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ORIGINAL ARTICLE

Age- and Sex-Dependent Impact of Repeated Social Stress on Intrinsic and Synaptic Excitability of the Rat Prefrontal Cortex

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Abstract

Stress is implicated in psychiatric illnesses that are characterized by impairments in cognitive functions that are mediated by the medial prefrontal cortex (mPFC). Because sex and age determine stress vulnerability, the effects of repeated social stress occurring during early adolescence, mid-adolescence, or adulthood on the cellular properties of male and female rat mPFC Layer V neurons in vitro were examined. Repeated resident–intruder stress produced age- and sex-specific effects on mPFC intrinsic and synaptic excitability. Mid-adolescents were particularly vulnerable to effects on intrinsic excitability. The maximum number of action potentials (APs) evoked by increasing current intensity was robustly decreased in stressed male and female mid-adolescent rats compared with age-matched controls. These effects were associated with stress-induced changes in AP half-width, amplitude, threshold, and input resistance. Social stress at all ages generally decreased synaptic excitability by decreasing the amplitude of spontaneous excitatory postsynaptic potentials. The results suggest that whereas social stress throughout life can diminish the influence of afferents driving the mPFC, social stress during mid-adolescence additionally affects intrinsic characteristics of mPFC neurons that determine excitability. The depressant effects of social stress on intrinsic and synaptic mPFC neurons may underlie its ability to affect executive functions and emotional responses, particularly during adolescence.

Key words: development, electrophysiology, prefrontal cortex, sex, stress

Introduction

The medial prefrontal cortex (mPFC) regulates executive functions such as attentional direction, judgment, behavioral inhibition, and cognitive flexibility. It allows for the representation and consideration of information not currently in the environment (Goldman-Rakic 1996). Because many psychiatric illnesses including depression, posttraumatic stress disorder (PTSD), schizophrenia, anxiety, and attention-deficit hyperactivity disorder are characterized by impairments in executive function, the mPFC has been implicated as a primary site of the pathophysiology of these disorders (Gorman et al. 1989, Brody et al. 2001, Arnsten 2011, Veeraiah et al. 2014). Many of these disorders have also been associated with stress, which impacts cognitive function (Mazure 1995, Kessler 1997, Marin et al. 2011, Carr et al. 2013). Stress effects on cognition can be either adaptive or detrimental, depending on the severity and length of stressor exposure and the endpoint. Acute stress has been shown to improve performance on cognitive tasks that require memory (Jelici et al. 2004; Andreano and

Cahill 2006; Steidl et al. 2006; Duncko et al. 2007). Repeated or chronic stress improves the performance of simple, routine tasks but impairs performance on tasks that require cognitive flexibility and complex problem-solving, suggesting a selective negative impact on mPFC function (Hockey 1970; Hartley and Adams 1974). Consistent with this, chronic stress alters mPFC neuronal morphology, causing dendritic stunting and spine loss and these structural changes correlate to decreased cognitive flexibility (Liston et al. 2006, 2009; Radley et al. 2006; Liu and Aghajanian 2008; Muhammad et al. 2012; Ota et al. 2014; Liu et al. 2015). Chronic stress-induced dendritic atrophy of mPFC neurons accompanied by hypertrophy of neurons in the orbitofrontal cortex and dorsal medial striatum has been associated with a shift from goal-directed to habitual behavioral strategies (Dias-Ferreira et al. 2009). These effects of repeated stress on mPFC structure may underlie the association of stress with psychiatric disorders characterized by impairments in executive function.

Substantial individual variability exists in the consequences of stress and elucidating the basis for this variability can reveal the underlying mechanisms of vulnerability to stress-related diseases. The age at which stress occurs is one determinant of its consequences. The mPFC is the last cortical region to mature and this late development provides an extended period of high plasticity for early-life experiences to affect mPFC function (Kolb et al. 2012). This may account for the association of early-life stress with an increased risk of psychiatric disorders (Graham et al. 1999; Yang and Clum 2000; Heim and Nemeroff 2001).

In addition to age, sex is a determinant of the consequences of stress. Females have higher baseline corticosteroid levels and display greater hypothalamic–pituitary–adrenal axis response to stressors compared with males (Kitay 1961; Kirschbaum et al. 1992; Handa et al. 1994; Rivier 1999; Bangasser and Valentino 2012). Females also exhibit increased neuronal sensitivity to the stress-related neuropeptide, corticotropin-releasing factor (CRF), as a result of enhanced CRF receptor signaling (Bangasser et al. 2010). These factors may contribute to the relative vulnerability of females to stress-related psychiatric disorders such as depression and PTSD (Kessler et al. 2005; Bekker and van Mens-Verhulst 2007).

Most basic studies of stress effects on cognition or on mPFC function have used physical stressors such as restraint stress or chronic unpredictable stress (Goldwater et al. 2009; Muller et al. 2011; Gadek-Michalska et al. 2013; Nikiforuk and Popik 2013; Negron-Oyarzo et al. 2014; van Zyl et al. 2015). However, for humans, stressors of a social nature are most common (Brown and Prudo 1981; Prudo et al. 1981; Adler et al. 1994; Sapolsky 2005). Social stress is especially relevant during adolescence, as this is a developmental stage of dynamic social growth and interactions. We previously demonstrated that repeated social stress has sex- and age-specific effects on strategy shifting, a task requiring cognitive flexibility and mPFC function (Snyder et al. 2014, 2015).

Given the evidence that stressors target the mPFC to affect cognitive functions that are impaired in stress-related psychiatric disorders, the present study examined the effects of repeated social stress occurring during 3 distinct developmental periods (early adolescence, mid-adolescence, and adulthood) in male and female rats on the electrophysiological properties of mPFC Layer V pyramidal neurons. These neurons are the primary output layer of the prefrontal cortex, and are uniquely plastic and susceptible to perturbations due to their high concentration of NR2B-containing NMDA receptors (Wang et al. 2008). The working hypothesis was that adolescent rats and female rats would be more sensitive to the effects of social stress on mPFC function.

Materials and Methods

Animals

Male and female Sprague-Dawley rats (Charles River) served as social stress "intruders" or matched controls. Rats had access to water and food ad libitum and were pair-housed for 1 week upon arrival to acclimate, then housed individually for the duration of testing. Retired breeder male Long-Evans hooded rats (Charles River) that were individually housed served as residents for male intruders. Lactating female Sprague-Dawley rats that were individually housed served as residents for female intruders. The care and use of animals was approved by the IACUC of the Children's Hospital of Philadelphia.

Experimental Design

Social stress or control manipulation was begun during 1 of 3 developmental periods: early adolescence (PD 30–36), mid-adolescence (PD 42–46), or adulthood (PD 69–76). These adolescent ages were selected to span the social and physical stages of early and mid-adolescence as designated previously (Spear 2000; McCormick and Mathews 2010; Sturman and Moghaddam 2011). Social stress and control rats were exposed to 5 days of their respective experimental manipulation. Twenty-four hours after completion of the final manipulation, rats were sacrificed using inhaled 5% isofluorane (Baxter Healthcare) until they no longer responded to toe pinch, perfused intracardially with ice-cold sucrose-rich artificial cerebrospinal fluid (aCSF) in order to improve tissue slice quality, decapitated, and brains were removed and prepared for slice recordings.

Social Stress

The social stress was a modification of the resident–intruder paradigm, as previously described (Miczek 1979; Chaijale et al. 2013; Snyder et al. 2014). Intruders were individually placed in the home cage of a resident rat (retired breeder males for male intruders and lactating females for female intruders). Pups were removed from the cage of lactating females for the duration of the social stress period, and returned following removal of the intruder. The resident and the intruder were allowed to freely interact until either the intruder assumed a submissive defeat posture (>2 s in a frozen supine position) or 15 min had elapsed. Upon satisfaction of one of these criteria, a mesh barrier was placed in the cage to separate the animals. This mesh barrier allows for visual, olfactory, and auditory contact for the remainder of the 30-min test period. Intruders were then returned to their home cages, and pups were returned to lactating mothers. This was repeated for 5 consecutive days with the intruder being randomly placed into the home cage of a different resident rat on each testing day. Control rats were placed into a novel cage the same size as resident home cages each day. They were allowed to freely explore the cage for 15 min and then placed behind the mesh barrier for an additional 15 min, providing an identical experience to that of the intruders with the exception of the resident exposure.

Prefrontal Cortex Slice Preparation

The mPFC was dissected and placed in ice-cold sucrose-rich aCSF containing 87 mM NaCl, 75 mM Sucrose, 25 mM $NaHCO_3$, 25 mm glucose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 7 mM $MgSO_4$ and bubbled with 95% O_2/5% CO_2. Horizontal 300-μm-thick slices were made using a Leica 1000S Vibratome

(Leica Microsystems). The slices were collected and incubated at 35.5 °C in sucrose-rich aCSF bubbled with 95% O_2/5% CO_2 for 1 h, and then stored at room temperature until being transferred to the recording chamber.

Electrophysiological Recordings

mPFC slices were bathed in a heated (36–37 °C) recording chamber with aerated (95% O_2/5% CO_2) Ringer's aCSF (125 mM NaCl, 25 mM $NaHCO_3$, 25 mM glucose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$). Layer V PFC neurons are glutamatergic pyramidal neurons or GABAergic interneurons. All recorded neurons were visually identified using the Zeiss Axioskop with attached camera. To identify Layer V pyramidal neurons, the presence of an apical dendrite projecting to Layer II/III was required, as interneurons do not possess this apical dendrite. Layer V was identified based on the organization of apical dendrites; in Layer V, all apical dendrites will project upwards to Layer II/III. Figure 1 shows the location of the recordings and a biocytin-stained pyramidal neuron. During recording, firing pattern and action potential (AP) shape were used to support neuronal type. Interneurons will possess a distinct steep afterhyperpolarization with fast recovery that results in fast firing rates. Pyramidal neuron afterhyperpolarization has lower amplitude and slower decay time.

Electrode pipettes were pulled using a Sutter P97 puller (Sutter Instruments) and filled with a potassium-gluconate intracellular solution (120 mM K-gluconate, 6 mM KCl, 0.3 mM GTP, 0.2 mM EGTA, 10 mM HEPES, and 4 mM ATP-Mg). Clampex 10.0 software (Axon Laboratories, Molecular Devices) was used to write and run recording programs; the Multi-Clamp Commander 700A was used to maintain patch seals and apply holding current or voltage (Molecular Devices). Only cells that formed a giga-Ohm seal were used for data collection. For passive membrane properties and excitability studies, a step-current (50 pA increment) protocol was applied for 90 s per sweep, with 15 sweeps, each sweep representing a 50-pA step from the previous sweep. AP spike numbers, resting membrane potential, input resistance, AP amplitude (taken as the distance between threshold and peak of the AP), half-width (taken as the time from halfway up the rise of the AP to halfway down the fall of the AP), and afterhyperpolarization amplitude (taken as the distance between threshold and maximum afterhyperpolarization) were recorded and analyzed using Clampfit 10.0 software (Axon Laboratories). Latency to first spike was quantified at 250 and 150 pA (for male mid-adolescents) and interspike interval was recorded as the average interval between all spikes for the 250-pA or 150-pA sweep. Cells that did not produce more than one spike at 250 and/or 150 pA were rejected from interspike interval and latency analysis. For synaptic transmission studies, cells were recorded in voltage clamp, held at −60 mV. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded for 5 min, and their frequency and amplitude was analyzed using Clampfit 10.0. For miniature excitatory postsynaptic currents (mEPSCs), 1 μM tetrodotoxin was added to the bath, and the resulting mEPSCs were recorded in voltage clamp for 5 min at −60 mV. Amplitude and frequency of mEPSCs was analyzed using Clampfit 10.0.

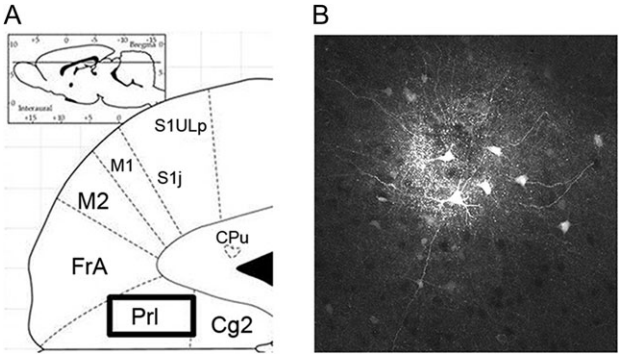

Figure 1. Placement of recordings. (A) Horizontally oriented Atlas plate (Bregma −3.1 mm, Interaural 6.9 mm; Adapted from Plate 116; Paxinos and Watson 2006) showing the region of prefrontal cortex from which recordings were obtained as indicated by the black box. Recordings were taken from Layer V prelimbic region of the prefrontal cortex. Cg2 = cingulate cortex area 2, Cpu = caudate putamen, FrA = frontal association cortex, M1 = primary motor cortex, M2 = supplementary motor cortex, S1j = primary somatosensory cortex, S1ULP = somatosensory cortex upper lip region, PrL = prelimbic prefrontal cortex. (B) PFC Layer V labeled with biocytin showing pyramidal neurons.

Data Analysis

Data were analyzed using the Clampfit 10.0 software package. Two-way ANOVAs were run to compare each parameter across all age points and stress or control. Separate ANOVAs were run for males and females. For excitability curves, the number of APs at each injected current was recorded, and a repeated measures ANOVA was used to compare each set of control and stressed rats. Post hoc comparisons were by Tukey's HSD test unless otherwise stated.

Results

Effects of Social Stress on Passive Membrane Properties of mPFC Neurons

To examine intrinsic excitability, whole cell recordings were made from early adolescent males (12 cells/5 rats control and 16 cells/5 rats stress), mid-adolescent males (9 cells/4 rats control and 8 cells/4 rats stress), adult males (9 cells/4 rats control and 8 cells/4 rats stress), early adolescent females (13 cells/4 rats control and 20 cells/4 rats stress), mid-adolescent females (15 cells/4 rats control and 13 cells/4 rats stress) and adult females (16 cells/5 rats control and 19 cells/4 rats stress). Table 1 shows the mean of all parameters for all groups. Social stress selectively increased the input resistance of mPFC neurons of mid-adolescent males (Main effect: $F_{5,80} = 5.3$, $P = 0.0003$; Age × Stress interaction: $F_{2,80} = 7.4$, $P = 0.0012$; $P < 0.05$ Tukey's HSD). There was no effect on input resistance in females (Main effect: $F_{5,100} = 1.6$, $P = 0.16$). Resting membrane potential was not altered by stress in either males or female (Main effect for males: $F_{5,80} = 1.8$, $P = 0.13$; Main effect for females: $F_{5,100} = 1.4$, $P = 0.23$).

Effects of Social Stress on AP Parameters

Social stress commonly increased AP half-width in mid-adolescent males and females (Table 1). A 2-factor ANOVA revealed a main effect ($F_{5,80} = 8.6$, $P < 0.0001$) for males and an Age × Stress interaction ($F_{2,80} = 7.5$, $P = 0.001$) and post hoc analysis revealed that this was driven by a stress-induced increase in mid-adolescent males compared with all other ages ($P < 0.05$; Tukey's HSD). Likewise for females, there was a main effect ($F_{5,100} = 5.3$, $P = 0.0002$) and an Age × Stress interaction ($F_{2,100} = 10.6$, $P < 0.0001$) such that stress increased AP half-width in female mid-adolescents compared with the corresponding mid-adolescent control group. Consistent with an increased AP half-width, there was a trend for social stress to decrease the peak AP amplitude for males (Main effect: $F_{5,80} = 2.0$, $P = 0.08$) and females

Table 1. Passive membrane properties of Layer V pyramidal neurons

Group	RMP (mV)	R_N (MΩ)	AP_{Thresh} (mV)	AP_{Amp} (mV)	$AP_{½\ width}$ (msec)	AHP_{Amp} (mV)
Male						
EA control (12)	−63.59 ± 2.3	159 ± 14	−36.1 ± 2.4	78.6 ± 4.8	2.33 ± 0.20	−37.0 ± 2.8
EA stress (16)	−61.47 ± 2.0	145 ± 12	−35.2 ± 2.1	73.6 ± 4.1	1.95 ± 0.17	−32.1 ± 2.4
MA control (9)	−60.99 ± 2.5	121 ± 16	−40.4 ± 2.7	85.7 ± 5.2	2.55 ± 0.21	−35.4 ± 3.1
MA stress (8)	−60.58 ± 2.1	**214 ± 13***	**−52.4 ± 2.2***	**65.0 ± 4.4***	**3.51 ± 0.18***	−35.7 ± 2.6
Adult control (9)	−63.32 ± 2.1	164 ± 13	−47.8 ± 2.2	75.6 ± 4.4	2.66 ± 0.18	−28.0 ± 2.6
Adult stress (8)	−55.81 ± 2.1	185 ± 13	−50.3 ± 2.2	75.4 ± 4.3	2.39 ± 0.18	**−36.4 ± 2.5***
Female						
EA control (13)	−63.37 ± 1.9	162 ± 16	−39.3 ± 2.8	75.0 ± 5.1	2.21 ± 0.12	**−52.9 ± 2.8***
EA stress (20)	−63.46 ± 1.4	147 ± 12	−44.1 ± 2.2	77.9 ± 3.9	2.15 ± 0.09	−35.6 ± 2.2
MA control (15)	−63.09 ± 1.7	156 ± 15	−38.3 ± 2.6	93.4 ± 5.1	1.81 ± 0.11	−35.5 ± 2.6
MA stress (13)	−67.43 ± 1.7	167 ± 15	−39.4 ± 2.5	76.9 ± 4.9	**2.59 ± 0.11***	−35.3 ± 2.6
Adult control (16)	61.50 ± 1.7	185 ± 15	−41.6 ± 2.5	78.5 ± 4.9	2.29 ± 0.11	−34.7 ± 2.5
Adult stress (19)	−64.50 ± 1.5	132 ± 13	−46.3 ± 2.3	86.6 ± 4.5	2.21 ± 0.10	−38.6 ± 2.3

Note: Data are mean ± standard error of the mean (SEM) for the number of cells in parentheses. Rat groups are as follows: EA, early adolescence; MA, mid-adolescence; Adult. Number of cells in parentheses. Values that are significantly different than their sex and aged matched experimental counterpart are indicated in bold and by an asterisk, *$P < 0.05$. RMP = resting membrane potential in millivolts, R_N = input resistance in megaohms, AP_{Thresh} = threshold for firing action potential in millivolts, AP_{Amp} = peak amplitude of action potential in millivolts, $AP_{1/2\ width}$ = half-width of the action potential in milliseconds, AHP_{Amp} = peak amplitude of the afterhyperpolarization in millivolts.

(Main effect: $F_{5,100} = 2.0$, $P = 0.08$). There was a significant decrement produced by stress for males (Stress effect: $F_{1,80} = 5.5$, $P = 0.02$) but not for females (Stress effect: $F_{1,100} = 0.2$, $P = 0.66$). For males, there was a trend for a Stress × Age interaction ($F_{2,80} = 2.7$, $P = 0.08$) and a Tukey's HSD post hoc test revealed that stress specifically occurring during mid-adolescence decreased AP amplitude ($P < 0.05$). For females, there was a Stress × Age interaction ($F_{2,100} = 3.5$, $P < 0.05$) but a Tukey's HSD post hoc test did not reveal significant differences between individual groups.

Certain other effects of social stress on AP properties were sex specific. For example, social stress selectively decreased the threshold for AP firing of mPFC neurons of male mid-adolescent rats (Main effect: $F_{5,80} = 11.1$, $P < 0.0001$; Age × Stress interaction: $F_{2,80} = 3.9$, $P = 0.02$). Only males that were stressed during mid-adolescence were different from their age-matched control group ($P < 0.05$, Tukey's HSD). There was no effect on AP threshold for females (Main effect: $F_{5,100} = 1.7$, $P = 0.15$).

Interestingly, stress reduced afterhyperpolarization amplitude in females ($F_{1,100} = 4.9804$, $P = 0.03$) and this was driven by early adolescent female controls having a significantly greater AHP amplitude compared with all other groups (Age × Stress interaction: $F_{2,100} = 10.2$, $P < 0.0001$, $P < 0.05$, Tukey's HSD).

Effects of Social Stress on Intrinsic Neuronal Excitability

Step-current recordings used to examine passive membrane properties were also used to generate APs. Figure 2A shows representative traces of AP firing elicited by the same current magnitude for male and female stressed and control rats. Social stress decreased mPFC neuronal excitability for both male and female mid-adolescents (males: $F_{1,15} = 13.2$, $P = 0.0025$; females: $F_{1,27} = 19.4$, $P = 0.0001$) and female adults ($F_{1,33} = 1.9$, $P = 0.009$) (Fig. 2B). Interestingly, the effects of stress were qualitatively different for mid-adolescents compared with adult females. Whereas social stress during mid-adolescence robustly decreased the maximum number of spikes generated by increasing current, during adulthood it shifted the entire curve to the right, suggesting that it affects excitability through distinct mechanisms at these 2 ages. Additionally, excitability was increased for male mid-adolescents at lower current intensities (Fig. 2B).

The effects of stress on intrinsic excitability were further analyzed for male and female mid-adolescents and female adults by examining interspike interval and spike latency. The interspike interval determined at 250 pA was significantly increased by stress in all groups ($P < 0.05$ for male and female mid-adolescents; $P = 0.0005$ for female adults, Student's *t*-test; Fig. 2C). Notably, for male mid-adolescents the interspike interval for firing elicited by 150 pA-injected current was decreased by social stress ($P < 0.05$, Student's *t*-test), revealing a biphasic alteration of interspike interval in male mid-adolescents that reflect the biphasic alterations in intrinsic excitability. Stress had no effect on spike latency ($P > 0.05$ for all groups) (Supplementary Fig. 1).

Effects of Social Stress on Synaptic Transmission

Representative traces of sEPSCs are shown in Figure 3A. The amplitude of sEPSCs increased with age in both males ($F_{2,68} = 10.0$, $P = 0.0002$) and females ($F_{2,86} = 6.0$, $P = 0.004$). Stress significantly reduced EPSC amplitude in males ($F_{1,68} = 5.6$, $P = 0.02$) and females ($F_{1,86} = 28.1$, $P < 0.0001$). There was an Age × Stress interaction for females such that female adult controls had higher sEPSC amplitude relative to all other groups ($F_{2,86} = 5.6$, 0 = 0.005, $P < 0.05$ Tukey's HSD). There was no Age × Stress interaction for males ($F_{2,68} = 0.37$; $P = 0.69$). Stress had no effect on sEPSC frequency in either sex (Main effect for males: $F_{5,68} = 0.84$, $P = 0.5$; Main effect for females: $F_{5,86} = 1.7$, $P = 0.14$).

Miniature Excitatory Postsynaptic Currents

In order to further examine the effects of social stress on synaptic transmission, miniature EPSCs (mEPSCs) were recorded for male ($n = 11$ for control and 10 for stress) and female ($n = 9$ for control and 14 for stress) mid-adolescent rats (Fig. 4A). There was an effect of sex on mEPSC frequency ($F_{1,43} = 6.7$, $P = 0.01$) such that frequency was greater in males. Stress significantly reduced mEPSC frequency ($F_{1,43} = 23.7$, $P < 0.0001$) and there was a Sex × Stress interaction ($F_{1,43} = 12.4$, $P = 0.001$; $P < 0.05$, Tukey's HSD), indicating that stress specifically decreased mEPSC frequency for males. The mEPSC amplitude was greater in males (Sex effect: $F_{1,43} = 34.4$, $P < 0.0001$) but there was no effect of stress ($F_{1,43} = 1.5$, $P = 0.2$) or Sex × Stress interaction ($F_{1,43} = 0.2$, $P = 0.6$).

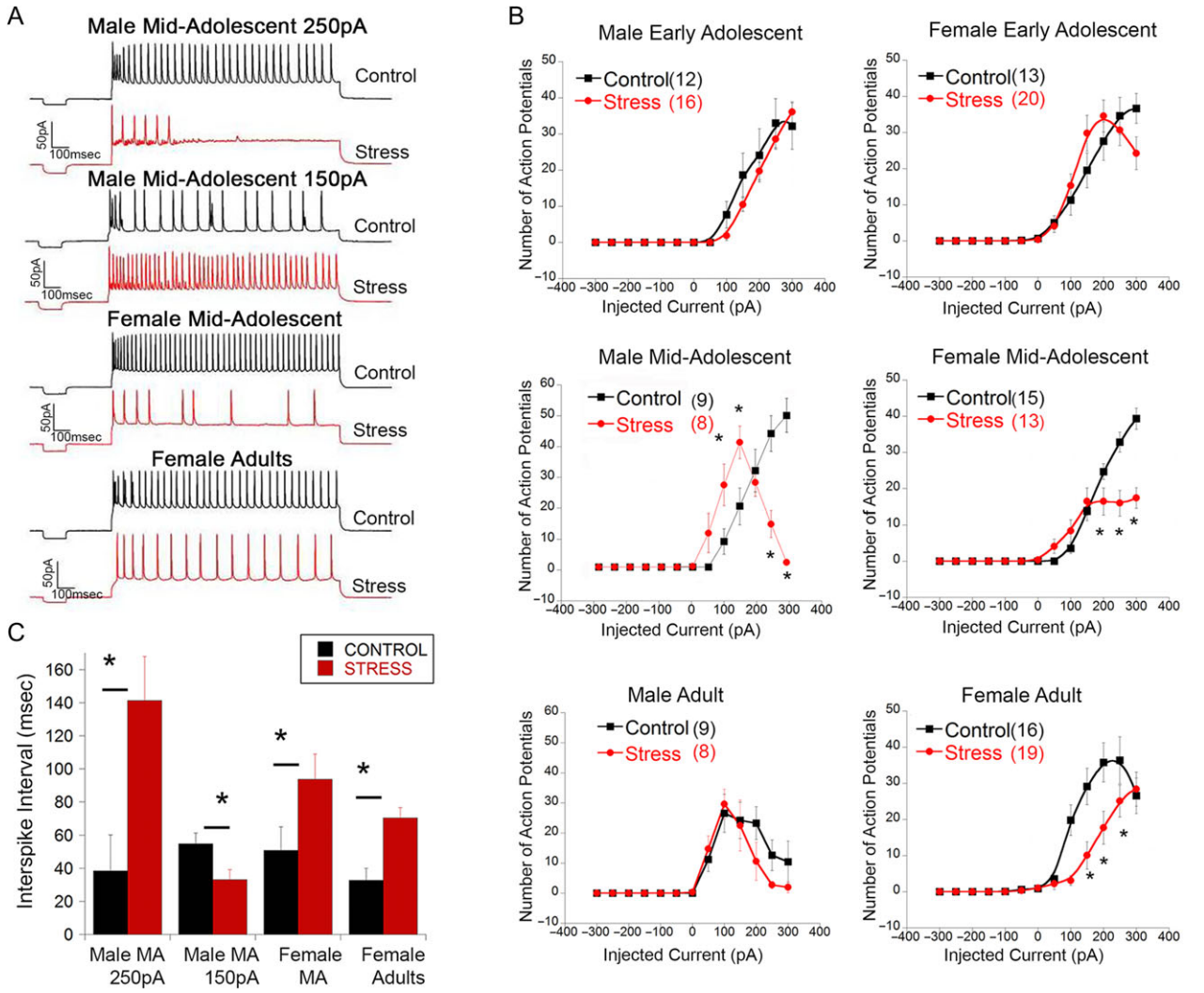

Figure 2. Effects of social stress on intrinsic excitability. (*A*) Representative traces from male and female mid-adolescent control and stressed rats showing the firing elicited by injected current (150 or 250 pA). (*B*) Current-response plots comparing control and stressed rats for each sex–age group. The number of cells for each group is indicated in the graph legends. Repeated measures ANOVA of number of spikes at injected currents for each group revealed that stress significantly altered neuronal excitability in male mid-adolescents ($F_{1,15} = 13.2$, $P = 0.003$), female mid-adolescents ($F_{1,27} = 19.4$, $P = 0.0001$), and female adults ($F_{1,33} = 1.9$, $P = 0.01$). Vertical lines indicate standard error of the mean (SEM). *$P < 0.05$ for individual points. (*C*) Quantification of interspike interval. Stress significantly increased interspike interval in male mid-adolescents ($n = 6$ cells for control and 6 for stress $P = 0.02$), female mid-adolescents ($n = 15$ cells for control and 13 for stress, $P < 0.05$), and female adults ($n = 15$ cells for control and 17 cells for stress, $P = 0.0005$) at a high current that reliably elicited spiking (250 pA). However, at a low injected current (150 pA), stress reduced interspike interval in male mid-adolescents ($n = 6$ cells for control and 7 cells for stress, $P = 0.02$). *$P < 0.05$.

Discussion

The present study is the first to characterize the effects of repeated social stress on the intrinsic and synaptic activity of rat mPFC Layer V neurons that govern executive function. Additionally, it examined the age of occurrence and sex as variables that determine the consequence of social stress. Repeated social stress generally decreased the impact of synaptic transmission regardless of age as a result of a decrement in postsynaptic responses. When it occurred during mid-adolescence, social stress additionally decreased intrinsic neuronal excitability of both males and females. Similar effects of social stress during mid-adolescence on AP half-width and amplitude implicate mid-adolescence as a developmental window during which certain ion channels that regulate mPFC neuronal excitability are particularly stress sensitive. In addition to these general and age-specific effects of social stress on mPFC neuronal properties, certain effects were sex specific. Together, the results are consistent with other structural and electrophysiological evidence for impairments in mPFC function produced by nonsocial stressors and provide novel information on age and sex dependence of these effects.

Relationship to Previous Studies

The majority of studies examining stress effects on the mPFC have focused on morphological changes. These demonstrated that repeated restraint stress induces dendritic retraction and spine loss of both Layer II/II and layer V mPFC neurons (Liston et al. 2006; Radley et al. 2006; Liu and Aghajanian 2008). Several studies have characterized the effects of stressors on mPFC physiology in vivo. These studies demonstrated that stress decreases long-term potentiation in pathways to the PFC,

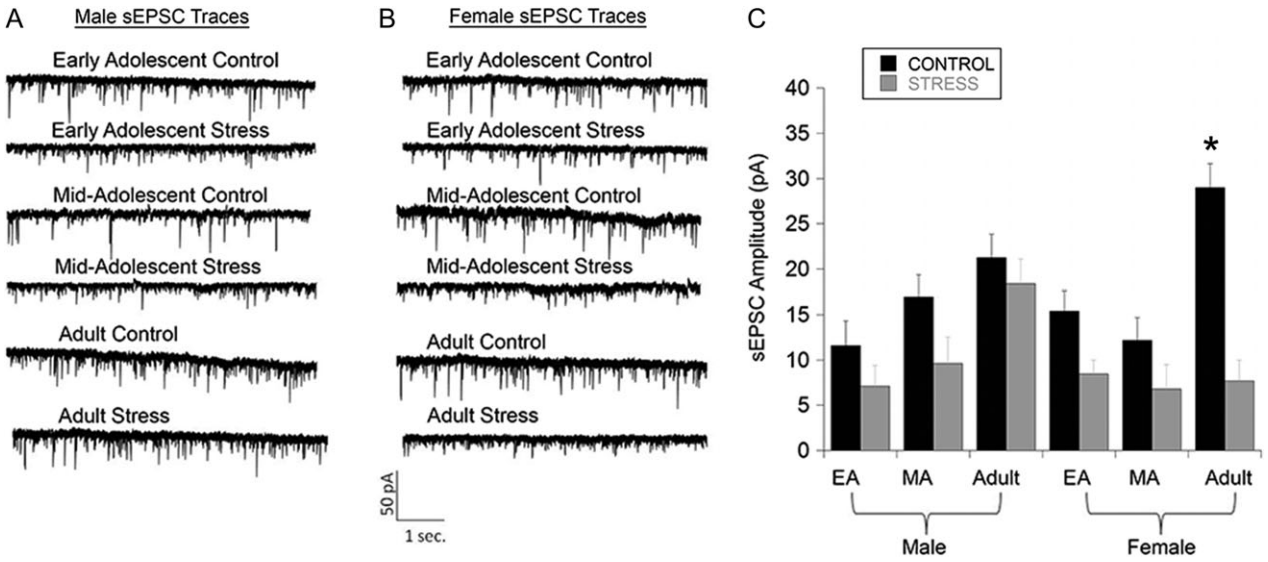

Figure 3. Stress reduces synaptic excitability. (*A*) Representative sEPSC traces from each male rat group. (*B*) Representative sEPSC traces from each female rat group. (*C*) Quantification of stress effects on sEPSC amplitude. Bars show the mean sEPSC amplitude in control (black) and stressed (gray) rats. Stress significantly reduced sEPSC amplitude in males ($F_{1,68} = 5.6$, $P = 0.02$) and females ($F_{1,86} = 28.1$, $P < 0.0001$). Vertical lines indicate standard error of the mean (SEM). *$P < 0.05$ post hoc Tukey's HSD. Male EA control ($n = 11$), Male EA stress ($n = 16$), Male MA control ($n = 9$), Male MA stress ($n = 10$), Male Adult control ($n = 12$), Male Adult stress ($n = 12$), Female EA control ($n = 17$), Female EA stress ($n = 16$), Female MA control ($n = 14$), Female MA stress ($n = 12$), Female Adult control ($n = 12$), Female Adult stress ($n = 16$).

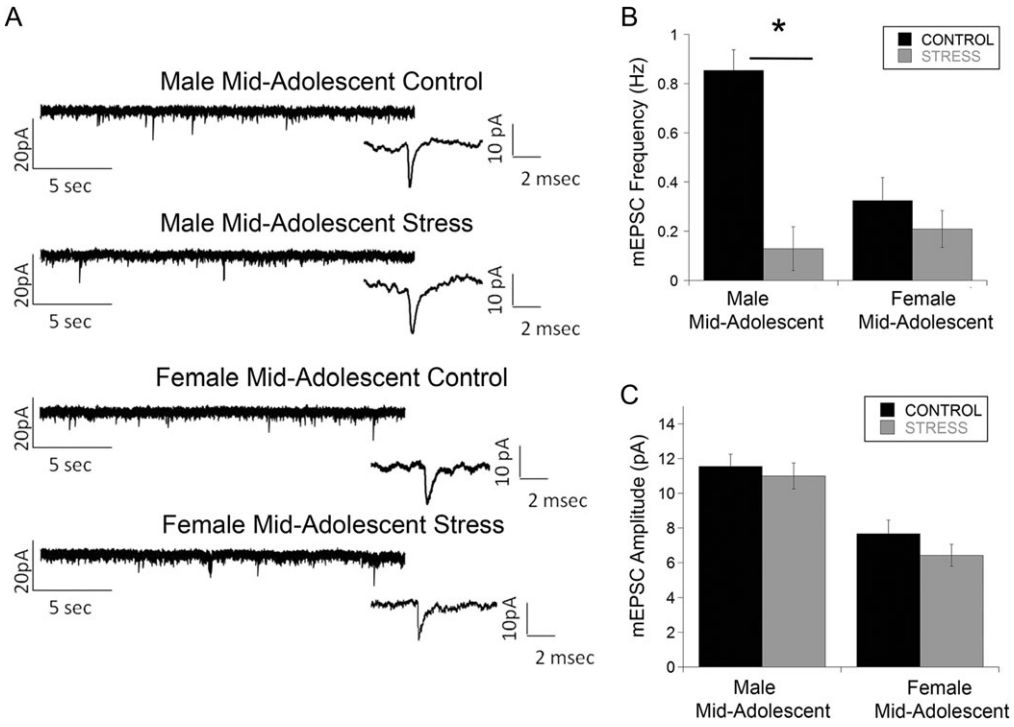

Figure 4. Stress reduces frequency of mEPSCs in male mid-adolescent rats. (*A*) representative mEPSC traces from male and female mid-adolescent rats. (*B*) Stress significantly reduced frequency of mEPSCs only in males (Stress effect: $F_{1,43} = 23.7$, $P < 0.001$; Stress × Sex effect: $F_{1,43} = 12.4$, $P = 0.001$). Vertical lines indicate standard error of the mean (SEM). *$P < 0.05$ post hoc Tukey's HSD. Stress had no effect on mEPSC amplitude. Male MA control ($n = 11$), Male MA stress ($n = 10$), Female MA control ($n = 9$), Female MA stress ($n = 14$).

indicating an impairment in synaptic plasticity by stress (Maroun and Richter-Levin 2003; Jay et al. 2004; Goldwater et al. 2009; Lee and Kirkwood 2011). Additionally, stress exposure impaired cortico-limbic coherence (Jay et al. 2004; Lee and Kirkwood 2011; Oliveira et al. 2013). In vitro studies of mPFC neurons in slice preparations are consistent with a decrease in synaptic function, demonstrating that repeated restraint stress decreases serotonin- and hypocretin-elicited EPSPs in Layer V mPFC neurons and this correlates with apical dendritic spine loss (Liu and Aghajanian 2008).

Most studies investigating the effects of stressors on mPFC neurons used adult male rats and both nonsocial and social stressors including chronic unpredictable stress, restraint stress, maternal separation, and social isolation (Liston et al. 2006, 2009; Liu and Aghajanian 2008; Dias-Ferreira et al. 2009; Varela et al. 2012). The present study substantially advanced the current state of knowledge by quantifying the effects of resident–intruder stress occurring during either adolescence or adulthood on the intrinsic properties of mPFC Layer V neurons and synaptic transmission in both sexes. Resident–intruder stress was used because for humans, stressors of a social nature are prevalent and this is considered a relevant model of human social stress (Bjorkqvist 2001; Blanchard et al. 2001). Despite the prevalent use of this model in the stress literature, the effects of this stress on mPFC cellular physiology have not been explored. This is, to our knowledge, the first comprehensive study of the effects of resident–intruder stress on mPFC cellular physiology in both males and females, at 3 distinct developmental time points chosen to span the course of mPFC maturation.

Repeated Social Stress and Intrinsic Excitability

Mid-adolescence was a specific developmental period during which social stress affected passive membrane properties and decreased intrinsic excitability of mPFC Layer V neurons. The common ability of social stress during mid-adolescence to increase AP half-width in both males and females is likely related to the common reduction in AP firing elicited by relatively high current intensities. Lengthening of the AP half-width results in slower repolarization, increased calcium influx, and maintenance of a depolarized state that would prevent voltage-activated sodium channels from reactivating. As AP half-width is determined by A-type potassium channels, the results suggest that this channel is targeted by social stress during mid-adolescence, either through decreased expression or function (Kim et al. 2005; Bean 2007). This could also account for the stress-related decrease in AP amplitude, which is more prominent in males. The finding that mPFC neurons of males exposed to social stress during mid-adolescence also have a higher input resistance is consistent with decreased potassium channel conductance and suggests that males may be more sensitive to this effect than females.

The stress-related increase in interspike interval in the absence of effects on spike latency in male and female mid-adolescents also implicates delayed-rectifier M-type potassium channels (Kv7), which regulate interspike interval and spiking patterns (Yue and Yaari 2004; Lawrence et al. 2006). Activation of M-type K+ channels (Kv7/KCNQ channels) reduces neuronal firing in multiple brain regions, including hippocampus, striatum, and infralimbic PFC (Aiken et al. 1995; Cooper et al. 2001; Brown and Passmore 2009; Song et al. 2009; Santini and Porter 2010; Lee and Kwag 2012; Perez-Ramirez et al. 2015). Notably, conditioned fear is associated with activation of M-type K+ channels and inhibition of infralimbic PFC neuronal excitability (Santini and Porter 2010).

Whereas the ability of social stress to decrease mPFC Layer V neuronal excitability elicited by high current intensity was shared by both male and female mid-adolescent rats, a sex difference was apparent at low intensity currents, which revealed increased excitability of stressed male rats. The effect of social stress to selectively increase excitability of male mid-adolescent mPFC Layer V neurons at relatively low currents may be related to its ability to lower AP threshold in this group. The basis for this sex difference, which must lie in ion channel expression and/or kinetics, is presently unknown. However, sex differences in ion channels of Layer V pyramidal neurons that are specific to adolescence have been documented and attributed to differential phosphorylation (Marron Fernandez de Velasco et al. 2015). Together, the results suggest that under conditions of high afferent drive, mPFC output neurons of both male and female mid-adolescent rats with a history of social stress would be less responsive compared with unstressed counterparts. However, under conditions of more moderate input, mPFC Layer V neurons of male mid-adolescent rats may be more responsive.

Although neuronal sensitivity to current injection was also decreased by social stress in adult females, the underlying mechanism for this population is likely to be different as they exhibited no alteration in AP characteristics. Moreover, in this case, the current-response curve was only shifted to the right and the same maximum number of APs was obtained with sufficient current.

Repeated Social Stress and Synaptic Excitability

Previous in vitro studies of mPFC Layer V neurons demonstrated that repeated restraint stress reduces serotonin- and hypocretin-evoked EPSCs and this was expressed as a decreased frequency (Liu and Aghajanian 2008; Yuen et al. 2012; Liu et al. 2015). Changes in sEPSCs were not assessed in that study. The current finding of age-related increases in sEPSC amplitude is indicative of the development of excitatory drive and expression of excitatory amino-acid receptors and consistent with the mPFC coming online later in development. The finding that social stress generally decreased EPSC amplitude without affecting frequency is indicative of a selective decrease in postsynaptic sensitivity. This could be explained by stress-induced loss of dendritic spines, which has been demonstrated to occur with other repeated stressors (Liston et al. 2006, 2009; Radley et al. 2006; Liu and Aghajanian 2008; Muhammad et al. 2012; Ota et al. 2014; Liu et al. 2015). Alternatively, this could reflect decreased expression of excitatory amino-acid receptors. The stress-induced decrease in EPSC amplitude is consistent with, and could contribute to, reported impairments in long-term potentiation and loss of coherence between the mPFC and other brain regions (Jay et al. 2004; Oliveira et al. 2013; Zitnik et al. 2016).

The present study identified potential sex differences in glutamatergic transmission in the mid-adolescent mPFC indicating a greater mEPSC frequency and amplitude in males compared with females. Notably, stress decreased mEPSC frequency in males such that it was comparable to that in females. This is consistent with previous studies using repeated restraint or chronic corticosterone administration, which also decreased mEPSC frequency of mPFC cells of male adolescent rats although mEPSC amplitude was also affected in these studies (Yuen et al. 2012).

Functional Implications

The Layer V pyramidal neurons that were examined in this study are thought to control behavioral flexibility and working memory through their uniquely high concentration of NR2B-containing NMDA receptors (Wang et al. 2008). By decreasing intrinsic and synaptic excitability in these neurons, the present findings predict that repeated social stress would impair these functions and that mid-adolescent animals would be more vulnerable. Previous studies demonstrated that social stress during male adolescence impairs working memory and conditioned fear learning when measured during adulthood and this was associated with a decrease in NMDA receptors in the mPFC (Novick et al. 2013,

2016). Similarly, social stress impaired performance of an operant strategy-shifting task in male rats that were stressed during mid-adolescence and tested during adulthood (Snyder et al. 2014). The lack of immediate behavioral consequences in this study was attributed to evidence that mPFC activity is not well correlated to operant strategy-shifting performance in adolescent males as it is in adults, suggesting that other circuits facilitate this function. A parallel study in females revealed decrements in strategy shifting in rats that were stressed and tested during early adolescence and decrements in simple discrimination in rats that were stressed and tested during mid-adolescence (Snyder et al. 2015). Although the present findings would have predicted more consistent decrements in strategy shifting in female rats stressed during mid-adolescence or adulthood, this may reflect the limitations of translating cellular findings in the in vitro slice to complex in vivo circuitry, and may indicate that cellular changes in mPFC precede behavioral impact.

In addition to executive function and working memory, the mPFC is involved in fear expression through its reciprocal connections to structures such as the dorsal raphe nucleus and the amygdala. For example, inhibition of serotonin neurons of the dorsal raphe nucleus by the mPFC mediates the enduring protective effects of perceived control over a stressor that prevents learned helplessness (Amat et al. 2006). In subjects with diminished mPFC excitability, this protective effect would be lost and learned helplessness, considered a rodent endpoint of depression, would be facilitated. Reciprocal communication between the mPFC and amygdala is important in the expression of conditioned fear and extinction (Quirk et al. 2008). mPFC drive to inhibitory intercalated cells in the amygdala inhibits conditioned fear and facilitates extinction. A decrement in this drive as a result of repeated social stress would interfere with the extinction of conditioned fear, a characteristic feature of PTSD.

Conclusion

This study revealed age- and sex-dependent effects of social stress on the physiology of mPFC Layer V pyramidal neurons. It extends previous work by examining intrinsic and synaptic excitability in an age- and sex-dependent manner. It is the first to directly compare the effects of social stress, a human-relevant stressor, on males and females at developmental time points during which mPFC maturation occurs. These findings underscore the roles of age and sex as determinants of the vulnerability of PFC function to social stress and reinforce that mid-adolescence is a particularly vulnerable developmental window for both sexes during which social stress can significantly impact structures related to cognitive function and emotional expression.

Supplementary Material

Supplementary material can be found at *Cerebral Cortex* online.

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