Sex Differences Distinguish Intracortical Glutamate Receptor-Mediated Regulation of Extracellular Dopamine Levels in the Prefrontal Cortex of Adult Rats

M. N. Locklear1,2, A. B. Cohen2, A. Jone1,2 and M. F. Kritzer2

1Graduate Program in Neuroscience and 2Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY 11794-5230, USA

Address correspondence to Mallory Locklear. Email: mallory.locklear@gmail.com

Executive functions of the prefrontal cortex (PFC) are sensitive to local dopamine (DA) levels. Although sex differences distinguish these functions and their dysfunction in disease, the basis for this is unknown. We asked whether sex differences might result from dimorphisms in the glutamatergic mechanisms that regulate PFC DA levels. Using antagonists selective for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors, we compared drug effects on in vivo microdialysis DA measurements in the PFC of adult male and female rats. We found that baseline DA levels were similar across sex, AMPA antagonism decreased PFC DA in both sexes, and NMDA antagonism increased DA in males but decreased DA in females. We also found that, at subseizure-producing drug levels, γ-aminobutyric acid (GABA)-A antagonism did not affect DA in either sex but that GABA-B antagonism transiently increased PFC DA in both sexes, albeit more so in females. Finally, when NMDA antagonism was coincident with GABA-B antagonism, PFC DA levels in males responded as if to GABA-B antagonism alone, whereas in females, DA effects mirrored those induced by NMDA antagonism. Taken together, these data suggest commonalities and fundamental differences in the intracortical amino acid transmitter mechanisms that regulate DA homeostasis in the male and female rat PFCs.

Keywords: hyperdopaminergia, hypodopaminergia, NMDA-R hypofunction, schizophrenia

Introduction

The prefrontal cortices (PFCs) of humans and animals mediate higher-order executive functions including working memory, behavioral flexibility, and decision-making (Goldman-Rakic et al. 1990; Dalley et al. 2004; Tandon 2013). Prefrontal dysfunction is also implicated in the cognitive deficits seen in neurological disorders including Parkinson’s disease (Dubois and Pillon 1997; Zgaljardic et al. 2003; Narayanan et al. 2013) and schizophrenia (Eisenberg and Berman 2009; Seeman 2009). At the same time, it is also known that executive functions and the incidence and/or severity of executive dysfunctions in disease are often significantly different in males and females. For example, in humans (Overman et al. 1996; Woolley et al. 2010), non-human primates (Goldman et al. 1974; Overman et al. 1996; Lacreuse et al. 1999), and rodents (Roof et al. 1993; Faraji et al. 2010), males tend to outperform females on spatial cognitive and working memory tasks while in disorders including Parkinson’s disease (Miller and Cronin-Golomb 2010) and schizophrenia (Leung and Chue 2000; Mendrek and Stip 2011), males are more susceptible to and more impaired by deficits in executive function than females.

The question arises as to whether sex differences in PFC-dependent behavioral operations might be related to sex differences in the functionally critical DA systems that underlie them. Recent tract tracing studies in rats showed that, for mesoprefrontal projections, there is a nearly 2-fold female over male difference in the proportions of DA to non-DA afferents that make up these pathways (Kritzer and Creutz 2008). However, PFC DA levels measured in tissue homogenates have been shown to be either similar across sex (Tanila et al. 1994; Duchesne et al. 2009) or higher in males (Dalla et al. 2008). These seemingly contradicting observations could be reconciled by sex differences in the regulatory mechanisms that set functional PFC DA levels. These include strategically placed, intracortical receptor subtype-specific glutamatergic (GLU) influences that tonically and flexibly regulate DA levels (Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Wu et al. 2002; Aubele and Kritzer 2012) and that have been recently shown to be highly sensitive to circulating gonadal hormone levels in adult male rats (Aubele and Kritzer 2012). Using in vivo microdialysis and reverse dialyses, drug and dual drug challenges we examined whether these receptor subtype-specific mechanisms of PFC DA regulation might also differ across sex.

Previous studies—carried out to date exclusively in the male brain—have shown that tonic regulation of PFC DA tone is achieved in part by a balance of offsetting receptor subtype-specific intracortical GLU influences (Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Wu et al. 2002; Balla et al. 2009; Balla et al. 2012). More specifically, studies combining techniques of in vivo microdialysis or electrophysiology with drug challenge have shown that N-methyl-D-aspartate (NMDA)-mediated GLU actions engage PFC interneurons to inhibit the PFC’s descending drive over the ventral midbrain and tonically suppress PFC DA levels (Del Arco and Mora 2002; Jackson et al. 2004; Homayoun and Moghaddam 2007; Aubele and Kritzer 2012; Povyysheva and Johnson 2012). In contrast, local α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-mediated systems excite PFC pyramidal neurons, including those projecting to the ventral tegmental area and tonically stimulate mesoprefrontal DA neurons and DA release back in the PFC (Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Wu et al. 2002; Aubele and Kritzer 2012). Taken
Materials and Methods

Animal Subjects
A total of 60 adult male and 53 female Sprague-Dawley rats (Taconic Farms, Germantown, NY, USA) were used. Animals were housed in a specific pathogen-free environment in same sex pairs under a 12:12 h light/dark cycle (lights on at 0700) with food (Purina PMI Lab Diet: Prolib RMH 3000) and water available ad libitum. Animals were 8–10 weeks of age and weighed between 200 and 400 g at the time of the microdialysis studies. For the females, vaginal lavage was used to identify the stage of the estrus cycle subjects were in on the day of the microdialysis experiments. All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Stony Brook University and were designed to minimize animal use and discomfort.

Stereotaxic Placement of Guide Cannulae
Craniotomies were performed 24 h before the microdialysis experiments under aseptic conditions and using intraperitoneal injections of ketamine (90 mg/kg) and xylazine (10 mg/kg) as anesthesia. Stereotaxic coordinates were used to place guide cannulae (14 mm, CMA Microdialysis, North Chelmsford, MA, USA) within the left pregenual medial PFC area corresponding to plate 8 of Paxinos and Watson (1998). Cannulae were secured to the skull with shallow anchor screws and dental cement. After surgeries, animals were given single doses of buprenorphine (0.03 mg/kg) and returned to home cages for recovery.

In Vivo Microdialysis, DA Detection, and DA Measurement
Microdialysis studies took place during animals’ subjective nights. Animals were typically asleep for the duration of the experiment. Rats were placed in clear bowls (Raturn, BioAnalytical Systems) and allowed to acclimate for 10 min. Microdialysis probes (100 000 Da cutoff, 2 mm Polyethersulfone exposed membrane tip, CMA Microdialysis) were then gently inserted through guide cannulae and perfused with artificial cerebrospinal fluid (aCSF; 145 mM NaCl; 2.8 mM KCl; 1.2 mM MgCl2; 0.25 mM ascorbic acid; 5.4 mM glucose; 1.2 mM CaCl2, pH 6.8) at a flow rate of 2 μL/min for a 2 h equilibration period. Sensitivity of PFC DA levels to tetrodotoxin (TTX) after the equilibration period and for the duration of the subsequent drug application period was established in a subset of test subjects (n = 7) by reverse dialysis delivery of 1 μM TTX (Fig. 1). While the low dose of TTX used established patency of stimulated DA terminal release for the duration of the experimental timelines, it does not fully rule out the possibility for a small pool of TTX-insensitive DA that may lead to an underestimation of drug effects. For all other subjects, baseline dialysates were collected (10 μL) and directly injected into the HPLC every 15 min using an online autoinjector (Pollen-8, BAS). Because of the comparative design of all within- and across-subject assessments and the hours-long experimental timelines involved, uncorrected rather than no-net flux methods were used to measure basal DA levels. After obtaining 3 consecutive stable, uncorrected baseline measures (DA levels within 5% of each other), drug was added to the aCSF [5–50 μM bicineulline (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), 30–60 μM CGP52432 (Tocris Bioscience), 100–150 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) (Tocris Bioscience), 100–670 μM D(-)-2-amino-5-phosphono pentanoic acid (APV) (Sigma-Aldrich Chemical Co.), or 50–200 μM picrotoxin (Sigma-Aldrich Chemical Co.)] and infused for 120 min while dialysates were collected (every 15 min); exceptions included the co-administration study where CGP52432 was administered for 45 min followed by a 90-min co-infusion of CGP52432 and APV and the dose-response studies in which each drug concentration of APV was administered for 75 min and each concentration of CGP52432 was administered for 45 min. With the exception of the dose response studies, after drug delivery, infusion of aCSF was resumed and dialysates were collected until DA levels returned to within 5% of pre-drug baseline values. It should be noted that a small number of subjects in the dose-response studies did not receive every drug concentration due to problems occasionally encountered over the long experimental timelines of these studies. Dialysate samples (10 μL) were directly injected into an HPLC system (PM 92-E pump, BAS, West Lafayette, IN, USA) and analyzed using a microbore column (Unijet, 1.0 mm inner diameter, 100 μm length, 3 μm Octadecylsilane particles; BAS) and a BioAnalytical Systems LC-Epsilon detector (BAS). The E_{appr} was +0.65 V versus the Ag/AgCl reference electrode. The mobile phase consisted of 14.5 mM NaH2PO4; 30 mM sodium citrate; 10 mM diethylamine HCl; 2.2 mM 1-octanesulfonic acid; 0.027 mM ethylenediaminetetraacetic acid; 7.2% acetonitrile (v/v); 1% tetrahydrofuran (v/v), pH 6.0 (with phosphoric acid). All chemicals used were purchased from Sigma-Aldrich Chemical Co.

DA peaks were isolated and quantified (ng/mL) in relation to a series of DA standards of known concentrations (2, 10 ng/mL) run before and after each microdialysis study. DA concentration (fmol/μL) was calculated using the ChromGraph Software (BAS) and measurements of “peak area.” Drug-induced changes in the DA level were also quantified as a percent of pre-drug baseline. Probe efficiency was determined to be 10–18%, and an overall detection limit of 8 fmol was achieved.

Estrous Stage Determination, Euthanasia, Histology, and Determination of Probe Placement
At the conclusion of microdialysis studies, female rats were vaginally lavaged and vaginal cytology was used to determine estrus cycle stage together, with what have largely been shown to be phasic, resetting actions of intracortical γ-aminobutyric acid (GABA)-A or GABA-B receptor-mediated influences (Yonezawa et al. 1998; Balla et al. 2009; Del Arco et al. 2011), these regulatory processes play important roles in modulating electrophysiological properties (Tong et al. 1996; Wang et al. 2010; Povysheva and Johnson 2012) and complex behaviors (Aultman and Moghadam 2001; Feenstra et al. 2002; Fejgin et al. 2009) associated with the PFC. Likewise, they have also been repeatedly implicated in the hyper- and hypodopaminergia associated with PFC dysfunction in disease (Lewis et al. 1999, 2012; Cryan and Kaupmann 2005; Kehrer et al. 2008; Kantrowitz and Javitt 2010; Gonzalez-Burgos and Lewis 2012). Here, we tested hypotheses for sex differences in the organization and/or operations of these regulatory circuits. This was done by combining in vivo microdialysis with reverse dialysis administration of receptor subtype-selective GLU (NMDA and AMPA) and GABA (GABA-A and GABA-B) antagonists and HPLC with electrophysiological detection to quantitatively compare drug effects, alone and in combination, on DA levels in the PFC of adult male and in female rats where estrous cycle stage was tracked.
white matter line drawing pairs. Scale bar = 1 mm. IL, infralimbic cortex; PrL, prelimbic cortex; wm, (I
power photomicrographs of these probe tracks show their relation to cortical layers
probe tracks are depicted as black lines. The antagonist drug study is identi
are as per plate 8 of the Paxinos and Watson rat atlas; the locations of microdialysis
Figure 2. Representative photomicrographs (left panels, A) and line drawings (right
panels, B) showing the locations of microdialysis probe tracks in relation to
cytoarchitectonic areas of the prefrontal cortex in male and female rats. The tissue
sections shown (A) are counterstained with cresyl violet. Visible damage from the
probe tracks is identified by white triangles (male) and white stars (female). Higher
power photomicrographs of these probe tracks show their relation to cortical layers
(I–VI) and white matter (wm). Line drawings (B) and their cytoarchitectonic boundaries
are as per plate 8 of the Paxinos and Watson rat atlas; the locations of microdialysis
probe tracks are depicted as black lines. The antagonist drug study is identified above
line drawing pairs. Scale bar = 1 mm. IL, infralimbic cortex; PrL, prelimbic cortex; wm, white matter
(Marcondes et al. 2002; Goldman et al. 2007). All rats were euthanized by
rapid decapitation. Brains were removed and post-fixed for 2–4
days in a 10% buffered formaldehyde solution containing 30% sucrose
for cryoprotection. Once fixed, brains were rapidly frozen in powdered
dry ice and serially sectioned in a coronal plane on a freezing micro-
tome (40 μm). A 1 of 4 series sections taken from the level of mid-
olfactory bulb to the genu of the corpus callosum were slide mounted
and counterstained with 0.5% cresyl violet (Fig. 2). Light microscopic
evaluation was used to map probe tracks in relation to cortical cyto-
architecture. Only those cases where dialysis probes were confirmed to
have spanned the deep layers of the left prelimbic and infralimbic
medial PFC were included in the analysis (Fig. 2).

Statistical Analysis
Uncorrected basal DA levels were compared across groups using a
one-way analysis of variance (ANOVA). Drug effects on PFC DA levels
were evaluated using two-way ANOVAs with repeated measures
design, where sex served as the independent factor and the 15-min
sample bins as the repeated measure. When significant sex, time, or
sex by time interactions were found, post hoc Bonferroni analyses
were used to identify when along study timelines drug effects on PFC
DA levels significantly diverged between males and females.

Additionally, within-sex one-way ANOVAs with repeated measures
design, with the 15-min sample bins serving as the within-subjects
factors, were run to determine at which time point during drug admin-
istration DA concentrations was significantly different from baseline.
In all cases, a P-value of < 0.05 was accepted as a significant and 0.05 <
P ≤ 0.09 was designated as a near significant.

Results
Baseline Extracellular PFC DA Levels in Male
and Female Rats Across the Estrous Cycle
Uncorrected, basal extracellular PFC DA levels were measured
prior to drug delivery in all animal subjects. In males rats,
these values ranged from 0.02 to 0.50 fmol/μL and had a group
average of 0.238 fmol/μL (±0.06 SEM, Fig. 3). A somewhat
broader and slightly lower range of uncorrected basal DA con-
centrations was found among the female rats. Although there
was substantial overlap, it was also noted that the highest DA
levels mapped to females in diestrus (from 0.01 to 0.50 fmol/
μL; group average 0.169 fmol/μL, ±0.04 SEM) that intermediate
uncorrected basal DA levels were found for proestrus females
(from 0.02 to 0.24 fmol/μL; group average 0.099 fmol/μL,
±0.03 SEM), and that the lowest values were associated with
the estrus females (from 0.02 to 0.12 fmol/μL; group average
0.063 fmol/μL, ±0.03 SEM, Fig. 3). One-way ANOVA, carried
out between males and all females and between males and
females separated by estrous cycle stage, identified no signifi-
cant main effects of group.

Comparisons of Glutamate Receptor Antagonism on
Extracellular PFC DA Levels in Male and Female Rats
AMPA Receptor Antagonism
The effects of the AMPA antagonist NBQX on PFC DA levels
were initially assessed across a range of concentrations in male
(n = 8) and female [diestrus (n = 6) and proestrus (n = 2)] sub-
jects. These studies showed that, at concentrations lower than
100 μM, NBQX had no discernable effects on PFC DA levels and
at concentrations higher than 150 μM, drug precipitation inter-
fered with the HPLC analysis. However, at 150 μM, infusion of
the AMPA antagonist NBQX reliably and similarly decreased
extracellular PFC DA in both sexes (Fig. 4). Thus, in males
(n = 5) and in females [in diestrus (n = 3) or proestrus
(n = 2)], uncorrected basal DA levels began to fall within 15 min
of drug application, and dropped to maximally depressed extra-
cellular DA concentrations that were roughly 30% below base-
line within 45 min (Fig. 4). DA levels remained depressed for
the remainder of the drug application period and upon drug
removal returned to pre-drug concentrations within 45–60 min
(Fig. 4). A two-way ANOVA (repeated measures) identified sig-
nificant main effects of NBQX on PFC DA level (F(1,112) = 11.62,
P < 0.0001). However, no significant main effects of sex and no
significant interactions between sex and drug treatment on PFC
DA levels. The main effects of drug were explored further in
one-way ANOVA that revealed significant main effects of time/
drug (F(9,63) = 6.245–8.479, P < 0.0001) and post hoc testing that
showed that PFC DA levels in males and females dropped to
values that were significantly lower than baseline within 45 min
of drug onset and remained significantly lower than baseline
until the time of drug removal (P ≤ 0.001–0.048).
NMDA Receptor Antagonism

The effects of reverse dialysis intra-PFC infusion of the NMDA antagonist APV on local DA levels were initially assessed across 5 concentrations of ranging from 100 to 670 μM in males (n = 13) and females [diestrus (n = 13), proestrus (grey circles), and estrus (black circles, A)]. Group means are depicted by horizontal black bars (A). No significant differences were found in basal dopamine levels among any of these groups in comparisons in which males were compared with a single combined pool of all female subjects. Scatter plots showing mean basal dopamine level concentrations (fmol/µL) in individual male (B) and female (D) subjects separated by the estrous cycle stage. A 2-h drug-infusion protocol using 500 μM APV further showed that, in males (n = 5), PFC DA levels began to rise within 15 min of drug onset, reached peak concentrations of 30–40% above baseline roughly 60 min later, remained elevated until drug offset and returned to pre-drug levels within approximately 30 min (Fig. 5 B). In females [diestrus (n = 5)], PFC DA levels were more sluggish in responding. However,
15–30 min after drug infusion, PFC DA concentrations dropped to levels of 30–40% below baseline, remained depressed until drug offset, and returned to baseline within 30 min (Fig. 5B). An initial two-way ANOVA (repeated measures) identified significant main effects of sex ($F_{1,9} = 16.05, P = 0.0039$), and significant interactions between sex and APV treatment ($F_{14,112} = 7.184, P < 0.0001$) on PFC DA levels. Within-sex, one-way ANOVAs further revealed significant main effects of time/drug on PFC DA levels in males and females ($F_{9,79} = 3.381–6.387, P ≤ 0.0001–0.002$). Finally, post hoc comparisons showed that, in males, APV stimulated DA levels that were significantly higher than baseline from 60 min of drug infusion until drug removal ($P = 0.000–0.0015$), that in females, APV decreased DA to levels that were significantly lower than baseline from 60 min of drug infusion to drug offset ($P = 0.040–0.087$), and that the effects of APV on PFC DA levels were significantly different in males versus females from 45 min after drug infusion until drug removal ($P ≤ 0.0001–0.0032$).
tested. However, when infused at either 50 μM or at 60 μM, CGP52432 had similar, albeit sex-specific effects on PFC DA levels, with no observable motor effects in any subjects (Fig. 7A). More specifically, in males (n = 5), roughly 30 min after drug onset (50 μM), DA concentrations transiently peaked at levels that were about 2 times higher than baseline. Over the next 15 min, DA levels dropped to concentrations of about 60% above baseline and continued to decline thereafter, returning to pre-drug baseline levels 15–30 min before drug offset (Fig. 7B). The effects of CGP52432 in female rats [diestrus (n = 5)] were similar but were larger overall (Fig. 7B). Thus, 30 min after drug application, DA levels showed a roughly 5-fold peak before quickly dropping to levels that were approximately 3 times higher than baseline and then undergoing a slow decrement for the remainder of the drug application period, and a return to baseline within 15–30 min of drug offset (Fig. 7B). An initial two-way ANOVA (repeated measures, data collected using 50 μM CGP52432) identified significant main effects of sex (F(1,8) = 6.938, P = 0.03), significant main effects of drug treatment/time (F(15,120) = 8.219, P < 0.0001), and significant interactions between the two (F(15,120) = 4.424, P < 0.0001) on PFC DA level. Separate within-sex, one-way ANOVA (repeated measures) also revealed significant main effects of drug/time on PFC DA level in both sexes (F(5,70) = 2.338–7.881, P ≤ 0.001–0.023). Finally, post hoc comparisons showed that, in males, peak DA concentrations were significantly higher than baseline (P = 0.008), that in females DA levels were significantly to near significantly higher than baseline from its peak until drug removal (P ≤ 0.001–0.079), and that the effects of CGP52432 on extracellular PFC DA levels were significantly to near significantly greater in females than in males for the majority of the drug application period (P = 0.0002–0.09, Fig. 7B).

**Combined NMDA/GABA-B Receptor Antagonism**

A dual drug reverse dialysis infusion challenge was carried out in which CGP52432 (50 μM) was introduced 45 min prior to adding 500 μM of APV to the dialysate and co-infusing both drugs for an additional 90 min (Fig. 8). In male rats (n = 5), the prior infusion of CGP52432 blocked the expected DA-stimulating actions of APV (Fig. 8A). Thus, 30 min after CGP52432 infusion, DA levels transiently peaked at levels that were about 2-fold higher than baseline but 15 min, these levels dropped back to within roughly 60% of baseline and continued to fall thereafter to near pre-drug baseline levels, despite the infusion of APV (Fig. 8A). In female rats [diestrus (n = 3), proestrus (n = 1), and estrus (n = 1)], the co-infusion protocol had no obvious effects on APV's suppression of PFC DA levels (Fig. 8B). Thus, while CGP52432 administration induced its expected peaks in DA level, when APV co-infusion commenced, PFC DA levels quickly dropped to levels that were 30–40% below baseline that were sustained until drug offset (Fig. 8B). An initial two-way ANOVA (repeated measures) identified significant main effects of sex (F(1,10) = 12.609, P = 0.005), significant main effects of drug treatment (F(15,150) = 3.114, P < 0.001), and significant interactions between sex and drug treatment (F(15,150) = 3.853, P < 0.0001) on PFC DA levels. Within-sex, one-way ANOVA further revealed significant main effects of time/drug on PFC DA levels in both sexes (F(10,95) = 3.509–4.737, P ≤ 0.001). Allowed post hoc analyses showed that, in males, only the DA peaks that followed CGP52432 application were significantly higher than baseline (P < 0.001). In females, the CGP52432-induced DA peaks were also significantly higher than baseline (P = 0.065), and following co-infusion of APV, DA levels dropped to concentrations that were nearly significantly lower than baseline (P = 0.077).

**Discussion**

The executive functions that are associated with the PFC are highly sensitive to local, extracellular DA concentration (Murphy et al. 1996; Verma and Moghaddam 1996; Zahr et al. 1997; Landau et al. 2009; Cools and D'Esposito 2011). The DA levels of the PFC are governed by regulatory circuits and mechanisms that tonically set and phasically re-set PFC DA tone. Not surprisingly, factors that interfere with these processes and move PFC DA levels away from optimal operational settings, for example, stress, drug stimulation, disease (Davis et al. 1991; Deutch 1992; Murphy et al. 1996; Zahr et al. 1997; Goldberg et al. 2003; Arnsten and Li 2005; Niwa et al. 2010; Dumontheil et al. 2011; Cervenka et al. 2012) negatively impact executive function. Stimulated in part by sex differences that characterize DA-sensitive executive functions and executive dysfunctions related to prefrontal hyper- or hypodopaminergia (Ott et al. 1996; Overman et al. 1996; Szymanski et al. 1996; Leung and Chue 2000; Goldstein et al. 2002; Petry et al. 2002; Locklear et al. 2011; Kim et al. 2013; Li and Arnsten 2013; Biederman et al. 2014) negatively impact executive function.

**Figure 7.** Bar graphs (A) showing the maximum effects of CGP52432 on extracellular prefrontal DA at different drug concentrations. Timeline/line graphs (B) showing the effects of reverse dialysis application effects of CGP52432 on extracellular prefrontal DA levels, expressed as mean percent change from baseline (± standard error of the mean) in male (white) and female (black) rats. The solid black line beneath the line graphs in B marks the drug-infusion period. (A) In both sexes, the lowest concentration of CGP52432 (30 μM) had no effect on extracellular DA levels and maximal, seizure-free effects were reached at the 50-μM concentration. (B) Within 30 min of application, CGP52432 (50 μM) increased in prefrontal DA levels in males (white squares) and more so in females (black circles).
Canuso and Pandina 2007; Usall et al. 2007; Miller and Cronin-Golomb 2010; Lai et al. 2012; Feinstein and Kritzer 2013), this study asked whether there might be also be dimorphisms in the intracortical GLU mechanisms that regulate PFC DA levels. Recent in vivo microdialysis, reverse dialysis drug application studies showed that the intracortical AMPA and NMDA receptor-mediated GLU mechanisms that are well known to tonically regulate PFC DA levels in the male brain (Feenstra et al. 1995, 2002; Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Aubele and Kritzer 2012) are sensitive to changes in circulating gonadal steroids in adult male rats (Aubele and Kritzer 2012). Here, similar microdialysis/drug challenge approaches were used for the first time to compare the effects of AMPA and NMDA receptor antagonists on extracellular PFC DA levels in male and female rats. These studies showed that the effects of AMPA antagonists on PFC DA levels were indistinguishable across sex. More specifically, AMPA antagonism, which has been repeatedly shown to depress extracellular PFC DA levels in males (Jedema and Moghaddam 1996; Jin 1997; Takahata and Moghaddam 1998; Aubele and Kritzer 2012), was found to have qualitatively and quantitatively similar effects in females. In contrast, NMDA antagonism had markedly different effects on PFC DA baseline levels in males and females. Thus, although NMDA antagonism increased extracellular DA in males as expected (Feenstra et al. 1995, 2002; Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Aubele and Kritzer 2012), its infusion in females significantly decreased PFC DA levels. These latter findings suggested that long-held views of intracortical NMDA-mediated influences as being levied on PFC interneurons (Yonezawa et al. 1998; Homayoun and Moghaddam 2007) describe DA regulation in the male, but not in the female PFC. Instead, our data suggest that NMDA antagonism in females acts independently of interneurons in regulating PFC DA levels. This was borne out in co-infusion studies where antagonism of intra-PFC GABAergic signaling blocked the DA-potentiating effects of the NMDA antagonist APV in males, but had no effect on APV’s DA-depressing actions in females. In the sections below, these findings along with those from foundation studies examining the effects of extra-PFC infusion of GABA-A and GABA-B antagonists are considered further in relation to the extant literature describing amino acid transmitter regulation of PFC DA tone and are incorporated into sex-specific models of the seemingly disparate ways in which the male and the female PFCs regulate basal DA levels.

Glutamatergic Regulation of PFC DA Levels in the Male and Female Brain

Prefrontal DA-regulating GLU systems were compared across sex using reverse dialysis intra-PFC infusion of the AMPA antagonist NBQX and the NMDA antagonist APV. These along with other antagonists of AMPA and NMDA GLU receptors have been extensively used in prior in vivo microdialysis studies of PFC DA regulation in rats. Carried out exclusively in males, these studies along with data from male subjects of the present all show that, in this sex, local AMPA antagonism depresses extracellular PFC DA levels (Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Wu et al. 2002; Aubele and Kritzer 2012), and that local NMDA antagonism has opposite, PFC DA-potentiating effects (Feenstra et al. 1995; Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Aubele and Kritzer 2012). These data have been interpreted as evidence for a tonic AMPA receptor-mediated drive and a tonic NMDA receptor-mediated suppression over mesocortical DA systems and DA overflow in the PFC. Our studies suggest that this is only half correct for the female brain. Thus, while intra-PFC infusion of NBQX dose-dependently decreased extracellular PFC DA levels in both sexes, APV also dose-dependently decreased extracellular PFC DA levels in females—the opposite of its stimulatory actions in males. Thus, while in males intracortical NMDA and AMPA receptor-mediated actions exert opposing, functionally balancing influences over mesocortical DA systems, in females both receptor subtypes appear to tonically regulate PFC DA levels. These latter findings suggested that long-held views of intracortical NMDA-mediated influences as being levied on PFC interneurons (Yonezawa et al. 1998; Homayoun and Moghaddam 2007) describe DA regulation in the male, but not in the female PFC. Instead, our data suggest that NMDA antagonism in females acts independently of interneurons in regulating PFC DA levels. This was borne out in co-infusion studies where antagonism of intra-PFC GABAergic signaling blocked the DA-potentiating effects of the NMDA antagonist APV in males, but had no effect on APV’s DA-depressing actions in females. In the sections below, these findings along with those from foundation studies examining the effects of extra-PFC infusion of GABA-A and GABA-B antagonists are considered further in relation to the extant literature describing amino acid transmitter regulation of PFC DA tone and are incorporated into sex-specific models of the seemingly disparate ways in which the male and the female PFCs regulate basal DA levels.

Glutamatergic Regulation of PFC DA Levels in the Male and Female Brain

Prefrontal DA-regulating GLU systems were compared across sex using reverse dialysis intra-PFC infusion of the AMPA antagonist NBQX and the NMDA antagonist APV. These along with other antagonists of AMPA and NMDA GLU receptors have been extensively used in prior in vivo microdialysis studies of PFC DA regulation in rats. Carried out exclusively in males, these studies along with data from male subjects of the present all show that, in this sex, local AMPA antagonism depresses extracellular PFC DA levels (Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Wu et al. 2002; Aubele and Kritzer 2012), and that local NMDA antagonism has opposite, PFC DA-potentiating effects (Feenstra et al. 1995; Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Aubele and Kritzer 2012). These data have been interpreted as evidence for a tonic AMPA receptor-mediated drive and a tonic NMDA receptor-mediated suppression over mesocortical DA systems and DA overflow in the PFC. Our studies suggest that this is only half correct for the female brain. Thus, while intra-PFC infusion of NBQX dose-dependently decreased extracellular PFC DA levels in both sexes, APV also dose-dependently decreased extracellular PFC DA levels in females—the opposite of its stimulatory actions in males. Thus, while in males intracortical NMDA and AMPA receptor-mediated actions exert opposing, functionally balancing influences over mesocortical DA systems, in females both receptor subtypes appear to tonically drive them.
The DA-depressing actions of APV observed here in females are similar to those previously seen in male rats that have undergone long-term gonadectomy (Aubele and Kritzer 2012). That the effects of gonadectomy (GDX) on APV were attenuated by supplementing GDX rats with testosterone propionate but not estradiol suggests that diminished androgen is responsible for the anomalous drug effects observed (Aubele and Kritzer 2012). It is thus tempting to speculate that the chronically low levels of androgen experienced by the female brain likewise contribute to their sex-specific mode of PFC DA homeostasis. Similar to what has been posited for GDX male rats (Aubele and Kritzer 2012), the impact of APV on PFC DA levels in female rats also suggests an NMDA-mediated influence that directly excites PFC pyramidal cells, including those projecting to the ventral midbrain. This is fundamentally different from all current, male-specific evidence of NMDA receptor-mediated effects on PFC DA homeostasis as being translated through GABAergic PFC interneurons (Yonezawa et al. 1998; Homayoun and Moghaddam 2007). These sex-specific circuit schemas are consistent with outcomes from the present CGP52432/APV co-infusion studies, wherein the effects of NMDA antagonism on PFC DA levels were found to be dependent on GABAergic signaling in males, but independent of GABAergic inhibition in females.

The list of activational hormone effects and/or sex differences among NMDA receptors and NMDA receptor-mediated actions in rat cerebral and hippocampal cortices is considerable and continues to grow. These include effects of estrogens and androgens on pyramidal cell spine densities (Gould et al. 1990; Woolley and McEwen 1993; Leranth et al. 2003, 2004; Hajsanz et al. 2008), on NMDA receptor numbers, affinities, and/or subunit compositions (Smith and McMahon 2006; Therianfard et al. 2012; Vedder et al. 2013), and on measures of NMDA-dependent toxicity and synaptic plasticity (Pozzo-Miller et al. 1999; Kajta et al. 2001; de Olmos et al. 2008; Smith et al. 2010). We hypothesize that the sex differences in NMDA-mediated PFC DA homeostatic mechanisms identified in this study have origins in a differential NMDA receptor-mediated activation of ventral tegmental area (VTA)-projecting PFC pyramidal cells. How this occurs is unknown but could be related to differences in genomic androgen actions as these corticofugal neurons are heavily invested with the requisite intracellular receptive machinery in both male and female rats (Aubele and Kritzer 2012). As previously argued for GDX male rats, targets of this genomic activation could include protein kinase C which is known to be activated by AR-dependent androgen signaling (Nguyen et al. 2009) and is capable of stimulating Ca-dependent NMDA receptor deactivation (Lu et al. 2000). However, other alternatives, including impact on NMDA receptor trafficking similar to that recently demonstrated for the neurosteroid pregnenolone (Kostakis et al. 2013), should also be considered.

**GABAergic Regulation of PFC DA Levels in the Male and Female Brain**

In setting up the dual drug-infusion paradigm, the impact of GABA-A and GABA-B antagonists on PFC DA levels was investigated in male and female rats using reverse dialysis infusion of picrotoxin, bicuculline, and CGP52432. Although sex differences and/or hormone effects have been described in susceptibility to ictal activity including that induced by bicuculline and picrotoxin (Pericic et al. 1996; Bujas et al. 1997; Frye 2008, 2010), we found that seizure-producing thresholds—defined by us as drug concentrations producing piloerection and/or motor twitches in at least some animals over the course of the 2-h drug application period—were similar in males and females for all 3 antagonists. For all 3 as well, these thresholds were substantially lower to half of the drug concentrations used in previous in vivo microdialysis/reverse dialysis studies focused on the male rat PFC, which also found no effects on basal DA levels or on GLU overflow in the ventral tegmentum (Santiago et al. 1993; Harte and O’Connor 2005; Fallon et al. 2007; Balla et al. 2009). Thus, although the range of drug concentrations tested in our studies is low, consensus cross-study findings that subsume a substantially larger range of drug doses at least provisionally suggest that the negative results reported here for bicuculline and picrotoxin in the male and female PFC may not be due to insufficient receptor occupancy.

Previous studies using drugs including CGP52432 at concentrations of up to 2-fold higher than those used here have also found no effects of GABA-B antagonism on either PFC DA or ventral midbrain GLU levels in the male brain (Takahata and Moghaddam 1998; Harte and O’Connor 2005; Balla et al. 2009). However, our data suggest a tonic GABA-B-mediated suppression of PFC DA levels in both male and female rats. This discrepancy may be related to the fact that, in our hands, GABA-B blockade produced surges in PFC DA concentration that in males appeared and returned to near-baseline levels within about 30 min. Thus, it is possible that the 30-min sampling periods combined with the exclusive use of male subjects in each of the prior studies (Harte and O’Connor 2005; Fallon et al. 2007; Balla et al. 2009) averaged out and obscured these transient GABA-B responses. We also found that CGP52432 produced transient spikes in PFC DA levels in female rats that were significantly larger, and that took significantly longer to return to baseline than those produced in males. In both cases, it must be considered that these peaks are driven by undetected, drug-induced seizure activity in the PFC. However, an alternative explanation is that the surges in the PFC DA level induced by CGP52432 trigger a GABA-B-independent, rectifying response(s). An attractive candidate for this resetting mechanism may be DA activation of intracortical DA D2 receptors. Although DA affects PFC excitability in complex, concentration and receptor subtype-dependent ways (Seamans et al. 2001; Xu et al. 2009), in the male brain DA D2 agonists, have been shown to activate fast-spiking PFC interneurons (Tseng and O’Donnell 2004; Williams and Castner 2006), in the male brain DA D2 agonists, have been shown to activate fast-spiking PFC interneurons (Tseng and O’Donnell 2004, 2007a, b), to stimulate local GABA overflow (Retaux et al. 1991; Grobin and Deutch 1998), to mediate DA potentiation of GLU-stimulated GABA overflow (Del Arco and Mora 2000), and to inhibit PFC pyramidal cell activity in part via GABA-A sensitive means (Tseng and O’Donnell 2007). These and other D2-mediated actions are concentration-dependent and typically emerge only under conditions of elevated to supranormal DA levels (Zheng et al. 1999; Del Arco and Mora 2000). Moreover, DA D2 stimulation’s net inhibitory actions have been shown, to date only in the male brain, to emerge after adolescence (O’Donnell 2010), suggesting intriguing links to circulating gonadal steroids. Given the importance of D2 signaling to PFC network operations (Seamans et al. 2001; Xu et al. 2009; Gruber et al. 2010) and to clinical therapeutics (Laruelle et al.
2005; Masana et al. 2012), it will be important to use indices of cortical excitability and/or additional drug challenge strategies to resolve the basis for the self-limiting spikes in DA level that we observed in males and in females following the intra-PFC infusion of CGP52432.

**Conclusion**

A large body of work sums to identify tonic intracortical AMPA-mediated stimulation of VTA-projecting PFC pyramidal cells that drives mesoprefrontal DA neurons and keeps PFC DA levels elevated and an opposing intracortical NMDA-mediated drive of GABAergic PFC interneurons that inhibits VTA-projecting pyramidal cells and holds PFC DA levels in check (Feenstra et al. 1995; Jedema and Moghaddam 1996; Takahata and Moghaddam 1998; Del Arco and Mora 1999; Wu et al. 2002; Aubele and Kritzer 2012). Taken together, these mechanisms form bases for contemporary computational models of PFC function (Berridge and Robinson 1998; Durstewitz et al. 2000; Durstewitz and Seamans 2002; O'Donnell 2003; Seamans and Yang 2004) that are strongly influential in shaping etiologic thinking about mental illness and its treatment (Lewis and Moghaddam 2006; Seeman 2009; Javitt 2010; Moghaddam and Javitt 2012; Stan and Lewis 2012). However, the foundation studies all share limitations of only examining male subjects. The present comparative studies add new information about NMDA receptor-mediated DA homeostatic mechanisms that appear to be oppositely tuned in the female compared with male PFC, and about tonic, DA-suppressing GABA-B receptor-mediated effects that appear to be significantly larger in females compared with males. It may be important to consider these findings further in 2 contexts. The first is the sexually dimorphic mesoprefrontal projections, where DAergic cells of origin are roughly 2 times more numerous in female rats than in males (Swanson 1982; Deutch et al. 1991; Carr and Sesack 2000; Margolis et al. 2006; Kritzer and Creutz 2008). The second is basal PFC DA concentrations that are by most accounts similar across sex (Tanila et al. 1994; Duchesne et al. 2009). From these we hypothesize that, in females, potent intra-PFC, DA-facilitating GLU influences, uniquely conferred by both of its major classes of cortical ionotropic receptors, drive mesoprefrontal systems that are characterized by a doubling of constituent DA cells of origin relative to males. While this could potenti ate DA levels in the female compared with male PFC, findings suggesting a significantly more powerful GABA-B-mediated DA inhibition in females could aid in maintaining basal DA concentrations that are more similar across sex. These sex-specific means of PFC DA homeostasis may also shape the sex differences that differentiate PFC hyperdopaminergia, and their relative protection from cognitive deficits associated with PFC hypodopaminergia in disorders such as schizophrenia.

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**Notes**

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