Please cite this article in press as: Stevenson CW, et al., Early life programming of hemispheric lateralization and synchronization in the adult medial prefrontal cortex, *Neuroscience* (2008), doi: 10.1016/j.neuroscience.2008.06.013

Neuroscience xx (2008) xxx

EARLY LIFE PROGRAMMING OF HEMISPHERIC LATERALIZATION AND SYNCHRONIZATION IN THE ADULT MEDIAL PREFRONTAL CORTEX

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Abstract—Neonatal maternal separation (MS) in the rat increases the vulnerability to stressors later in life. In contrast, brief handling (H) in early life confers resilience to stressors in adulthood. Early life programming of stress reactivity may involve the medial prefrontal cortex (mPFC), a region which modulates various stress responses. Moreover, hemispheric specialization in mPFC may mediate adaptive coping responses to stress. In the present study, neuronal activity was examined simultaneously in left and right mPFC in adult rats previously subjected to MS, H or animal facility rearing (AFR). *In vivo* electrophysiology, under isoflurane anesthesia, was used to conduct acute recordings of unit and local field potential (LFP) activity in response to systemic administration of *N*-methyl- β -carboline-3-carboxamide (FG-7142), a benzodiazepine receptor partial inverse agonist which mimics various stress responses. MS decreased basal unit activity selectively in right mPFC. Basal LFP activity was reduced with MS in left and right mPFC, compared to AFR and H, respectively. Hemispheric synchronization of basal LFP activity was also attenuated by MS at lower frequencies. FG-7142 elicited lateralized effects on mPFC activity with different early rearing conditions. Activity in left mPFC was greater with AFR and MS (AFR>MS), whereas activity was predominantly greater with H in right mPFC. Finally, compared to AFR, MS reduced and H enhanced hemispheric synchronization of LFP activity with FG-7142 treatment in a dose-dependent manner.

These results indicate that functionally-relevant alterations in mPFC GABA transmission are programmed by the early rearing environment in a hemisphere-dependent manner. These findings may model the hemispheric specialization of mPFC function thought to mediate adaptive coping responses to stressors. They also suggest the possibility that early environmental programming of hemispheric functional coupling in mPFC is involved in conferring vulnerability or resilience to stressors later in life. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: asymmetry, functional connectivity, handling, laterality, maternal separation, stress.

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E-mail address: carlwstevenson@googlemail.com (C. W. Stevenson). **Abbreviations:** ANOVA, analysis of variance; AFR, animal facility rearing; CC, corpus callosum; FG-7142, *N*-methyl- β -carboline-3-carboxamide; H, handling; HPA, hypothalamic–pituitary–adrenal; LFP, local field potential; mPFC, medial prefrontal cortex; MS, maternal separation.

The early rearing environment plays a critical role in mediating stress reactivity later in life (Macri and Würbel, 2006). Separating rat pups from their mothers repeatedly during the postnatal period has different effects on responsiveness to stressors in adulthood depending on the duration of separation. Pups subjected to brief separations in early life, known as handling (H), show reduced behavioral measures of fear and attenuated neuroendocrine activation in response to stressors, compared to rats left undisturbed as pups (see Meaney et al., 1996 for review). In contrast, pups repeatedly subjected to prolonged maternal separation (MS) during this period display increased anxiety, compared to those subjected to H (Caldji et al., 2000; Huot et al., 2001; Francis et al., 2002). MS also enhances hypothalamic corticotropin releasing factor expression and potentiates the release of adrenocorticotropin and corticosterone in response to stressors, compared to H (Huot et al., 2001; Francis et al., 2002; Plotsky et al., 2005).

The medial prefrontal cortex (mPFC) plays an important role in modulating various stress responses. This region is involved in mediating behavioral coping responses to stressors (Amat et al., 2005, 2006). Activation of glucocorticoid receptors within mPFC also regulates the neuroendocrine stress response by modulating negative feedback of the hypothalamic–pituitary–adrenal (HPA) axis (Radley et al., 2006). Various neurotransmitters in mPFC have been implicated in mediating these behavioral and neuroendocrine responses to stressors, including GABA (Martijena et al., 2002; Tan et al., 2004) and dopamine (Bland et al., 2003). Importantly, activation of mPFC and its dopamine innervation have been shown to occur asymmetrically in response to stressors, suggesting a role for lateralized mPFC and mesocortical dopamine function in mediating adaptive coping responses to stress (Sullivan, 2004).

Early postnatal life is a critical period for the development of mPFC (Benes et al., 2000). Maturation of mPFC GABA neurons and mesocortical dopamine projections occurs during this period (Vincent et al., 1995; Benes et al., 1996). The early rearing environment exerts plasticity on the developing mPFC which likely affects its function later in life. Dendritic morphology in mPFC is altered by maternal deprivation and H, compared to animals left undisturbed in early life (Helmeke et al., 2001a,b). Compared to H, MS reduces the expression of glucocorticoid receptors in mPFC (Ladd et al., 2004), suggesting a possible mechanism by which negative feedback of HPA axis function is impaired in these animals. Furthermore, MS may alter dopamine and GABA transmission in mPFC (Matthews et al., 2001; Helmeke et al., 2008). Compared to undisturbed controls, basal mPFC do-

pamine levels are increased and stress-induced dopamine release in mPFC is attenuated with maternal deprivation (Jeziński et al., 2007). MS decreases GABA_A and benzodiazepine receptor expression in mPFC, compared to H, suggesting changes in GABA_A receptor signaling (Caldji et al., 2000). Alterations in mPFC function may therefore contribute to differences in stress responsivity mediated by the early environment.

We have recently demonstrated reduced neuronal activity in mPFC with MS in response to systemic administration of the benzodiazepine receptor partial inverse agonist *N*-methyl- β -carboline-3-carboxamide (FG-7142), suggesting altered mPFC GABA transmission with MS (Stevenson et al., 2008). This drug causes anxiety, impairs working memory and induces corticosterone release (Pellow and File, 1985, 1986; Murphy et al., 1996). FG-7142 also activates mPFC (Singewald et al., 2003; Stevenson et al., 2007, 2008) and dopamine transmission in this region (Murphy et al., 1996; Dazzi et al., 2001). Given that FG-7142 mimics various effects of stressors, our previous findings may also model an impairment of stress-induced mPFC function with MS. However, the extent to which the early rearing environment is involved in mediating lateralized mPFC function in response to stressors remains unclear. Furthermore, the early environment may mediate alterations in cortical hemispheric connectivity (Denenberg et al., 1981) relevant to modulating stress reactivity. Thus, the present study was conducted to examine the potential role of early rearing conditions in mediating lateralized activation and hemispheric functional coupling of mPFC. *In vivo* electrophysiology, under isoflurane anesthesia, was used to conduct acute neuronal activity recordings in left and right mPFC in response to FG-7142 in adult rats previously subjected to MS, H or animal facility rearing (AFR) as pups.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed in Lister hooded rats. Animals were housed on a 12-h light/dark cycle (lights on at 7 AM) with free access to food and water. All experimental procedures were carried out with approval from the University of Nottingham ethics committee and in accordance with the Animals (Scientific Procedures) Act 1986, UK (Project License number 40/2715). Every effort was made to minimize both the number of animals used and the animals' suffering in the experiments described below.

MS procedure

Adult females (Charles River, UK) were bred with in-house colony males (Biomedical Services Unit, University of Nottingham Medical School). After breeding, females were group housed (three to four/cage) for approximately 2 weeks. Pregnant females were then singly housed for approximately 1 week prior to parturition. On the day of birth (postnatal day 0), dams and pups were left undisturbed. The day after birth (postnatal day 1), litters were culled to five male and five female pups where possible. MS consisted in separating pups from their respective dams for 6 h/day on postnatal days 2–14. Dams were removed from their home cages and placed in adjacent cages with free access to food and water throughout the duration of separation. Pups were then removed and placed, as a litter, in an incubator in the same room

and maintained at a constant temperature (30–32 °C) throughout the duration of separation. Separations were conducted during the light cycle. Although several previous studies have used shorter separation durations (up to 3 h; Caldji et al., 2000; Huot et al., 2001; Francis et al., 2002), rat dams in the wild are known to forage away from their pups for up to 3 h at a time (Calhoun, 1962). Thus a longer duration of MS was deliberately chosen as it would presumably be more stressful for both dams and pups. Previous studies also indicate that 6 h of MS induces alterations in offspring behavior (Matthews et al., 1996, 1999; Gustafsson and Nylander, 2006), neurochemistry (Matthews et al., 2001; Gartside et al., 2003) and neuronal activity (Stevenson et al., 2008). Pups subjected to H were also used and underwent the same separation procedure except for a much briefer duration (15 min/day on postnatal days 2–14). Pups not separated from their dams but subjected to standard AFR practices (e.g. regular cage cleaning) served as controls. Pups were weaned on postnatal day 23 and housed two to four/cage by sex and early rearing group. Adult (60–90 days) male offspring of each early rearing group from at least two different litters were used in the experiments described below.

Surgery

All drugs and chemicals were obtained from Sigma (MO, USA) unless otherwise stated. Anesthesia was induced with 3.5% isoflurane (IVAX Pharmaceuticals, UK) in a 50% N₂O:50% O₂ mixture. The isoflurane level was reduced progressively and maintained at 2.0% throughout surgery to ensure complete inhibition of the hind-paw withdrawal reflex. Body temperature was monitored and maintained at ~37 °C using a homeothermic heating pad. The femoral vein was cannulated with Portex fine bore polythene tubing (0.28 mm ID) for i.v. administration of drug (see below) prior to placing the animal in a stereotaxic frame. The incisor bar was adjusted to maintain the skull horizontal and a scalp incision was made. A small portion of the skull (~2 mm²) was removed prior to excision of the dura mater over the left and right mPFC. Two eight-microwire electrode arrays (NB Laboratories, TX, USA), configured as bundles, were used to record unit activity from multiple neurons and local field potential (LFP) activity simultaneously in the left and right mPFC. Electrode arrays were lowered into the left and right infralimbic cortices (3.2 mm anterior and 0.5–0.7 mm lateral to bregma; 4.3–4.5 mm ventral to the cortical surface) using the atlas coordinates of Paxinos and Watson (1997).

Recording procedure

Microwire electrodes (Teflon-coated stainless steel, 50 μ m diameter/wire, NB Laboratories) had an impedance of ~100 k Ω measured at 1 kHz (Robinson, 1968). Electrode arrays were connected via a unity-gain multi-channel headstage (HST/8m-G1, Plexon Inc., TX, USA) to a multi-channel preamplifier. Extracellular action potential spikes and LFPs (gain 1000 $\times$; band-pass filtered at 250 Hz–8 kHz (spikes) and 0.7–170 Hz (LFPs); Plexon Inc.) were fed to a Multichannel Acquisition Processor system (Plexon Inc.) linked to a host PC (Dell 1.5 GHz; Windows 2000), providing simultaneous 40 kHz (25 μ s) A/D conversion on each channel at 12 bit resolution. The system provided further additional programmable amplification and filtering of spikes (final gain up to 32,000 $\times$, final bandwidth 400 Hz–5 kHz). Unit activity was displayed on D11 5000 series dual-beam (Tektronix, OR, USA) and 507 analog-digital (Hameg Instruments, Germany) oscilloscopes and also monitored aurally with the aid of a loudspeaker. LFP signals were monitored from one microwire in each array and were digitized at 1 kHz.

Drug administration

Electrode arrays were allowed to settle for ~30 min after being lowered into mPFC and BLA prior to recording basal activity for 20–30 min in each region. Following basal recordings, animals

received repeated systemic (i.v.) injections of FG-7142. Animals were injected with vehicle (10% Cremophor EL in 0.5 mL/kg; Hart et al., 1998) and three doses of FG-7142 (0.1, 1.0 and 10.0 mg/kg in 0.5 mL/kg) every 5 min. Each dose was followed immediately by injection of heparinized saline (50 μ L; CP Pharmaceuticals Ltd., UK) to flush the cannula and thus ensure that drug was administered completely. Drug and saline administration, combined, occurred over 30 s. Given the relatively short half-life ($\sim$ 30 min) of FG-7142 (Dorow et al., 1983; Yuan and Manabe, 1996), this dosing regimen was used to ensure that the effect of a particular dose did not subside prior to administration of the subsequent dose.

Histology

The total duration of each experiment was approximately 4–5 h. At the end of each experiment, current (0.1 mA) was passed through microwires in each electrode array for 5–7 s to deposit ferric ions at the electrode tips. Transcardial perfusion with 0.9% saline, followed by a 4% paraformaldehyde/4% potassium ferrocyanide solution, was conducted under deep anesthesia, allowing for the marking of recording sites using the Prussian Blue reaction (Green, 1958). Brains were removed and stored in perfusion medium until sliced. Brains were sectioned (200 μ m) with a vibratome to determine electrode array placements within left and right mPFC.

Data analysis

Spike sorting. Spike discrimination was achieved with Off-Line Sorter software (Plexon Inc.) using both automatic and manual sorting. Principal component analysis was used to display the waveforms recorded from each electrode in two-dimensional space. Each electrode was checked for artifacts (e.g. noise) which were removed manually. Automatic sorting (valley-seeking) methods were then used to separate the waveforms into individual units. The resulting clusters were inspected and the units were considered to be separate only if the cluster borders did not overlap. Furthermore, waveforms which were not consistent with the shape of action potentials and occurred within the absolute refractory period (1.1 ms; Homayoun et al., 2005) were also manually removed. Finally, clusters were considered to be single units only if the autocorrelogram showed that no significant errors occurred in sorting as a result of noise. Although the majority of electrodes showed only one discriminated unit, up to three units were observed on some electrodes.

Neuronal subtypes in mPFC have previously been characterized based on differences in firing rate and action potential waveform characteristics. Studies have shown that regular-spiking neurons, presumed to be glutamatergic pyramidal cells, have a firing rate <10 Hz; fast-spiking neurons, presumed to be local GABA interneurons, fire at a rate >10 Hz (Jung et al., 1998; Homayoun et al., 2005; Laviolette et al., 2005). None of the neurons recorded from mPFC had firing rates >10 Hz and were all therefore initially presumed to be pyramidal cells. Evidence also indicates that the majority of putative pyramidal mPFC neurons have a biphasic waveform, with an initial negative deflection followed by a positive deflection in their action potential waveform. The remaining neurons show triphasic waveforms, with a small positive deflection preceding the initial negative deflection, and are presumed to be interneurons (Sesack and Bunney, 1989; Gronier and Rasmusen, 2003). Examination of the average waveform shape of the mPFC units recorded in the present study revealed that 182 of the 187 recorded mPFC units exhibited waveform characteristics consistent with pyramidal cells. The five units which were putatively characterized as interneurons were omitted from the data analysis. A paucity of recordings from interneurons in mPFC has been observed in other studies (Homayoun et al., 2005; Laviolette et al., 2005; Stevenson et al., 2007, 2008) and is likely due to both anatomical and technical considerations. Anatomical studies indi-

cate that the majority of neurons in mPFC are pyramidal cells (Gabbott et al., 1997). Furthermore, the large diameter (50 μ m) metal electrodes used in the present study preferentially detect activity in larger pyramidal cells compared to smaller interneurons (Snodderly, 1973).

Unit activity. Electrophysiological data were analyzed using NeuroExplorer (NEX Technologies, TX, USA). Basal firing rate was defined as the mean ($\pm$ S.E.M.) firing rate (Hz) during the final 300 s of the baseline recording period (i.e. immediately preceding vehicle infusion). The effects of the different early rearing environments on the mean firing rate of mPFC units under basal conditions were analyzed separately in the left and right hemispheres using a one-way analysis of variance (ANOVA), with early rearing group as the between-subject factor. As previously reported, systemic (i.v.) administration of FG-7142 had immediate and sustained effects on unit activity (Stevenson et al., 2007, 2008). Thus the mean ($\pm$ S.E.M.) firing rate was determined for the 300 s period immediately following vehicle or drug injection. The effects of the different early rearing environments on FG-7142-induced changes in the mean firing rate of mPFC units were analyzed separately in the left and right hemispheres using a two-way ANOVA, with early rearing group as the between-subject factor and FG-7142 dose as the within-subject factor. When indicated, post hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. The level of significance was set at $P<0.05$.

LFP activity. The LFP is thought to represent the vector sum of all (i.e. dendritic, somatic, axonal, synaptic) electrical activity in a relatively large volume (up to 1 mm³) of a given brain region and is therefore attributable to both pre- and post-synaptic activity of a neuronal population (Bullock, 1997). Power spectra of LFP activity were generated using periodogram-based spectral estimation techniques (Halliday et al., 1995). LFP power spectra from left and right mPFC were generated for individual animals from the 300 s epochs associated with basal activity and each dose of FG-7142. Individual power spectra from animals in each early rearing group were then combined to give pooled power spectral estimates of LFP activity in left and right mPFC across the population of animals in each group (Amjad et al., 1997). Comparisons of LFP power between the early rearing groups in left or right mPFC were assessed under basal conditions and separately in response to each dose of FG-7142. Differences between early rearing groups were initially quantified using a χ^2 extended difference test. This test is based on the hypothesis of equal LFP power across the groups, with significant values of χ^2 indicating that this null hypothesis does not provide a plausible interpretation of the data. The χ^2 extended difference test is applied separately at each frequency of interest. Significance is assessed through inclusion of an upper 95% confidence limit, based on the null hypothesis (Amjad et al., 1997; Farmer et al., 2007). At frequencies where χ^2 exceeded the confidence limit, differences in LFP power between early rearing groups were further quantified using a log ratio comparison of spectra test (Diggle, 1990). Confidence intervals (95%) for these ratios were then used to characterize any statistically significant differences in LFP power between groups.

LFP synchronization. Early rearing group differences in functional coupling between left and right mPFC were determined by assessing LFP coherence under basal conditions and in response to FG-7142. Coherence is a measure of linear association between two signals in the frequency domain (Halliday et al., 1995). This measure is dimensionless and is bounded from 0 to 1, with a value of zero indicating no linear relationship and a value of one indicating two identical signals at a particular frequency. Coherence spectra were calculated from the periodogram-based spectral estimates described above and generated for individual animals from the 300 s epochs associated with basal activity and

Fig. 1. (A) Schematic representation of multi-electrode array placements within left and right mPFC. The distance (mm) anterior to bregma is indicated beside each coronal section (Paxinos and Watson, 1997). (B) Photomicrograph of array placements within left and right mPFC. Arrows show the ventral extent of the Prussian Blue reaction, indicating the placement of the arrays.

each dose of FG-7142. Coherence from individual animals in each early rearing group was then combined to give pooled coherence estimates of LFP synchronization between left and right mPFC across the population of animals in each group (Amjad et al.,

1997). Comparison of pooled coherence spectra between early rearing groups was assessed under basal conditions and separately for each dose of FG-7142. As with LFP power, differences in coherence between early rearing groups were initially quantified using the χ^2 extended difference test applied separately at each frequency of interest. At frequencies where χ^2 exceeded the confidence limit, differences in LFP coherence between early rearing groups were further quantified using a comparison of coherence test (Rosenberg et al., 1989). Confidence intervals (95%) for these comparisons were used to examine any statistically significant differences in LFP coherence between groups.

RESULTS

The locations of multi-electrode array recording sites within left and right mPFC are shown in Fig. 1. Only animals with histologically-confirmed placements in left and right mPFC (ventral prelimbic/infralimbic cortices) were included in the data analysis ($n=5$ rats/group). Similarly, only activity recorded from mPFC neurons ($n=23-38$ units/hemisphere/group) putatively classified as glutamatergic pyramidal cells was included in the data analysis (see above). An example of discriminated unit activity recorded from two putative glutamatergic pyramidal neurons is illustrated in Fig. 2.

Multiple unit and LFP activity in left and right mPFC under basal conditions from one experiment is represented

Fig. 2. An example of discriminated unit activity from two putative glutamatergic neurons recorded from one microwire of a multi-electrode array. (A) Cumulative waveforms (black and gray) and the resulting average waveforms (white and black) of unit activity recorded from two neurons. (B) Cluster analysis of unit activity from two neurons (black and gray) in two-dimensional space using principal component analysis.

Fig. 3. Representative unit rasters and LFP plots recorded under basal conditions in one experiment. The first seven raster plots correspond to left mPFC units and the last seven plots represent units in right mPFC. Unit activity in both hemispheres was characterized by irregular burst firing. Similarly, LFPs in left and right mPFC displayed synchronized activity characterized by irregular deflections in potential corresponding to unit firing.

in Fig. 3. Neurons in both hemispheres exhibited an irregular firing pattern characterized by periods of low tonic activity coupled with phasic burst firing, in animals from each early rearing group. This pattern of neuronal activity has been reported previously in mPFC (Gronier and Rasmussen, 2003; Jackson et al., 2004; Homayoun et al., 2005; Stevenson et al., 2007). The LFP activity pattern mirrored that of neuronal firing in both hemispheres such that the initial negative deflection in potential coincided with neuronal activity in left and right mPFC. This temporal association between unit and LFP activity has also been previously observed in cortical regions (Steriade, 1997), including mPFC (Stevenson et al., 2007).

Role of early environment on basal mPFC activity

Basal unit activity in left and right mPFC in each of the early rearing groups is shown in Fig. 4. A one-way ANOVA showed no significant differences in mean firing rate between early rearing groups in left mPFC ($F_{(2, 96)}=0.07$; $P=0.93$). Conversely, a one-way ANOVA revealed a significant main effect of early rearing group on mean firing rate in right mPFC ($F_{(2, 85)}=3.68$; $P<0.05$). Moreover, post hoc analysis showed that MS significantly decreased mean firing rate, compared to AFR and H ($P<0.05$), indicating

Fig. 4. Effects of early rearing environment on basal unit firing rate in left and right mPFC. There were no differences between early rearing groups in left mPFC. However, MS reduced unit activity in right mPFC, compared to H and AFR (* $P<0.05$).

that MS induced a reduction in basal mPFC unit activity which was lateralized to the right hemisphere.

Fig. 5 shows LFP power in left and right mPFC under basal conditions in the different early rearing groups. In left mPFC, the χ^2 extended difference test revealed significant differences in power between early rearing groups over the entire frequency range examined (0–30 Hz), showing robust differences at lower (<12 Hz) frequencies and modest differences at higher (>12 Hz) frequencies ($P<0.05$). Further pairwise comparisons using log ratio tests showed significantly enhanced power at predominantly lower frequencies (<10 Hz) with AFR, compared to H and MS ($P<0.05$). Slight increases in power were also observed with MS, compared to H ($P<0.05$). In right mPFC, the χ^2 extended difference test also revealed highly significant differences between early rearing groups over the entire frequency range ($P<0.05$). In contrast to left mPFC, log ratio tests showed significantly greater power at all frequencies with H, compared to AFR and MS ($P<0.05$). Modest differences between the AFR and MS groups were also apparent at various frequencies ($P<0.05$). Therefore differences in basal LFP power were observed in each mPFC hemisphere which depended on the early rearing conditions.

Basal LFP coherence between left and right mPFC is also shown in Fig. 5. The χ^2 extended difference test revealed significant differences in left–right coherence between early rearing groups at various lower (<4 Hz), intermediate (8–16 Hz) and higher (22–28 Hz) frequency ranges ($P<0.05$). Pairwise comparison of coherence tests applied within these frequency ranges showed significantly augmented coherence with H, compared to MS, at lower frequencies. Modest differences between the AFR and H groups were also observed within these frequency ranges ($P<0.05$), as were differences between the AFR and MS groups ($P<0.05$). Thus hemispheric synchronization in

Fig. 5. Effects of early rearing environment on basal LFP power in left and right mPFC and LFP coherence between left and right mPFC. (A) Log spectral estimates of LFP power with different early rearing conditions (AFR: solid lines; H: dashed lines; MS: dotted lines). (B) Significant differences in power were observed at various frequencies between the early rearing groups in both hemispheres ($P < 0.05$) as revealed by χ^2 extended difference tests, where the horizontal dashed lines at the bottom of the plots indicate the 95% confidence limit. (C) Log ratio plots for comparing power spectra. The two solid horizontal lines indicate the upper and lower 95% confidence limits. In left mPFC, pairwise comparisons showed increased power with AFR, compared to H and MS ($P < 0.05$). In contrast, increased power was observed with H, compared to AFR and MS ($P < 0.05$), in right mPFC. (D) LFP coherence estimates for the AFR (solid), H (dashed) and MS (dotted) groups. (E) Significant differences in coherence between early rearing groups were observed ($P < 0.05$). (F) Comparison of coherence plots, where the two solid horizontal lines represent the upper and lower 95% confidence limits. Pairwise comparisons showed predominantly increased coherence with H, compared to AFR and MS ($P < 0.05$).

mPFC was altered by the early rearing environment under basal conditions.

FG-7142 induces hemisphere-dependent mPFC activation: Role of early environment

Changes in unit activity in response to FG-7142 in left and right mPFC in each of the early rearing groups are shown in Fig. 6. In left mPFC, a two-way ANOVA revealed significant main effects of early rearing group ($F_{(2, 96)} = 3.96$; $P < 0.05$) and FG-7142 dose ($F_{(3, 96)} = 17.72$; $P < 0.0001$), as well as a significant group $\times$ dose interaction ($F_{(6, 288)} = 13.64$; $P < 0.0001$). Post hoc analysis indicated significantly increased mean firing rates with AFR (Vehicle vs. 1.0 and 10.0 mg/kg; $P < 0.0001$) and MS (Vehicle vs. 0.1 and 1.0 mg/kg; $P < 0.01$) elicited by FG-7142; however, no such effect occurred with H ($P = 0.29$). Furthermore, FG-7142

induced significantly greater unit firing with AFR, compared to MS (1.0 mg/kg: $P < 0.01$; 10.0 mg/kg: $P < 0.0001$). In right mPFC, a two-way ANOVA also revealed significant main effects of early rearing group ($F_{(2, 85)} = 6.47$; $P < 0.01$) and FG-7142 dose ($F_{(3, 85)} = 8.93$; $P < 0.0001$), as well as a significant group $\times$ dose interaction ($F_{(6, 255)} = 5.71$; $P < 0.0001$). In contrast to left mPFC, post hoc analysis indicated that FG-7142 significantly increased mean firing rate only with H (Vehicle vs. 0.1, 1.0 and 10.0 mg/kg; $P < 0.0001$), but not AFR ($P = 0.82$) or MS ($P = 0.66$), in right mPFC. Therefore FG-7142-induced unit activity in mPFC occurred in a hemisphere-dependent manner with different early rearing conditions.

FG-7142-induced alterations in LFP power in left and right mPFC in the different early rearing groups are shown in Fig. 7. In left mPFC, χ^2 extended difference tests re-

Fig. 6. Effects of early rearing environment on unit firing rate in left and right mPFC elicited by FG-7142. In left mPFC, FG-7142 increased unit firing rate with MS and AFR (* FG-7142 vs. vehicle; $P < 0.01$), but not H. Moreover, this increase was greater with AFR, compared to MS ($^{\dagger} P < 0.01$). However, in right mPFC, FG-7142 increased unit firing rate with H (* FG-7142 vs. vehicle; $P < 0.0001$), but not AFR or MS.

revealed significant differences in power between early rearing groups at each dose of FG-7142, over the majority of the frequency range examined ($P < 0.05$). In general, pairwise log ratio tests demonstrated the following significant differences between early rearing groups: AFR > MS > H ($P < 0.05$). These differences in power were apparent at all FG-7142 doses over most frequencies. In right mPFC, χ^2 extended difference tests also revealed significant differences between early rearing groups at each dose of FG-7142, again over most of the frequency range ($P < 0.05$). Compared to left mPFC, pairwise log ratio tests showed

more complex differences in power between early rearing groups which depended on FG-7142 dose and the frequencies examined. At the lower (0.1 and 1 mg/kg) doses of FG-7142, the following significant differences between early rearing groups were generally observed at most frequencies: H > MS > AFR ($P < 0.05$). Frequency-dependent differences in power were also apparent between early rearing groups at the highest (10 mg/kg) dose of FG-7142 ($P < 0.05$). These differences in LFP power between early rearing groups in response to FG-7142 were broadly similar to those observed for unit activity in left and, to a lesser extent, right mPFC. Thus, as with unit activity, altered LFP power in mPFC induced by FG-7142 occurred in a hemisphere-dependent manner with different early rearing conditions.

Fig. 8 shows FG-7142-induced changes in LFP coherence between left and right mPFC in the different early rearing groups. Qualitative inspection of the data indicated that FG-7142 enhanced left-right LFP coherence in a dose-related manner. Significant differences between early rearing groups were also revealed with χ^2 extended difference tests ($P < 0.05$), indicating that progressively greater differences were observed between the groups over a wider range of frequencies with escalating doses of FG-7142. Pairwise comparison of coherence tests showed

Fig. 7. Effects of early rearing environment on LFP power in left and right mPFC in response to FG-7142. (A) Power spectral estimates for the AFR (solid), H (dashed) and MS (dotted) groups in response to different doses of FG-7142. (B) Significant differences in power between early rearing groups elicited by FG-7142 were observed at various frequencies in both hemispheres ($P < 0.05$). (C) In left mPFC, pairwise comparisons showed the following differences in power with each dose of FG-7142: AFR > MS > H ($P < 0.05$). In right mPFC, the following differences in power were generally observed at the lower (0.1 and 1 mg/kg) doses of FG-7142: H > MS > AFR ($P < 0.05$).

Fig. 8. Effects of early rearing environment on LFP coherence between left and right mPFC induced by FG-7142. (A) Coherence estimates for the AFR (solid), H (dashed) and MS (dotted) groups in response to FG-7142. (B) Significant differences between early rearing groups were observed which became more pronounced with increasing doses of FG-7142 ($P < 0.05$). (C) Pairwise comparisons showed the following differences in coherence between early rearing groups: $H > AFR > MS$ ($P < 0.05$); these differences were also more apparent with increasing doses of FG-7142.

the following significant differences between early rearing groups: $H > AFR > MS$ ($P < 0.05$). Again, these differences in coherence became more pronounced with increasing doses of FG-7142. Therefore, altered hemispheric synchronization in mPFC was observed with different early rearing conditions in response to FG-7142.

DISCUSSION

The present results confirm that the early environment mediates hemispheric lateralization and synchronization of mPFC function in adulthood. Basal unit activity was decreased with MS selectively in right mPFC. Basal LFP activity was reduced by MS in left mPFC, compared to AFR, and right mPFC, compared to H. Hemispheric synchronization of basal LFP activity was also attenuated by MS at lower frequencies. FG-7142 induced lateralized effects on mPFC activity with different early rearing conditions. Unit activity in left mPFC was elicited selectively with AFR and MS, an effect which occurred to a lesser extent with MS compared to AFR. LFP activity was also greater in left mPFC with AFR and MS, with attenuated activity again observed with MS compared to AFR. In contrast, unit activity in right mPFC was increased selectively with H. Similarly, LFP activity was predominantly greater with H in right mPFC. Finally, MS reduced and H enhanced left-right coupling of mPFC LFP activity with FG-7142 treatment in a dose-dependent manner.

We have recently shown that basal unit activity in mPFC is reduced by MS (Stevenson et al., 2008). The present results of attenuated unit and LFP activity with MS confirm and extend these previous findings, suggesting that MS impairs adult mPFC function. We have also previously demonstrated that activity in right mPFC neurons is attenuated with MS in response to FG-7142 (Stevenson et al., 2008), indicating that functionally-relevant alterations in mPFC GABA transmission are mediated by the early environment. This benzodiazepine receptor partial inverse agonist induces various behavioral and physiological effects which are also elicited by stressors (Pellow and File, 1985, 1986; Murphy et al., 1996; Dazzi et al., 2001). Thus the results presented here confirm our previous findings and lend further support to the suggestion that FG-7142 may be useful in modeling certain aspects of mPFC dysfunction induced by stressors (Stevenson et al., 2007, 2008). Moreover, these results may also model early environmental programming of stress reactivity in adulthood by mediating lateralized activation and hemispheric coupling of mPFC function.

The finding of increased neuronal activity in mPFC with FG-7142 is congruent with evidence from *in vivo* electrophysiology (Stevenson et al., 2007, 2008) and Fos labeling (Singewald et al., 2003) studies showing FG-7142-induced neuronal activation in this region. FG-7142 is a partial inverse agonist which acts on the benzodiazepine binding

site of the GABA_A receptor. This drug would therefore be expected to decrease inhibition mediated by local GABA interneurons and consequently disinhibit activity in glutamate pyramidal cells (Palmer et al., 1988). In addition to its effects on GABAergic inhibitory tone, FG-7142 also activates other neurotransmitter systems indirectly, including mesocortical dopamine projections (Murphy et al., 1996; Dazzi et al., 2001). Thus it is possible that the effects of FG-7142 on neuronal activity in mPFC observed in the present study are due to both direct and indirect effects on GABA_A and dopamine receptor signaling in mPFC.

It should be noted that the increase in mPFC neuronal activity in response to FG-7142 reported here appears to be greater than that reported previously (Stevenson et al., 2007, 2008). While the reasons for this apparent discrepancy are not obvious, subtle variations in the dosing regimens used in these studies may account for this difference. Although cumulative dosing and i.v. administration were used both here and in our previous studies, the doses used and the interval between successive doses differed. Previously we had shown that, although the lowest dose (0.33 mg/kg) of FG-7142 increased the activity of mPFC neurons, there was little if any effect of subsequent doses on neuronal activity. In the present study we sought to assess the effects of a lower dose (0.1 mg/kg) of FG-7142 and a longer interval (5 min) between drug doses than used previously (3 min) to ensure that FG-7142 had sufficient time to exert its effect. Compared to our previous studies, the present results suggest that a lower (0.1 mg/kg) initial dose of FG-7142 has a greater effect on mPFC activity compared to a higher (0.33 mg/kg) dose. However, as we observed previously, there was little or no additional effect on neuronal activation with subsequent doses of FG-7142 despite the longer interval used here. We have recently hypothesized that low doses of FG-7142 increase neuronal activity by acting directly on the GABA_A receptor, whereas higher doses of drug may have opposing effects on neuronal firing via indirect actions on other neuromodulators such as dopamine (Stevenson et al., 2007). Future studies directly comparing the effects of different doses of FG-7142 using both between- and within-subject designs could prove useful in addressing this issue.

Maturation of GABA neurons and their dopamine innervation occurs in mPFC during the postnatal period (Vincent et al., 1995; Benes et al., 1996), suggesting that GABA and dopamine transmission in mPFC are subject to plasticity in early life (Benes et al., 2000). Indeed, the expression of GABA_A and benzodiazepine receptors in mPFC is reduced by MS (Caldji et al., 2000). Basal levels of dopamine in mPFC are elevated by maternal deprivation (Jezierski et al., 2007), possibly as a consequence of reduced dopamine metabolism (Matthews et al., 2001). Moreover, the early environment may mediate asymmetries in mPFC neurotransmission and consequently lateralized mPFC function later in life. While there is a paucity of evidence concerning asymmetry of mPFC GABA transmission (Mora et al., 1984), dopamine transmission in mPFC shows significant lateralization (Slopsema et al., 1982; Carlson et al., 1988). Furthermore, studies have

shown that alterations in hemispheric asymmetry of mPFC dopamine transmission are programmed during various developmental stages in early life (Fride and Weinstock, 1988; Brake et al., 2000), including the postnatal period (Zhang et al., 2005; Sullivan and Dufresne, 2006). Thus it is possible that the hemisphere-dependent effects of FG-7142 on mPFC activity with different early rearing conditions reported here are due in part to lateralized alterations in dopamine transmission in this region.

Perhaps the most intriguing finding of the present study is that of altered functional coupling between left and right mPFC with different early rearing conditions. Inter-hemispheric communication between mPFC neurons is mediated predominantly by the corpus callosum (CC). Pyramidal cells in left and right mPFC project via the CC to both pyramidal cells and GABA interneurons in the contralateral hemisphere (Carr and Sesack, 1998). Mesocortical dopamine afferents also synapse with these contralaterally projecting pyramidal neurons (Carr and Sesack, 2000). This anatomical arrangement suggests that GABA and dopamine transmission may regulate left–right mPFC coupling. Interestingly, the early rearing environment has been shown to influence CC development. Early H increases the width of the CC (Berrebi et al., 1988). In contrast, a reduction in neuronal number has been observed in the CC with maternal deprivation (Poeggel et al., 2000). Early life stress also decreases CC size (Sanchez et al., 1998). Taken together with previous findings, the present results indicate that early rearing conditions regulate functionally-relevant alterations in CC structure later in life (Denenberg et al., 1981). Moreover, it is possible that the differences in hemispheric functional connectivity observed between the early rearing groups in response to FG-7142 are due to the effects of this drug on GABA and dopamine transmission in mPFC.

The mPFC and its dopamine innervation are importantly involved in mediating cognition and executive function and, as such, modulate stress reactivity (Robbins and Roberts, 2007). Behavioral and neuroendocrine responsivity to stressors are regulated by mPFC (Amat et al., 2005, 2006; Diorio et al., 1993; Radley et al., 2006) and mesocortical dopamine projections (Sullivan and Gratton, 1998; Bland et al., 2003). Furthermore, activation of mPFC and dopamine transmission in this region occurs in a lateralized manner in response to stressful stimuli, such that the right hemisphere is thought to play a preferential role in mediating adaptive coping responses to stressors (Sullivan, 2004). Studies have shown that behavioral and neuroendocrine reactivity to stressors is mediated selectively by right mPFC (Sullivan and Gratton 1999, 2002) and mesocortical dopamine afferents to this hemisphere (Carlson et al., 1991, 1993; Sullivan and Szechtman, 1995; Sullivan and Gratton, 1998; Berridge et al., 1999; Thiel and Schwarting, 2001). Thus the role of the early rearing environment in regulating stress reactivity later in life could be mediated by asymmetric alterations in mPFC function.

In the present study, reduced basal unit activity in mPFC neurons was observed only in the right hemisphere with MS. In response to FG-7142, these animals also

showed activation of left but not right mPFC neurons. This deficit in right mPFC function with MS could result in impaired adaptive coping responses to stressors which may account, at least in part, for the enhanced vulnerability to stress observed in these animals (Caldji et al., 2000; Huot et al., 2001; Francis et al., 2002; Ladd et al., 2004; Plotsky et al., 2005; Gardner et al., 2005). Furthermore, the finding of selective activation of right mPFC neurons by FG-7142 with H may model the enhanced resilience to stress seen in these animals (Meaney et al., 1996). Finally, hemispheric synchronization was attenuated by MS and potentiated by H. It is therefore tempting to speculate that early environmental programming of left–right mPFC coupling may also play a role in mediating adaptive or maladaptive coping responses to stressors and, as such, in conferring resilience or vulnerability to stress, respectively. One potentially relevant confound is that the neuronal recordings obtained in the present study were conducted under anesthesia in response to a drug which mimics various effects of stressors. Directly assessing the effects of MS on stress-induced mPFC activity in the freely-behaving animal should prove useful in clarifying this issue.

The present results demonstrate clear differences between H and MS on basal activity in mPFC, lateralization of mPFC activity in response to FG-7142, and hemispheric synchronization of mPFC activity. However, while the effects of AFR were similar to H on some measures (e.g. unit activity and hemispheric coupling under basal conditions), they resembled MS on others (e.g. FG-7142-induced activity). These findings are in general agreement with those reported in previous studies and add to a growing body of evidence suggesting that AFR may represent an intermediate phenotype between H and MS (Huot et al., 2001; Jaworski et al., 2005; Gustafson and Nylander 2006; Stevenson et al., 2008).

The findings reported here indicate that the early environment programs hemispheric lateralization and synchronization of mPFC function which may impact on stress reactivity in adulthood. These results may help to further elucidate the neurobiological mechanisms by which early adversity enhances the vulnerability to stressors later in life (Heim et al., 2004). The early postnatal period is critical for the development of the brain in general and the frontal lobe in particular (Case, 1992; Thatcher, 1992). Interestingly, evidence obtained from examining changes in intra-hemispheric synchronization throughout childhood indicates that differences in the onset and rate of frontal development are observed between the left and right hemispheres which appear to be functionally relevant (Thatcher et al., 1987). Moreover, childhood abuse or neglect is associated with enhanced HPA reactivity to stressors, decreased frontal cortical volume, abnormal cortical development in the left hemisphere, and reduced CC size in adulthood (Ito et al., 1998; Heim et al., 2000; Teicher et al., 2004; Cohen et al., 2006), suggesting that alterations in asymmetric activation and hemispheric coupling of frontal cortical function may also be mediated by early life stress in humans.

Acknowledgments—The authors would like to thank Clare Spicer for her expert technical assistance. This research was supported by a Marie Curie Incoming International Fellowship and a NARSAD Young Investigator Award to C.W.S. and C.A.M.

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(Accepted 6 June 2008)