and an interaction between type of memory testing and prenatal stress ($F_{1,71} = 11.76$; $P = 0.0010$) on the latency to find the escape box (Fig. 1D). Bonferroni post hoc comparison confirmed that 10 days after memory acquisition, but not in the recent memory testing, prenatally stressed animals required significantly less time than control mice to find the escape box ($P < 0.01$; Fig. 1D). We further examined the behavior of every tested animal (Supplementary Fig. 4B). While most of the Control animals increased the time to find the escape box from recent to remote memory tests,

PNS animals did not change, or even reduced, the time to find the escape box. We also quantified the number of pretarget errors and detected a significant effect of type of memory ($F_{1,71} = 10.16$; $P = 0.0030$; 2-way ANOVA), a nonsignificant effect of prenatal stress ($F_{1,71} = 1.72$; $P = 0.2102$), and a significant interaction between time of memory testing and prenatal stress ($F_{1,71} = 8.905$; $P = 0.0053$) in the number of pretarget errors (Supplementary Fig. 4C). Indeed, PNS animals made significantly less pretarget errors compared to Control mice in the remote memory testing ($P < 0.01$, Bonferroni post hoc; Supplementary Fig. 4C). Similarly, most Control animals increased the number of pretarget errors in response to the type of memory testing, whereas PNS animals reduced the number of pretarget errors (Supplementary Fig. 4D). We found no differences between groups in the maximum speed during recent or remote memory testing (Supplementary Fig. 4E), discarding an effect of altered locomotor activity in the observed differences. Altogether, these results suggest that prenatal stress did not affect recent recall of spatial memory in an aversive context. Instead, prenatal stress produced persistence of remote spatial memory.

Restraint Stress Results in Physiological Alterations Associated to Chronic Stress in Pregnant Mice, but not in Their Adult Offspring

We observed that pregnant mothers of both groups, Control and stressed, increased their body weight with the course of the days (Supplementary Fig. 2A). However, repeated restraint significantly reduced body weight gain in pregnant mothers (effect of restraint stress, $F_{1,84} = 19.92$; $P < 0.001$, 2-way ANOVA; Control, $n = 8$; PNS, $n = 8$; Supplementary Fig. 2A). These data show that repeated restraint during the last week of pregnancy effectively stressed pregnant mothers.

We then examined whether the offspring of the mothers stressed during pregnancy displayed behavioral and physiological changes associated to chronic stress. We observed in both groups of mice an increase of body weight since weaning (PND21) to adulthood (PND60, Supplementary Fig. 2B). However, we found no significant effect of prenatal stress in the body weight gain ($F_{1,203} = 2.561$; $P = 0.111$, 2-way ANOVA; Control, $n = 15$; PNS, $n = 15$). Likewise, we found no significant differences between groups in the adrenal gland weight at adulthood ($t = 0.884$, df = 14, $P = 0.3915$, unpaired t-test; Control, $n = 6$; PNS, $n = 7$; Supplementary Fig. 2C), another well-established marker of stress (Ulrich-Lai et al. 2006). Prenatal stress did not affect locomotor activity, since parameters such as distance traveled ($U = 26.00$, $P = 0.1709$; Mann–Whitney U test; control, $n = 11$; PNS, $n = 11$), mean speed ($U = 29.50$, $P = 0.3513$) and maximum speed ($U = 17.50$, $P = 0.0831$) were similar for both groups (Supplementary Fig. 3B–D). In addition, prenatal stress did not affect anxiety-like behavior, as the time spent in the open and closed arms ($F_{1,40} = 0.502$; $P = 0.482$, 2-way ANOVA; Supplementary Fig. 3E) and the ratio of open arm entries/total entries ($U = 33.50$, $P = 0.846$, Mann–Whitney U test; Supplementary Fig. 3F) remained similar for both groups. Altogether, these data suggest that prenatal stress did not activate the stress response in the adult offspring.

Prenatal Stress Does not Modify Major Network Activity Patterns in the Hippocampus or mPFC

Consistent with previous studies (Clement et al. 2008), we observed prominent activity in the delta frequency band in the
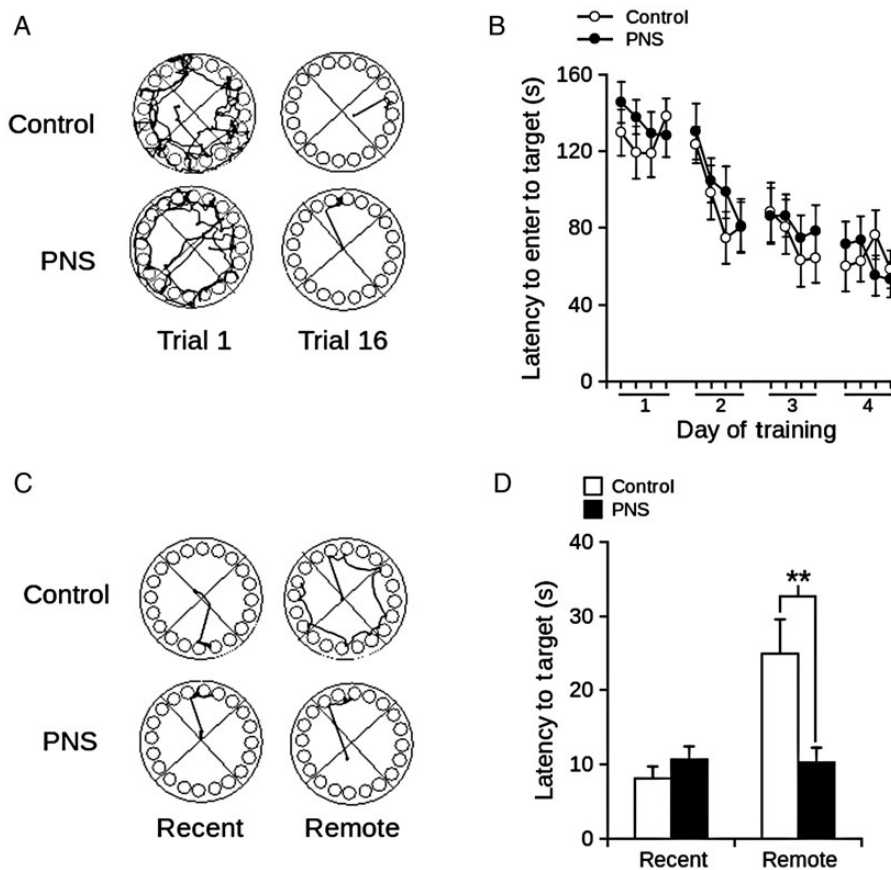
Figure 1. Prenatal stress produced long-term persistence of spatial memory. (*A*) Representative example of tracking plots for 2 mice (Control and PNS) in the Barnes maze for the first (Trial 1—Day 1) and last trials (Trial 16—Day 4) during the training period. (*B*) Learning curve of the spatial memory task for the 2 groups of mice measured as latency to find target in chronological blocks. (*C*) Example of tracking plots for 2 mice (Control and PNS) in the Barnes maze during recent (one day after training) and remote memory testing (10 days after training). (*D*) Bar chart showing the latency to escape for both groups of mice in the 2 memory conditions (*$P < 0.05$; Bonferroni post hoc after 2-way ANOVA). Data are shown as mean ± SEM.

hippocampus and mPFC of anesthetized mice (Supplementary Fig. 5*A* and *D*). Such activity pattern was evident in both the raw recordings and spectrograms, as well as in the power spectral density analysis (Supplementary Fig. 5*B* and *E*). We did not detect significant differences between groups in power density in the delta band, either in the hippocampus or mPFC, after both recent (hippocampus: $U = 960.0$, $P = 0.147$; mPFC: $U = 1192$; $P = 0.954$; Mann–Whitney U test; Control, $n = 7$; PNS, $n = 10$; Supplementary Fig. 5*C*) and remote memory testing (hippocampus: $U = 1100$, $P = 0.303$; mPFC: $U = 1062$; $P = 0.326$; Mann–Whitney U test; Control $n = 10$; PNS, $n = 10$; Supplementary Fig. 5*F*). These results suggest that the neuronal mechanisms underlying the generation of delta waves in the hippocampus and the mPFC remain largely intact in prenatally stressed animals. We evaluated the effect of prenatal stress on SWRs recorded in the CA1 area of the dorsal hippocampus after recent and remote memory testing. An example of SWR is shown in Figure 2*A*. We found no significant effect of the type of memory testing ($F_{1,20} = 0.694$; $P = 0.544$, 2-way ANOVA), prenatal stress ($F_{1,20} = 0.137$; $P = 0.745$, 2-way ANOVA) on the density of SWRs (Fig. 2*B*). We also compared intrinsic properties of SWR, as frequency and duration (see spectrogram of Fig. 2*C* as an example of all SWR recorded of individual animals). We found no significant effect of type of memory testing ($F_{1,36} = 0.393$, $P = 0.547$; 2-way ANOVA) or prenatal

stress ($F_{1,36} = 0.0066$, $P = 0.935$) in the duration of the SWRs (Fig. 2*D*). Neither did we find a significant effect of type of memory testing ($F_{1,36} = 0.00002$, $P = 0.996$; 2-way ANOVA) or of prenatal stress ($F_{1,36} = 0.132$; $P = 0.718$) on the intrinsic SWRs frequency (Fig. 2*E*). These results suggest that the main synaptic output from the hippocampus to the neocortex was not modified by prenatal stress.

During SWS the neocortex presents transient waxing-and-waning oscillations patterns of ~7–14 Hz known as spindles. These patterns depend of the thalamo-cortical network (Steriade 2006), and are correlated with memory consolidation (Fogel and Smith 2011). We analyzed the effect of prenatal stress and type of memory in spindles in the mPFC. Representative traces of spindles are shown in Supplementary Figure 6*A*. We found a significant effect of the type of memory testing ($F_{1,268} = 4.564$, $P = 0.034$, 2-way ANOVA) but not of prenatal stress ($F_{1,268} = 2.634$, $P = 0.106$), and no significant interaction between type of memory and prenatal stress ($F_{1,268} = 1.052$, $P = 0.332$) on the density of spindles (Supplementary Fig. 6*B*). We did not find a significant effect of type of memory testing ($F_{1,267} = 0.3798$, $P = 0.538$; 2-way ANOVA), but not of prenatal stress ($F_{1,267} = 0.0018$, $P = 0.9656$) in the duration of the spindles (Supplementary Fig. 6*C* and *D*). Finally, we did not find a significant effect of type of memory testing ($F_{1,267} = 0.00001$, $P = 0.996$; 2-way ANOVA) nor of prenatal stress ($F_{1,267} = 0.108$,
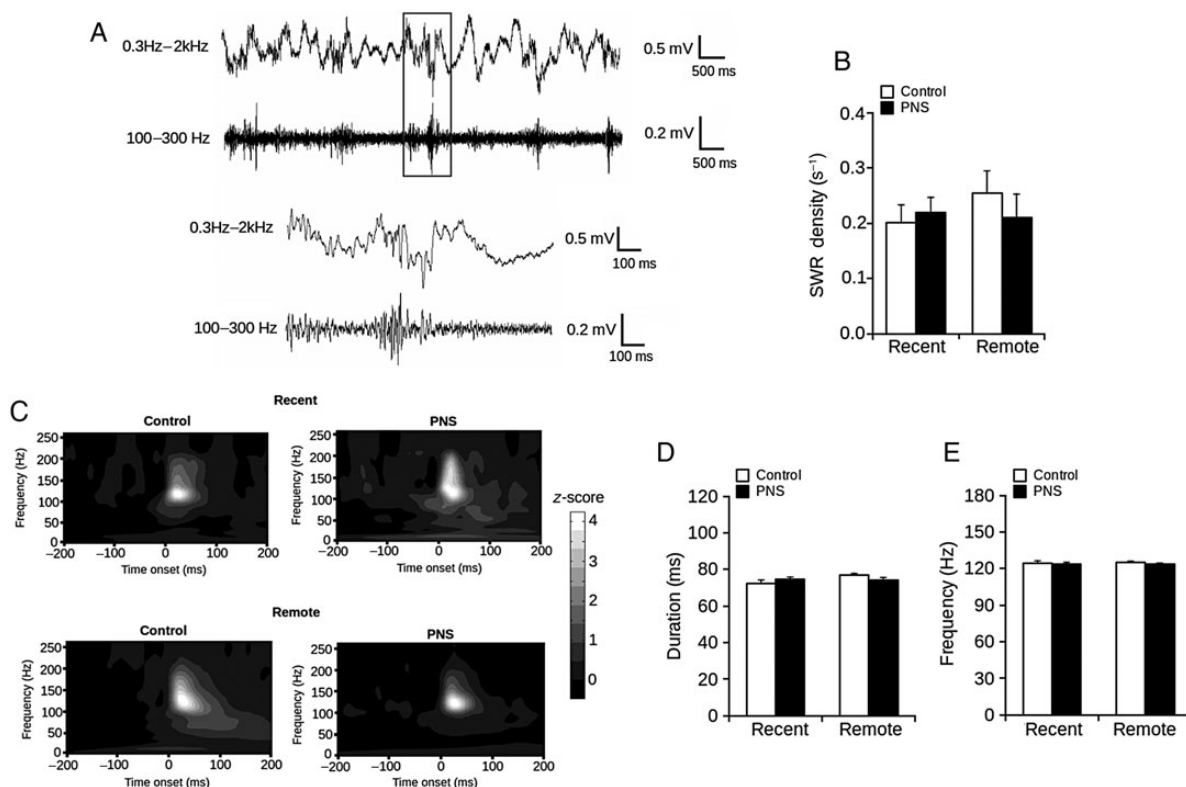
Figure 2. Prenatal stress did not affect hippocampal SWRs. (*A*) Upper panel: example recording from the dorsal CA1 area of the hippocampus at wideband (top, LFP) and high-pass filtered (bottom, SWRs). Lower panel: individual SWR from top panel at wideband and high-pass filtered (ID: IN23cell3). (*B*) Bar chart of the SWR density in the hippocampus of control and prenatally stressed animals after recent and remote memory testing. (*C*) Overall average spectrogram showing time–frequency distribution of SWR events recorded from Control and PNS animals after recent and remote memory testing. (*D* and *E*) Bar charts for the intrinsic parameters of SWRs. Data are shown as mean ± SEM.

$P = 0.7418$) on the intrinsic spindles frequency (Supplementary Fig. 6*C* and *E*). These results suggest that mPFC thalamo-cortical spindles were affected by memory consolidation, but not by prenatal stress.

Prenatal Stress Decreases Neuronal Firing in the mPFC Selectively During Memory Consolidation

We recorded spontaneous single-cell activity (total $n = 230$ single unit) after recent and remote memory testing in both groups. All recorded neurons were anatomically located in the mPFC (Supplementary Fig. 7*A*).

The mPFC includes both pyramidal and GABAergic cells (Kawaguchi and Kubota 1997; Jung et al. 1998). To estimate the neuronal identity of the recorded cells, we measured the half-width duration of the extracellularly recorded spikes. The ranges of spikes half-width were between 175 and 800 μs, generating only one single peak (Supplementary Fig. 7*B*), whereas interneurons typically display a spike half-width duration shorter than 100 μs (Jung et al. 1998). Another feature of inter-neurons is the high firing rate (average near 15 Hz, Jung et al. 1998). However, the average firing rate of the recorded single units was 1.30 ± 0.14 Hz. Finally, 33 of the 230 recorded neurons were juxtalabeled with neurobiotin and visualized under fluorescence microscopy, where all labeled neurons corresponded to pyramidal cells (example in Supplementary Fig. 7*C*). Altogether, our data suggest that all recorded neurons corresponded to pyramidal neurons. Additionally, we found no major morphological differences in the mPFC of Nissl stained

sections obtained from Control and PNS adult mice (Supplementary Fig. 7*D*).

We evaluated neuronal activity in the mPFC by comparing the mean firing rate between groups. As shown in Figure 3*B*, we found no significant differences in the mean firing rate of cells for both groups in recent memory testing (Control = 1.340 ± 0.288 Hz; PNS = 1.331 ± 0.201 Hz; $U = 1499$, $P = 0.8857$, Mann–Whitney *U* test). In contrast, after remote memory testing, we found a significantly lower mean firing rate in PNS mice when compared with Control mice (Control = 1.598 ± 0.299 Hz; PNS = 0.807 ± 0.292 Hz; $U = 1088$, $P = 0.0028$, Mann–Whitney *U* test; Fig. 3*B*). We further characterized differences in the distributions of firing rates in both groups of mice. Figure 3*D* shows that there was no significant difference between groups in the distribution of firing rates of neurons recorded after recent memory testing ($D = 0.039$, $P = 1.00$, Kolmogorov–Smirnov test). Nonetheless, distributions of firing rates were significantly different when compared after remote memory testing ($D = 0.229$, $P = 0.034$; Kolmogorov–Smirnov test), with nearly 70% of neurons in prenatally stressed animals firing at frequencies <0.5 Hz (Fig. 3*D*). In addition, we estimated the relative number of active neurons by counting the number of spontaneously firing neurons encountered by the electrode in every penetration in the mPFC (Fig. 3*C*). We found a significant effect of prenatal stress in the number of neurons active ($F_{1,30} = 5.379$, $P = 0.027$, 2-way ANOVA; Bonferroni post hoc, $P < 0.01$), but no effect of type of memory testing ($F_{1,30} = 0.5647$; $P = 0.4582$). Altogether, these data suggest that pre-natal stress selectively impaired mPFC function during the
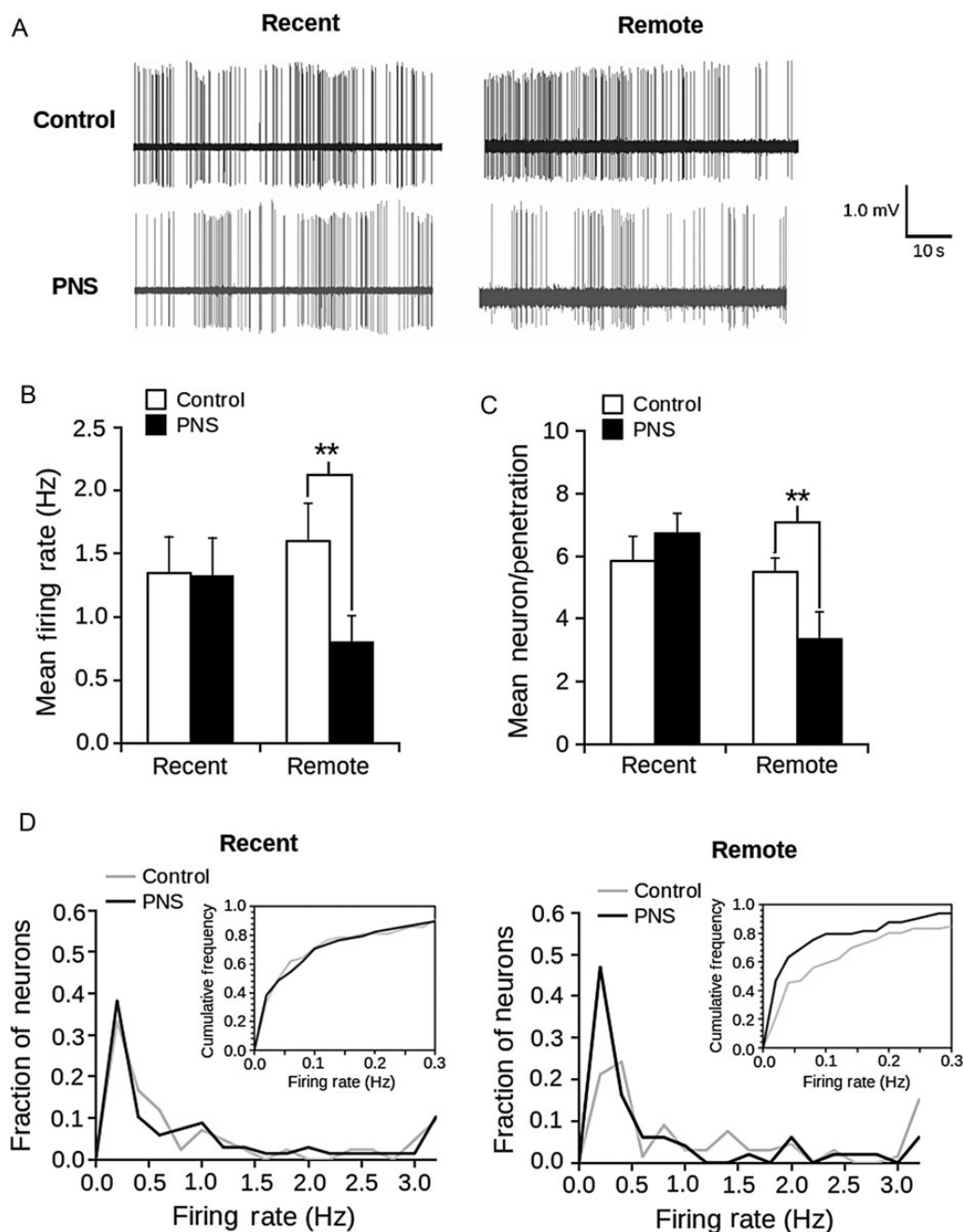
Figure 3. Prenatal stress decreased spontaneous firing in the prefrontal cortex after remote memory testing. (*A*) Example single-unit recordings from the mPFC (filtered at 300 Hz–5 kHz) for each group and condition (Recording ID: REC-CT: IN26cell3a; REC-PNS: IN29cell2a; REM-CT: IN38cell1a; REM-PNS: IN49cell1a). (*B*) Mean number of single units recorded per electrode penetration (**$P < 0.01$; Bonferroni post hoc comparison after 2-way ANOVA). (*C*) Mean firing rate of spontaneously firing neurons (**$P < 0.01$; Mann–Whitney U test). Data are shown as mean ± SEM. (*D*) Distribution of firing rates in the mPFC after recent and remote memory testing. Differences were significant in remote memory testing ($D = 0.228$; $P < 0.05$; Kolmogorov–Smirnov test), but not in recent memory testing ($D = 0.039$; $P = 1.0$; Kolmogorov–Smirnov test). Inset: cumulative distribution for both conditions.

period of memory consolidation, from recent-to-remote memory formation.

Prenatal Stress Disrupts Functional Connectivity Between Hippocampal SWRs and Neuronal Discharge in the mPFC

We observed in anesthetized mice that neuronal discharge in the mPFC and SWRs in the hippocampus were synchronized (Fig. 4A). To assess the effect of prenatal stress on

hippocampo–cortical synchrony, we computed the normalized cross-correlation function between simultaneously recorded hippocampal SWRs and neuronal spikes in the mPFC. As shown in the cross-correlograms of Figure 4B, we observed a central peak near zero delay in all conditions, indicating the simultaneous activation of mPFC neuronal discharge and hippocampal SWRs (Siapas and Wilson 1998). In recent memory testing, the shape of the cross-correlation function was not altered between Control and PNS animals (Fig. 4B). However, in remote memory testing, mPFC neurons decreased their
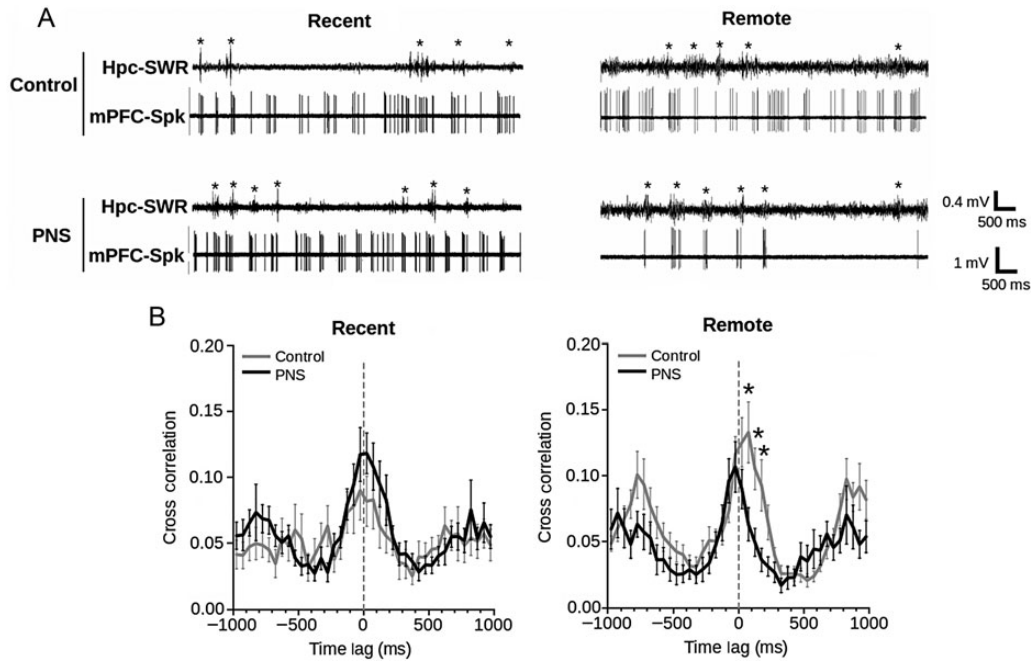
Figure 4. Prenatal stress modified cross-correlation of medial prefrontal spikes to hippocampal SWRs after remote memory testing. (*A*) Example recording for each group and condition displaying hippocampal SWRs (top) and mPFC single unit recording (lower). Asterisks indicate SWR cross-correlated with mPFC neuronal spikes. (REC-CT: IN20cell1a; REC-PNS: IN36cell2a; REM-CT: IN38cell10; REM-PNS: IN31cell6). (*B*) Mean normalized cross-correlation between significantly correlated mPFC single units to hippocampal SWRs (*: $P < 0.05$; Wilcoxon signed-rank test). Number of neurons selected per group: Recent-Control = 26; Recent-PNS = 50; Remote-Control = 58; Remote-PNS = 24. Data are expressed as mean ± SEM.

discharge probability during SWRs in PNS animals as compared with Control animals (Fig. 4*B*). Indeed, statistical analysis revealed a significant reduction of cross-correlations at positive lags, between 50 and 200 ms after the onset of SWRs in the PNS group compared to Controls (Fig. 4*B*). These differences were not related to firing rate, given that we found neither an effect of group ($F_{1,181} = 0.030$, $P = 0.862$, 2-way ANOVA) nor of type of memory testing ($F_{1,181} = 0.056$, $P = 0.8128$, Supplementary Fig. 7*A*) in the mean firing rate of mPFC neurons. These data suggest that PNS impairs functional connectivity in the hippocampo–cortical pathway during memory consolidation.

Slow rhythms, characteristic of SWS, organize local and distant neuronal networks (Sirota et al. 2003; Mölle et al. 2006). Hence, we evaluated the effect of prenatal stress on the synchrony between the mPFC and hippocampus in the delta frequency band. We found no treatment differences in the hippocampal–mPFC spectral coherence in the delta frequency range, either in recent ($U = 90$, $P = 0.158$; Mann–Whitney U test) or remote memory testing ($U = 71$, $P = 0.086$; Mann–Whitney U test) (see Supplementary Fig. 9*A* and *B*). In addition, we measured the spike-field coherence, an estimation of the relation between neuronal discharge and the LFP in the mPFC network activity in the frequency domain. Again, we found no significant differences in the spike-field coherence of mPFC neuronal discharge during local delta oscillation between groups after recent ($U = 105$, $P = 0.364$; Mann–Whitney U test; Supplementary Fig. 9*C*) or remote memory testing ($U = 97$, $P = 0.250$). Finally, given the cortical origin of slow-wave activity (Steriade et al. 1993), we evaluated the modulation of mPFC discharge and hippocampal SWRs by mPFC delta oscillations. We found that prenatal stress did not affect the phase-locking strength (measured as MRL) of mPFC neuronal discharge to delta oscillations ($F_{1,168} = 0.1401$, $P = 0.709$, 2-way ANOVA;

Supplementary Fig. 10*A*), or the modulation of SWRs to mPFC delta waves ($F_{1,181} = 0.214$, $P = 0.115$; Supplementary Fig. 10*B*). Altogether, these results suggest that prenatal stress did not modify oscillatory synchrony during slow cortical rhythms between the hippocampus and mPFC. However, even when mPFC neurons from both groups display a similar tendency to fire in synchrony with SWRs, prenatal stress decreased the temporal association between mPFC neuronal discharge to hippocampal SWRs after remote memory testing.

Discussion

In the present study we found that prenatal stress induced persistence of remote spatial memory under an aversive context, without affecting the acquisition or recall of recent memory. Prenatal stress did not affect major cortical rhythms like delta waves, hippocampal SWRs or mPFC spindles. On the other hand, prenatal stress reduced the firing rate in the mPFC specifically after remote memory testing. We also found that, selectively after remote memory testing, prenatal stress reduced the temporal coupling between mPFC neuronal discharge and hippocampal SWRs. Altogether, these results suggest that the persistence of aversive-spatial memory induced by prenatal stress could be related to alterations of local circuit activity in the mPFC and its functional connectivity with the hippocampus, selectively during the consolidation of memories.

Prenatal Stress Induced Persistence of Spatial Memory in an Aversive Context
Prenatal stress is considered a predisposition factor for the development of affective disorders (Huizink et al. 2004; Koenen et al. 2007; Green et al. 2011), in which an excessive and

maladaptive persistence of aversive-related memories is a key feature (American Psychiatric Association 2013; Parsons and Ressler 2013). We found that mice stressed prenatally during the last week of gestation displayed a persistence of spatial memory at adulthood measured in the Barnes maze (Fig. 2), without affecting acquisition or recent memory. To our knowledge, only Mueller and Bale (2007), using the same mice strain, stress procedure, and behavioral task as we used, have analyzed the effect of prenatal stress on recent and remote spatial memory. In that study, results were consistent with our findings. Thus, while Control animals extinguished spatial memory 10 days after acquisition (Mueller and Bale 2007; Heo et al. 2011), PNS animals exhibited persistence of memory.

Several factors induced by prenatal stress can be involved in the persistence of remote memory. For example, basal chronic stress can induce the persistence of aversive-related memories (Conrad et al. 1999). However, we believe it unlikely since we did not find significant differences on chronic stress markers, such as weight gain, adrenal glands weight, and basal anxiety-like behavior (Supplementary Figs 2 and 3) (Vyas and Chattarji 2004; Kim et al. 2013). Although it has been shown that prenatal stress (Miyagawa et al. 2011) or corticosterone administration to pregnant mothers (Ceci et al. 2013) can induce elevated anxiety-like behavior in adult offspring, this effect seems to depend on the intensity of the stressor. Finally, an enhanced acquisition can increase remote memory; however, we did not find an effect of prenatal stress, either on the training performance or in recent memory testing (Fig. 1). Thus, we propose that the persistence of remote memory could be the result of hypofunctionality of mPFC and disrupted extinction induced by prenatal stress. Importantly, evaluating drug-seeking behavior, Kippin et al. (2008) showed that adult rats exposed to PNS displayed higher initial responding to cocaine during extinction compared to Control, and took more sessions to reach the extinction criterion, suggesting that PNS could also induce persistence of appetitive memories.

In addition, it has been suggested that the increased persistence of aversive memory observed in patients suffering from affective disorders, such as post-traumatic stress disorders, could be related with an increased consolidation of emotionally aversive memories (Parsons and Ressler 2013).

Prenatal Stress Selectively Decreased Neuronal Firing in the mPFC During Memory Consolidation

It has been proposed that the mPFC is involved in the consolidation of memories (Wiltgen et al. 2004; Frankland and Bontempi 2005; Nieuwenhuis and Takashima 2011). Interestingly, we found that prenatal stress produced, in addition to increased persistence of memory, a decrease in neuronal activity in the mPFC during slow-wave activity, specifically after remote memory testing; that is, during the period of memory consolidation (Fig. 3). An interesting feature of the behavioral and neurophysiological effects of prenatal stress in the mPFC is that they were manifested after remote memory testing. On the other hand, it has been shown in humans that the mPFC is progressively recruited at least 72 h after memory encoding (Sterpenich et al. 2007) suggesting that memory becomes progressively dependent of neocortical structures. Accordingly, we propose that the effects of prenatal stress are manifested when the mPFC is recruited to consolidate spatial memory, suggesting that prenatal stress affected exclusively the consolidation mPFC-dependent stage. This effect was not related to

differences in the basal brain activation, either induced by differences on anesthesia level, or by prenatal stress, since we did not find differences between groups in the spectral power distribution in the mPFC (Supplementary Fig. 5).

What physiological mechanisms induced by prenatal stress might affect neuronal activity in the mPFC, specifically during memory consolidation? First, it is likely that at least some of the effects of prenatal stress on the offspring may be mediated by the increased and sustained secretion of corticosterone by the mother during pregnancy, which can cross both placental and fetal brain–blood barriers, altering prenatal brain development (Ward and Weisz 1984; Weinstock 2005; Ceci et al. 2013; Macrì et al. 2013). Given that glucocorticoids retard the radial migration of postmitotic neural progenitors (Fukumoto et al. 2009), corticosterone may alter the prenatal development of cortical structures, including the mPFC. However, we did not detect gross developmental alterations in the cytoarchitecture of the mPFC (Supplementary Fig. 7D), or major changes in the oscillatory activity patterns in the mPFC (Goto and Grace 2006; Supplementary Figure 5). Another important factor may be the sexual susceptibility to the effects of prenatal stress. The current hypothesis proposes that PNS masculinizes the female brain, and demasculinizes and feminizes behavioral features in males (Bowman et al. 2004). Prenatal restraint stress decreases circulating testosterone levels in fetuses (Ward and Weisz 1980), and in pregnant mothers, stress increased levels of corticosterone and estradiol during pregnancy (Ward and Weisz 1984; MacNiven et al. 1992; Misdrahi et al. 2005). Interestingly, estradiol can affect brain development at prenatal life, affecting, for example, the catecholaminergic systems in the frontal cortex in male rats (Stewart and Rajabi 1994). Thus, the elevated levels of estradiol and corticosterone, together with the reduction of testosterone in fetuses during gestation induced by prenatal stress, could be implicated in the alterations observed in the mPFC at adulthood. There are few studies investigating the sexual differences on the effect of prenatal stress on the persistence of aversive memories. Mueller and Bale (2007) showed that the persistence of memory associated to prenatal stress observed in the Barnes maze test was similar between male and female animals. However, it is important to point out that they found differences between genders in the strategy to locate the escape box. That is, PNS males used random and serial strategies, whereas PNS females used a cue-based strategy. Interestingly, male Control animals also used a cue-based strategy, supporting the hypothesis that prenatal stress masculinizes the brain of females, and demasculinizes many sexually dimorphic behavioral features in males (Bowman et al. 2004).

PNS could induce subtle developmental alterations in the mPFC. Interestingly, dopaminergic afferents, which starts to develop during the last week of gestation (Berger and Verney 1987; Kalsbeek et al. 1988) are crucial for the regulation of neuronal activity and synaptic plasticity in the mPFC (Otani et al. 2003). Thus, dopaminergic input to the mPFC is a likely target of prenatal stress. A previous report showed that prenatal stress reduced dopamine release in the neocortex (Takahashi et al. 1992), and the number of spontaneously active ventral tegmental area dopaminergic neurons, the principal dopaminergic afferent of the mPFC (Hausknecht et al. 2013). Accordingly, prenatal stress may modulate long-term synaptic plasticity in the hippocampo–cortical synapses through dopamine release (Otani et al. 2003). For instance, it has been shown that a single stimulation of the ventral tegmental area

induces long-term depression (LTD) in vivo in the hippocampo–mPFC monosynaptic projection, whereas tetanic stimulation (50 Hz) of the ventral tegmental area results in long-term potentiation (LTP) (Gurden et al. 1999). Moreover, low levels of dopamine induce LTD in vitro, whereas the incubation of slices with dopamine before high-frequency stimulation results in LTP (Matsuda et al. 2006). Thus, a plausible frame to explain our results is that low levels of dopamine induced by prenatal stress (Takahashi et al. 1992), especially during SWS (Dzirasa et al. 2006), combined with the high-frequency stimulation produced by hippocampal SWRs, might result in the induction of LTD in the mPFC, reducing basal levels of activity of pyramidal neurons (Daoudal et al. 2002). Further experiments will have to test this hypothesis.

Finally, is important to take into account that chronically stressed caregivers, as the pregnant mothers, may display abusive behaviors to the infants, leading to epigenetic alterations in the mPFC that persist to adulthood (Moore and Power 1986; Roth et al. 2009). Thus, we cannot discard an effect of maltreatment during early childhood in the alterations observed in prenatally stressed mice. Although adoption by birth has been proposed to control for this factor, there is evidence that this procedure has its own consequences on brain development and tends to revert the effects of prenatal stress on glucocorticoid secretion, neurotransmitter receptor density and other parameters (Maccari et al. 1995; Barros et al. 2004; Weinstock 2008). Furthermore, the most usual condition is that stressed mothers will keep their infants and raise them, possibly prolonging the effects of prenatal stress.

Prenatal Stress Altered Synchrony in the Hippocampus–mPFC Pathway

SWRs and neuronal discharge in the mPFC are synchronized during SWS (Siapas and Wilson 1998; Wierzynski et al. 2009). Interestingly, learning generates cell assemblies in the mPFC (Benchenane et al. 2010), which are subsequently reactivated during SWS, coordinately with hippocampal SWRs (Peyrache et al. 2009). Thus, SWRs may serve to select which neocortical neurons are preferentially activated based on the information placed into hippocampal networks by past experience (Siapas and Wilson 1998). We observed that neuronal discharge in the mPFC and SWRs were synchronized during slow-wave activity (Fig. 4A and B). However, we found that prenatal stress reduced the neuronal coupling between SWRs and mPFC discharging neurons, selectively after remote memory testing (Fig. 4B). We propose that this reduction of the temporal window, together with the reduced firing rate in the mPFC, may organize mPFC neuronal spikes around the onset of the SWR, allowing to filter the information received from the hippocampus through the SWRs, and making more efficient the process of consolidation. Future experiments in freely moving animals during SWS will be required in order to clarify this issue.

Concluding Remarks

Cognitive dysfunction, including persistence of aversive-related memories, is an important component of stress-related psychiatric disorders, including depression and post-traumatic stress disorder (American Psychiatric Association 2013; Parsons and Ressler 2013). This cognitive dysfunction might be related with an increased consolidation of aversive memories (Parsons and Ressler 2013). Interestingly, consolidation of aversive memories does not usually occur during traumatic events, but hours or days after events have occurred (Parsons and Ressler 2013), presumably during sleep (Diekelmann and Born 2010). Importantly, prenatal stress is considered a predisposition factor for these disorders (Huizink et al. 2004; Koenen et al. 2007; Green et al. 2011). In the present work we have shown that prenatal stress induced persistence of spatial memory under an aversive context. We suggest that the reduced neuronal activity in the mPFC induced by prenatal stress, which is selectively manifested during memory consolidation, and results in disrupted connectivity with the hippocampus, could be involved in the persistence of aversive long-term memories. Thus, prenatal stress is considered a predisposition factor for stress-related psychiatric affective disorders. Our results could contribute to the understanding of the stress-related psychiatric disorders.

Supplementary Material

Supplementary Material can be found at http://www.cercor.oxfordjournals.org/ online.

Funding

Notes

References

Abeles M, Gerstein GL. 1988. Detecting spatiotemporal firing patterns among simultaneously recorded single neurons. J Neurophysiol. 60 (3):909–924.

American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Publishing.

Axmacher N, Mormann F, Fernández G, Elger CE, Fell J. 2006. Memory formation by neuronal synchronization. Brain Res Rev. 52(1): 170–182.

Barnes CA. 1979. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol. 93:74–104.

Barros VG, Berger MA, Martijena ID, Sarchi MI, Pérez AA, Molina VA, Tarazi FI, Antonelli MC. 2004. Early adoption modifies the effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. J Neurosci Res. 76(4):488–496.

Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, Wiener SI. 2010. Coherent theta oscillations and reorganization of spike timing in the hippocampal–prefrontal network upon learning. Neuron. 66:921–936.

Berger B, Verney C. 1987. Recent contributions concerning the development and distribution of dopaminergic innervation in the cerebral cortex of the rat. Cellule. 74:291–305.

Bontempi B, Laurent-Demir C, Destrade C, Jaffard R. 1999. Time-dependent reorganization of brain circuitry underlying long-term memory storage. Nature. 400(6745):671–675.

Bowman RE, MacLusky NJ, Sarmiento Y, Frankfurt M, Gordon M, Luine VN. 2004. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology. 145(8):3778–3787.

Buss C, Davis EP, Hobel CJ, Sandman CA. 2011. Maternal pregnancy-specific anxiety is associated with child executive function at 6–9 years age. Stress. 14(6):665–676.

Buzsáki G. 1986. Hippocampal sharp waves: their origin and significance. Brain Res. 398(2):242–252.

Buzsáki G. 1989. Two-stage model of memory trace formation: a role for "noisy" brainstates. Neuroscience. 31:551–570.

Ceci C, Mela V, Macrì S, Marco EM, Viveros MP, Laviola G. 2013. Prenatal corticosterone and adolescent URB597 administration modulate emotionality and CB1 receptor expression in mice. Psychopharmacology (Berl). 231(10):2131–2144.

Clement EA, Richard A, Thwaites M, Ailon J, Peters S, Dickson CT. 2008. Cyclic and sleep-like spontaneous alternations of brain state under urethane anesthesia. PLoS One. 3(4):e2004.

Conrad CD, LeDoux JE, Magarinos AM, McEwen BS. 1999. Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behav Neurosci. 113(5):902–913.

Daoudal G, Hanada Y, Debanne D. 2002. Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proc Natl Acad Sci USA. 99(22):14512–7.

Diekelmann S, Born J. 2010. The memory function of sleep. Nat Rev Neurosci. 2:114–126.

Dzirasa K, Ribeiro S, Costa R, Santos LM, Lin SC, Grosmark A, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA. 2006. Dopaminergic control of sleep–wake states. J Neurosci. 26(41):10577–10589.

Entringer S, Buss C, Kumsta R, Hellhammer DH, Wadhwa PD, Wüst S. 2009. Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. Behav Neurosci. 123(4):886–893.

Fell J, Axmacher N. 2011. The role of phase synchronization in memory processes. Nat Rev Neurosci. 12(2):105–118.

Fogel SM, Smith CT. 2011. The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. Neurosci Biobehav Rev. 35(5):1154–1165.

Frankland PW, Bontempi B. 2005. The organization of recent and remote memories. Nat Rev Neurosci. 6(2):119–130.

Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ. 2004. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science. 304(5672):881–883.

Franklin KBJ, Paxinos G. 2007. The Mouse Brain in Stereotaxic Coordinates, 3rd Edition. Academic Press: San Diego.

Fukumoto K, Morita T, Mayanagi T, Tanokashira D, Yoshida T, Sakai A, Sobue K. 2009. Detrimental effects of glucocorticoids on neuronal migration during brain development. Mol Psychiatry. 14(12):1119–1131.

Girardeau G, Zugaro M. 2011. Hippocampal ripples and memory consolidation. Curr Opin Neurobiol. 21(3):452–459.

Goto Y, Grace AA. 2006. Alterations in medial prefrontal cortical activity and plasticity in rats with disruption of cortical development. Biol Psychiatry. 60(11):1259–1267.

Green MK, Rani CS, Joshi A, Soto-Piña AE, Martinez PA, Frazer A, Strong R, Morilak DA. 2011. Prenatal stress induces long term stress vulnerability, compromising stress response systems in the brain and impairing extinction of conditioned fear after adult stress. Neuroscience. 192:438–451.

Gurden H, Tassin J-P, Jay TM. 1999. Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal–prefrontal cortex long-term potentiation. Neuroscience. 94:1019–1027.

Hammen C. 2005. Stress and depression. Annu Rev Clin Psychol. 1:293–319.

Harrison FE, Hosseini AH, McDonald MP. 2009. Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. Behav Brain Res. 198(1):247–251.

Hausknecht K, Haj-Dahmane S, Shen RY. 2013. Prenatal stress exposure increases the excitation of dopamine neurons in the ventral tegmental area and alters their responses to psychostimulants. Neuropsychopharmacology. 38(2):293–301.

Heo S, Patil SS, Jung G, Höger H, Lubec G. 2011. A serotonin receptor 1A containing complex in hippocampus of PWD/PhJ mice is linked to training effects in the Barnes maze. Behav Brain Res. 216(1):389–395.

Huizink AC, Mulder EJ, Buitelaar JK. 2004. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychol Bull. 130(1):115–142.

Jarrard LE. 1995. What does the hippocampus really do? Behav Brain Res. 71(1–2):1–10.

Jay TM, Witter MP. 1991. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol. 313(4):574–586.

Jia N, Yang K, Sun Q, Cai Q, Li H, Cheng D, Fan X, Zhu Z. 2010. Prenatal stress causes dendritic atrophy of pyramidal neurons in hippocampal CA3 region by glutamate in offspring rats. Dev Neurobiol. 70(2):114–125.

Jones MW, Wilson MA. 2005. Theta rhythms coordinate hippocampal–prefrontal interactions in a spatial memory task. PLoS Biol. 3(12):e402–e400.

Jung MW, Qin Y, McNaughton BL, Barnes CA. 1998. Firing characteristics of deep layer neurons in prefrontal cortex in rats performing spatial working memory tasks. Cereb Cortex. 8(5):437–450.

Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB. 1988. Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol. 269(1):58–72.

Kawaguchi Y, Kubota Y. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex. 7(6):476–486.

Kim JG, Jung HS, Kim KJ, Min SS, Yoon BJ. 2013. Basal blood corticosterone level is correlated with susceptibility to chronic restraint stress in mice. Neurosci Lett. 555:137–142. S0304-3940(13)00847-1.

Kippin TE, Szumlinski KK, Kapasova Z, Rezner B, See RE. 2008. Prenatal stress enhances responsiveness to cocaine. Neuropsychopharmacology. 33(4):769–782.

Koenen KC, Moffitt TE, Poulton R, Martin J, Caspi A. 2007. Early childhood factors associated with the development of post-traumatic stress disorder: results from a longitudinal birth cohort. Psychol Med. 37:181–192.

Laroche S, Jay TM, Thierry AM. 1990. Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. Neurosci Lett. 114(2):184–190.

Logothetis NK, Eschenko O, Murayama Y, Augath M, Steudel T, Evrard HC, Besserve M, Oeltermann A. 2012. Hippocampal–cortical interaction during periods of subcortical silence. Nature. 491(7425):547–553.

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M. 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J Neurosci. 15(1 Pt 1):110–116.

MacNiven E, deCatanzaro D, Younglai EV. 1992. Chronic stress increases estrogen and other steroids in inseminated rats. Physiol Behav. 52(1):159–162.

Macrì S, Lanuzza L, Merola G, Ceci C, Gentili S, Valli A, Macchia T, Laviola G. 2013. Behavioral responses to acute and sub-chronic administration of the synthetic cannabinoid JWH-018 in adult mice prenatally exposed to corticosterone. Neurotox Res. 24(1):15–28.

Markham JA, Taylor AR, Taylor SB, Bell DB, Koenig JI. 2010. Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat. Front Behav Neurosci. 4:173.

Matsuda Y, Marzo A, Otani S. 2006. The presence of background dopamine signal converts long-term synaptic depression to potentiation in rat prefrontal cortex. J Neurosci. 26(18):4803–4810.

Maviel T, Durkin TP, Menzaghi F, Bontempi B. 2004. Sites of neocortical reorganization critical for remote spatial memory. Science. 305(5680):96–99.

McEwen BS. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. 87(3):873–904.

McEwen BS. 1998. Stress, adaptation, and disease. Allostasis and allostatic load. Ann NY Acad Sci. 840:33–44.

Mennes M, Stiers P, Lagae L, Van den Bergh B. 2006. Long-term cognitive sequelae of antenatal maternal anxiety: involvement of the orbitofrontal cortex. Neurosci Biobehav Rev. 30(8):1078–1086.

Misdrahi D, Pardon MC, Pérez-Diaz F, Hanoun N, Cohen-Salmon C. 2005. Prepartum chronic ultramild stress increases corticosterone and estradiol levels in gestating mice: implications for postpartum depressive disorders. Psychiatry Res. 137(1–2):123–130.

Mitra PP, Pesaran B. 1999. Analysis of dynamic brain imaging data. Biophys J. 76(2):691–708.

Miyagawa K, Tsuji M, Fujimori K, Saito Y, Takeda H. 2011. Prenatal stress induces anxiety-like behavior together with the disruption of central serotonin neurons in mice. Neurosci Res. 70(1):111–117.

Mölle M, Yeshenko O, Marshall L, Sara SJ, Born J. 2006. Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. J Neurophysiol. 96(1):62–70.

Moore CL, Power KL. 1986. Prenatal stress affects mother-infant interaction in Norway rats. Dev Psychobiol. 19(3):235–245.

Mueller BR, Bale TL. 2007. Early prenatal stress impact on coping strategies and learning performance is sex dependent. Physiol Behav. 91(1):55–65.

Muhammad A, Carroll C, Kolb B. 2012. Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex. Neuroscience. 216:103–109.

Mychasiuk R, Gibb R, Kolb B. 2012. Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. Synapse. 66(4):308–314.

Nádasdy Z, Hirase H, Czurkó A, Csicsvari J, Buzsáki G. 1999. Replay and time compression of recurring spike sequences in the hippocampus. J Neurosci. 19(21):9497–9507.

Nieuwenhuis IL, Takashima A. 2011. The role of the ventromedial prefrontal cortex in memory consolidation. Behav Brain Res. 218(2):325–334.

Otani S, Daniel H, Roisin MP, Crepel F. 2003. Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. Cereb Cortex. 13(11):1251–1256.

Parsons RG, Ressler KJ. 2013. Implications of memory modulation for post-traumatic stress and fear disorders. Nat Neurosci. 16(2):146–153.

Peyrache A, Khamassi M, Benchenane K, Wiener SI, Battaglia FP. 2009. Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. Nat Neurosci. 12(7):919–926.

Pinault D. 1996. A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly thalamic cells and other central neurons with biocytin or Neurobiotin. J Neurosci Methods. 65(2):113–136.

Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, Milad MR, Liberzon I. 2012. Biological studies of post-traumatic stress disorder. Nat Rev Neurosci. 13(11):769–787.

Roth TL, Lubin FD, Funk AJ, Sweatt JD. 2009. Lasting epigenetic influence of early-life adversity on the BDNF gene. Biol Psychiatry. 65(9):760–769.

Scherer IJ, Holmes PV, Harris RB. 2011. The importance of corticosterone in mediating restraint-induced weight loss in rats. Physiol Behav. 102(2):225–233.

Schwabe L, Bohbot VD, Wolf OT. 2012. Prenatal stress changes learning strategies in adulthood. Hippocampus. 22(11):2136–2143.

Siapas AG, Lubenov EV, Wilson MA. 2005. Prefrontal phase locking to hippocampal theta oscillations. Neuron. 46(1):141–151.

Siapas AG, Wilson MA. 1998. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. Neuron. 21(5):1123–1128.

Sirota A, Csicsvari J, Buhl D, Buzsáki G. 2003. Communication between neocortex and hippocampus during sleep in rodents. Proc Natl Acad Sci U S A. 100(4):2065–2069.

Sirota A, Montgomery S, Fujisawa S, Isomura Y, Zugaro M, Buzsáki G. 2008. Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm. Neuron. 60(4):683–697.

Squire LR, Alvarez P. 1995. Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr Opin Neurobiol. 5(2):169–177.

Squire LR, Slater PC, Chace PM. 1975. Retrograde amnesia: temporal gradient in very long term memory following electroconvulsive therapy. Science. 187(4171):77–79.

Steriade M. 2006. Grouping of brain rhythms in corticothalamic systems. Neuroscience. 137(4):1087–1106.

Steriade M, Contreras D, Curró Dossi R, Nuñez A. 1993. The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J Neurosci. 13(8):3284–3299.

Sterpenich V, Albouy G, Boly M, Vandewalle G, Darsaud A, Balteau E, Dang-Vu TT, Desseilles M, D'Argembeau A, Gais S et al. 2007. Sleep-related hippocampo–cortical interplay during emotional memory recollection. PLoS Biol. 5(11):e282.

Stewart J, Rajabi H. 1994. Estradiol derived from testosterone in prenatal life affects the development of catecholamine systems in the frontal cortex in the male rat. Brain Res. 646(1):157–160.

Takahashi LK, Turner JG, Kalin NH. 1992. Prenatal stress alters brain catecholaminergic activity and potentiates stress-induced behavior in adult rats. Brain Res. 574(1–2):131–137.

Takashima A, Petersson KM, Rutters F, Tendolkar I, Jensen O, Zwarts MJ, McNaughton BL, Fernández G. 2006. Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study. Proc Natl Acad Sci USA. 103(3):756–761.

Takehara K, Kawahara S, Kirino Y. 2003. Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. J Neurosci. 23(30):9897–9905.

Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. 2006. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab. 291(5):E965–E973.

Vyas A, Chattarji S. 2004. Modulation of different states of anxiety-like behavior by chronic stress. Behav Neurosci. 118(6):1450–1454.

Ward IL, Weisz J. 1984. Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology. 114:1635–1644.

Ward IL, Weisz J. 1980. Maternal stress alters plasma testosterone in fetal males. Science. 07(4428):328–329.

Weinstock M. 2008. The long-term behavioral consequences of prenatal stress. Neurosci Biobehav Rev. 32(6):1073–1086.

Weinstock M. 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav Immun. 19:296–308.

White AO, Wood MA. 2013. Does stress remove the HDAC brakes for the formation and persistence of long-term memory? Neurobiol Learn Mem. S1074-7427(13)00202-5.

Wierzynski CM, Lubenov EV, Gu M, Siapas AG. 2009. State-dependent spike-timing relationships between hippocampal and prefrontal circuits during sleep. Neuron. 61(4):587–596.

Wiltgen BJ, Brown RA, Talton LE, Silva AJ. 2004. New circuits for old memories: the role of the neocortex in consolidation. Neuron. 44(1):101–108.