

Impoverished Rearing Environment Alters Metabotropic Glutamate Receptor Expression and Function in the Prefrontal Cortex

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Rearing rats in impoverished (IC) and enriched (EC) environmental conditions alters synaptic plasticity and cognitive processes. Metabotropic glutamate receptors (mGluRs) are known to play a key role in synaptic and behavioral plasticity. In the present study, the effect of rearing conditions on the expression of mGluR proteins in the prefrontal cortex (PFC) was assessed by immunoblotting. A significant difference in the content of prefrontal mGluR1 and mGluR5 (ie group I) and mGluR2/3 (ie group II) was observed between IC and EC rats. To functionally characterize this difference, *in vivo* microdialysis was used to verify differences in mGluR regulation of extracellular glutamate in the PFC. The results indicate that the capacity of group I and II mGluRs to elevate extracellular glutamate levels was significantly blunted in the PFC of IC rats compared to either EC subjects, or rats reared in normal environmental conditions (ie NIH standards). Group II mGluR receptors regulate performance in a forced T-maze spatial memory task that involves the PFC, and IC rats demonstrated deficits in this task relative to EC rats. These data suggest that reduced mGluR transmission in the PFC produced by impoverished, relative to enriched, rearing environments may contribute to cognitive deficits.

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INTRODUCTION

Rats reared in complex, challenging environments ('enriched condition' or EC) develop reliable neurobiological differences from littermates reared alone without social or object interaction ('impoverished/isolated condition' or IC; Renner and Rosenzweig, 1987). Rearing in a relatively complex environment induces synaptogenesis (eg higher density of dendritic spines, enlargement of synaptic boutons, and other putative correlates of altered neurotransmission) throughout the cortex, as well as in the striatum and hippocampus (reviewed by Van Praag *et al*, 2000; Kolb and Whishaw, 1998). Conversely, IC animals are deprived of stimuli critical to behavioral and neurobiological development, resulting in altered neuronal morphology and deficits in complex problem-solving tasks (reviewed by Hall, 1998), including tasks requiring spatial

working memory (Martinez-Cue *et al*, 2002; Winterfeld *et al*, 1998).

Optimal performance in tasks requiring spatial working memory depends on intact dorsolateral prefrontal cortex in primates and medial prefrontal cortex (PFC) in rats (Goldman-Rakic, 1996; Aultman and Moghaddam, 2001). Within the PFC, both dopamine and glutamate transmission modulate working memory (Goldman-Rakic, 1996; Moghaddam *et al*, 1997; Romanides *et al*, 1999). Various studies show that group I and group II mGluRs are present in high density within the PFC (Romano *et al*, 1995; Wright *et al*, 2001; Xi *et al*, 2002b) and systemic or intra-PFC administration of drugs modulating group II metabotropic glutamate receptors (mGluR2/3) alters performance in a forced T-maze task requiring spatial working memory (Gregory *et al*, 2003; Aultman and Moghaddam, 2001; Moghaddam and Adams, 1998).

Metabotropic glutamate receptors are a family of G-protein-coupled receptors that have been divided into groups based on sequence similarities, coupling to intracellular messengers, and pharmacology (reviewed by Cartmell and Schoepp, 2000). Group I receptors (mGluR1 and mGluR5) are positively coupled to phospholipase C and are associated with the Homer family of intracellular proteins (Brakeman *et al*, 1997). Group II receptors (mGluR2/3) are negatively coupled to adenylate cyclase

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and regulate presynaptic glutamate release (Anwyl, 1999). Both electrophysiological and neurochemical studies show that stimulating group I or inhibiting group II mGluRs promotes the release of glutamate from presynaptic terminals and/or glia (Chen *et al*, 1997; Manzoni *et al*, 1997; Moroni *et al*, 1998; Cochilla and Alford, 1998; Fagni *et al*, 2000; Swanson *et al*, 2001; Xi *et al*, 2002a).

Given the differences between IC and EC subjects in memory tasks requiring intact PFC and the role of glutamate transmission in the PFC to regulate spatial working memory, the present study examined the effect of rearing environments on mGluR1, mGluR5, and mGluR2/3 protein immunoreactivity in the PFC. In addition, *in vivo* microdialysis was used to determine rearing impact on the capacity of mGluRs to regulate extracellular glutamate levels.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) arrived in the laboratory at 21 days of age. The animal colony was in an AAALAC-approved animal facility with access to food and water *ad libitum*. Rooms were set on a 12/12-h light cycle, with lights on at 0700h and all experimentation was conducted during the light period. All protocols were approved by the Institutional Care and Animal Use Committee in compliance with NIH's Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington DC, USA, 1996).

Environmental Conditions

The rationale for establishing each environmental condition was adapted as previously described by Bardo *et al* (2002). Upon arrival, rats were randomly assigned to an isolated (IC) or enriched (EC) environmental condition. IC rats were individually housed in a standard hanging metal cage ($16 \times 24 \times 18 \text{ cm}^3$) with wire mesh floor and front panel, and solid metal sides, back and top. IC rats were never exposed to toys or novel objects, nor handled during the exposure period. EC rats were housed in large metal cages ($60 \times 120 \times 45 \text{ cm}^3$) with cohorts (10–12 per cage). The EC environment contained pine chips on the floor and various toys made of metal or hard plastic. The objects were rearranged 4–5 days a week, with novel objects being introduced each day. Rats reared in normal environmental conditions (ie NC rats) were also used in the microdialysis experiment. NC rats were double-housed in a plastic tub ($16 \times 43 \times 18 \text{ cm}^3$) with pine chips on the floor, but no toys or novel objects available (ie standard NIH housing conditions). The exposure period for all the three environmental conditions was from 21 to 60 days of age prior to protein measurements, microdialysis, and behavioral testing. Separate groups of rats were used for the immunoblotting, dialysis, and behavioral experiments, and NC rats were available only for the microdialysis experiment.

Tissue Preparation and Immunoblotting

IC and EC rats ($n=10/\text{group}$) were decapitated and the brains were rapidly removed and placed into an ice-cold brain mold (Braintree Scientific, Braintree, MA) for coronal sectioning. Coronal sections were laid on an ice-cold dissection plate and a 15-gauge blunt tissue punch was used to sample the medial PFC (dorsal or ventral; 2.7–3.7 mm from bregma) and striatum (1.2–1.7 mm from bregma). Dorsal PFC punches consisted primarily of anterior cingulate and prelimbic cortex, and ventral PFC punches consisted primarily of infralimbic cortex (Figure 1a; Paxinos and Watson, 1998). Striatal samples included the dorsolateral quadrant of the rostral half of the striatum. Samples were frozen at -80°C until homogenized for protein analysis.

Tissue homogenization, electrophoresis and immunoblotting were conducted as described by Xi *et al* (2002b). Primary rabbit anti-rat antibodies for mGluR1, mGluR2/3 and mGluR5 (Upstate Biotechnology, Lake Placid, NY) were used in dilutions of 1:1000, 1:1000 or 1:40 000, respectively. Labeled proteins were detected using an HRP-conjugated anti-rabbit secondary IgG (Upstate) diluted to either 1:5000 or 1:10 000. Levels of actin were detected using goat anti-rat polyclonal IgG (Santa Cruz Biotech, Santa Cruz, CA) at 1:250 dilution and HRP-conjugated donkey anti-goat IgG (Jackson ImmunoRes Labs, West Grove, PA) at 1:10 000 dilution. Immunoreactivity was quantified by integrating band density \times area using a photoimage scanner and densitometry software (NIH Image 1.60). Samples from IC rats were normalized to the average from EC rats obtained from the same gel.

In Vivo Microdialysis

IC, NC, and EC rats ($n=5\text{--}6/\text{group}$) were bilaterally implanted with dialysis guide cannulae (20 gauge, 14 mm; Small Parts, Roanoke, VA) over the PFC using the following coordinates according to the atlas of Paxinos and Watson (1998), using a 6° angle from the vertical (in mm; +2.7 anterior; +1.1 lateral, -2.0 ventral). After surgery, rats were given 5 days to recover before testing. Microdialysis probes were constructed as described by Robinson and Whishaw (1988), except that both the inlet and outlet tubing consisted of fused silica. The active region of the dialysis membrane was between 2 and 3 mm in length and $\sim 0.22 \text{ mm}$ in diameter. The night before the dialysis experiment, the probes were inserted through the guide cannulae into the prefrontal cortex. The next morning, the dialysis buffer (in mM: 5 glucose, 5 KCl, 140 NaCl, 1.4 CaCl_2 , and 1.2 MgCl_2 , and 0.5% phosphate-buffered saline to give a pH of 7.4) was advanced through the probe at a rate of $2 \mu\text{l}/\text{min}$ via a syringe pump (Bioanalytical Systems, West Lafayette, IN). After 2 h, baseline samples were collected. Liquid switches were used to minimize the pressure fluctuations while changing dialysis buffers with varying concentrations of drug.

The standard protocol used for microdialysis experiments involved the collection of three 20 min baseline samples, followed by three additional 20 min samples for each concentration of a given drug. Thus, multiple doses of each mGluR compound were administered in each rat. Dosage

ranges of the various drugs were based upon the relative IC_{50} values for binding to the respective receptors (Schoepp et al, 1999). The mGluR1/5 agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG; $IC_{50} = 10 \mu M$) and the mGluR2/3 antagonist LY 341495 ($IC_{50} = 10 nM$) were purchased from Tocris (Ballwin, MO). All compounds were freshly prepared on the day of the experiment.

Quantification of Glutamate

Microdialysis samples were collected into vials containing $10 \mu l$ of 0.05 M HCl. The concentration of glutamate in the dialysis samples was determined using HPLC with fluorometric detection. Precolumn derivatization of glutamate with *O*-phthalaldehyde was performed using a Gilson 231 XL autosampler (Middleton, WI). The mobile phase consisted of 11% acetonitrile (v/v), 100 mM Na_2HPO_4 , 0.1 mM EDTA, pH 6.04. Glutamate was separated using a reversed-phase column ($3 \mu m$; $100 \times 4.2 mm$; Bioanalytical Systems, West Lafayette, IN), and was detected using a Shimadzu (Columbia, MD) 10RF-A fluorescence detector with an excitation wavelength of 320 nm and an emission wavelength of 400 nm. The concentration of glutamate in the dialysis samples was quantified by comparing peak heights from samples and external standards.

Histology

After completion of the microdialysis experiments, rats were deeply anesthetized with an overdose of CO_2 inhalation. The brains were removed and stored in 1% formalin for at least 1 week before sectioning. The tissue was then blocked, and coronal sections ($100 \mu m$) were cut and stained with cresyl violet to verify probe placements. Only animals with probes located in the medial prefrontal cortex were included in the data analysis.

Working Memory Task

IC and EC rats ($n = 10/\text{group}$) were trained to perform a forced-choice delayed-alternation task in a T maze (see Aultman and Moghaddam (2001) for details). The maze was constructed from black Plexiglas (alleys 30 cm high and 15 cm wide, main alley 50 cm long, start box 20 cm long, side arms 40 cm long). The start box was separated from the main arm by a guillotine door and the side arms were separated from the main arm by sliding doors. Movable doors were positioned between the start box and main alley and between the main alley and both side arms. Animals were habituated to the maze over a 3-day period in which each animal was allowed to roam throughout the maze freely for 10 min, followed by 3 days of pre-training during which the animals were closed off in each side arm for 5 min. Each rat was trained for 10 trials/day, with each trial consisting of two phases, the forced choice phase and free choice phase, separated by an intertrial delay (retention interval) ranging from 10 to 40 s. At the beginning of each trial, both side arms contain sucrose pellet treats. For the forced phase, the rat was placed in the start box and allowed to enter the main alley. The door to only one of the side arms was opened, allowing the rat to enter and consume a sucrose pellet. During this time, the animal was enclosed for

10 s and then returned to the start box for the intertrial delay. The rat was then allowed to re-enter the main arm for the free choice phase, when both side arms were open with a treat remaining in the side arm opposite to the arm previously selected. The rat made a choice and was enclosed in the selected arm for 10 s before being removed to a side cage for an additional 10 s before beginning a new trial (intertrial interval). The series of forced arm choices was 50% left arm and 50% right arm selected at random daily. Animals were tested at each intertrial delay interval for 7 days, starting at 10 s and increasing by 10 s increments through a 40 s intertrial delay. Both EC and IC rats were tested on the same delay intervals in the same order. Values for each delay were obtained by averaging the performances of each group over the last 3 days of each delay.

Statistical Analysis

Western blot data were normalized to EC values in each blot, and statistically evaluated using separate one-way ANOVAs. The dialysis data were normalized to percent change from the average concentration in the baseline glutamate samples. Normalized data were analyzed using a two-way ANOVA (Group \times Time) with repeated measures over time. Results showing significant interactions were followed by one-way ANOVA across Group at each 20-min interval, followed by Tukey's *b* test. Significant changes from baseline were determined by separate one-way ANOVAs with repeated measures over Time, followed by Fisher's LSD test. For the behavioral data, the percent correct responses were averaged over the last 3 days of each intertrial interval, and the EC and IC responses were compared using a two-way ANOVA with repeated measures over the intertrial interval. A confidence interval of 95% (0.05 alpha level) was used throughout.

RESULTS

Group I and II mGluR Proteins are Different in the PFC of IC vs EC Rats

Normalized densitometry measurements of the expression of mGluR content in the PFC and striatum are shown in Table 1. Figure 1a illustrates the region of the dorsal PFC punches, which consisted primarily of anterior cingulate and prelimbic cortex; ventral PFC punches consisted primarily of infralimbic cortex (Paxinos and Watson, 1998). The dimer form of mGluR1 and mGluR5 proteins was significantly reduced in dorsal PFC ($F(1,14) > 4.1$, $p < 0.02$) of IC compared with EC rats (Figure 2a). In contrast, no difference between IC and EC subjects was measured in the ventral PFC of IC rats. For mGluR1 protein, IC rats also had more mGluR1 monomer band than EC rats in dorsal PFC ($F(1,24) = 4.1$, $p < 0.05$), and levels of mGluR5 monomer were equivalent. For mGluR2/3 protein, IC rats had less monomer than EC rats in the ventral PFC ($F(1,27) = 5.1$, $p < 0.03$), but not the dorsal PFC (Figure 2b). In addition to the PFC, levels of mGluR were determined in the striatum and no significant difference was measured between EC and IC rats for the monomers or dimers of mGluR1, mGluR5, or mGluR2/3 (Table 1). Actin levels were equivalent between EC and IC subjects within

Table 1 Impoverished Rearing Environment Alters Metabotropic Glutamate Receptor Immunoreactivity in the Medial Prefrontal Cortex

Region	mGluR1	mGluR1	mGluR5	mGluR5	mGluR2/3	mGluR2/3
	Dimer	Monomer	Dimer	Monomer	Dimer	Monomer
dPFC	58 ± 8*	121 ± 5*	75 ± 7*	106 ± 4	107 ± 7	105 ± 3
vPFC	81 ± 9	99 ± 9	87 ± 16	101 ± 4	104 ± 3	77 ± 6*
Striatum	84 ± 33	110 ± 5	88 ± 26	91 ± 7	102 ± 7	99 ± 5

Results from densitometry measurements are presented as a percentage of rats reared in an enriched (EC) environment ± SEM (*n* = 6–14 determinations). mGluR, metabotropic glutamate receptors; dPFC, dorsal prefrontal cortex; vPFC, ventral prefrontal cortex.
**p* < 0.05 compared to EC rats.

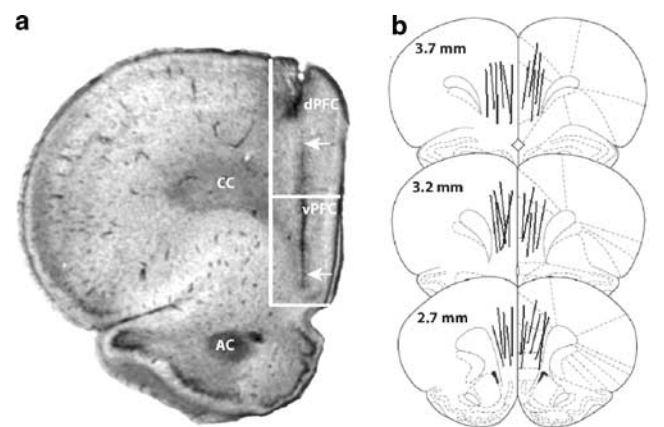


Figure 1 (a) Representative brain section of the medial prefrontal cortex of rats reared in EC and IC. Arrows indicate the beginning and ending of the dialysis membrane, which transverses along the dorsal and ventral region of the medial PFC. (b) Representative location of the microdialysis probes used in the study. Lines indicate the active portion of the dialysis membrane. The numbers indicate millimeters rostral to bregma according to the atlas of Paxinos and Watson (1998). Probe placements revealed that the probes traversed the dorsal (anterior cingulate and prelimbic) and ventral (infralimbic) region of the medial PFC. AC, anterior commissure; CC, corpus callosum; dPFC, dorsal prefrontal cortex; vPFC, ventral prefrontal cortex.

the dorsal and ventral PFC, and within the striatum (Figure 2a).

Group I and II mGluR-Mediated Increase in Extracellular Glutamate Levels is Blunted in IC Rats

Figure 1a shows the micrograph of a typical probe placement which transverses both the dorsal and ventral regions of the medial PFC. The representative locations of the dialysis membranes in the medial PFC of IC, NC, and EC rats used for analysis are shown in Figure 1b. Basal dialysate extracellular glutamate concentrations for the IC (*n* = 11), NC (*n* = 10), and EC (*n* = 12) groups were 40.1 ± 3, 30.2 ± 5, and 39.1 ± 6 pmol/sample, respectively. There were no significant differences in the basal levels of extracellular glutamate between groups (*F*(2,30) = 1.47, *p* = 0.24).
Time-course analysis of percent basal extracellular levels of glutamate during reverse microdialysis of the group I mGluR agonist DHPG is shown in Figure 3a. A two-way ANOVA with repeated measures over Time revealed a significant effect of Time (*F*(13,182) = 10.3, *p* < 0.001), Group (*F*(2,14) = 3.5, *p* < 0.05), and Time × Group interac-

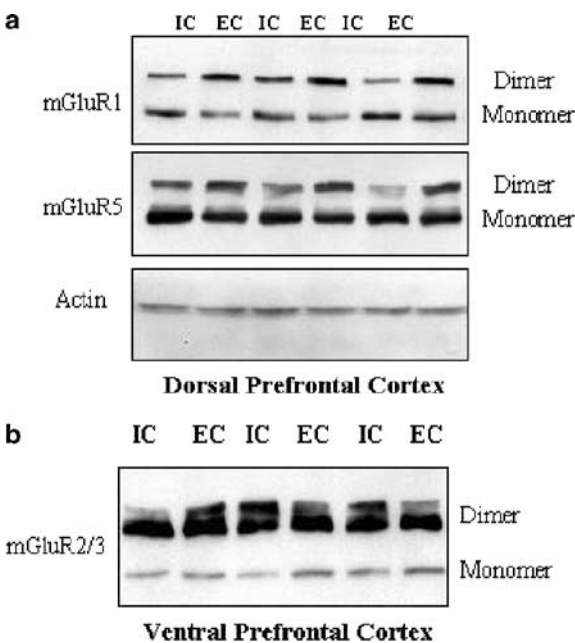


Figure 2 Western blots of rats reared in EC and IC for group I and group II mGluRs in the medial PFC. (a) Representative immunoblots for mGluR1, mGluR5, and actin. Regardless of environmental treatment, immunoblots for both mGluR1 and mGluR5 proteins yielded two distinct bands for each subtype (~150 and ~300 kDa). A significant reduction in the dimer form of mGluR1 (*n* = 8) and mGluR5 (*n* = 12) and a significant increase in the monomer form of mGluR1 (*n* = 14) within the dorsal region of the prefrontal cortex of IC rats were revealed (Table 1). (b) Representative immunoblots for mGluR2/3. Regardless of environmental treatment, immunoblots for mGluR2/3 protein yielded two distinct bands (~100 and ~200 kDa) reflecting monomer and dimerized forms of mGluR2/3 (Xi et al, 2002b). A significant reduction was seen in the monomer form of mGluR2/3 (*n* = 14) within the ventral region of the prefrontal cortex.

tion (*F*(26,182) = 1.6, *p* < 0.04). Further analysis of the Time × Group interaction revealed that, compared to IC rats, 5 and 50 μM DHPG significantly elevated the extracellular levels of glutamate in EC and NC rats (*p* < 0.05; Tukey's *b*). Analysis by separate one-way ANOVAs with repeated measures across Time revealed that stimulation of group I mGluRs by DHPG (5 and 50 μM) induced a significant concentration-dependent increase above basal extracellular glutamate levels in EC (*n* = 6) (*F*(13,65) = 5.4, *p* < 0.01) and NC (*n* = 5) (*F*(13,52) = 4.4, *p* < 0.01) rats. In contrast, IC rats (*n* = 6) showed no significant increase in extracellular glutamate in response to DHPG when com-

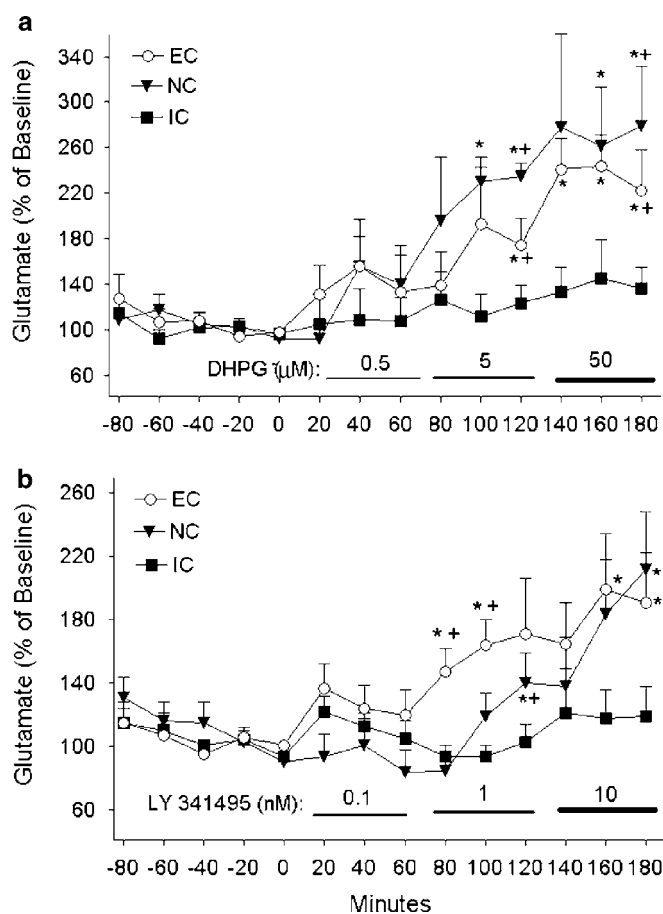


Figure 3 DHPG- and LY 341495-induced elevation in extracellular levels of glutamate is blunted in the medial prefrontal cortex of rats reared in IC. (a) Using percent differences from baseline, analysis by two-way ANOVA with repeated measures on Time revealed that reverse microdialysis of DHPG dose-dependently increased extracellular glutamate levels in the PFC of rats reared in enriched conditions (EC; $n=6$) and normal conditions (NC; $n=5$), but not in IC ($n=6$) rats. Further analysis revealed that EC and NC rats displayed significantly higher extracellular glutamate levels at 5 and 50 μ M DHPG. (b) Using percent differences from baseline, analysis by two-way ANOVA with repeated measures on Time revealed that reverse microdialysis of LY 341495 dose-dependently increased extracellular glutamate levels in the prefrontal cortex of EC ($n=6$) and NC ($n=6$) rats, but not in IC ($n=5$) rats. Further analysis revealed that EC and NC rats displayed significantly higher extracellular glutamate levels at 1 nM LY 341495. * $p < 0.05$, compared to baseline (post hoc LSD). + $p < 0.05$ compared to IC rats (post hoc Tukey's b).

pared with baseline values over the concentration range tested ($F(13,65) = 0.9$, $p < 0.6$).

Time-course analysis of percent basal extracellular levels of glutamate during reverse microdialysis of the mGluR 2/3 antagonist LY 341495 is shown in Figure 3b. A two-way ANOVA with repeated measures over Time revealed a significant effect of Time ($F(13,169) = 6.5$, $p < 0.001$) and Time \times Group interaction ($F(26,169) = 1.9$, $p < 0.02$). Further analysis of the Time \times Group interaction revealed that, compared to IC rats, 1 nM LY 341495 significantly elevated the extracellular levels of glutamate in EC and NC rats ($p < 0.05$; Tukey's b). Analysis by separate one-way ANOVAs with repeated measures over Time revealed that inhibition of group II mGluRs by LY 341495 (1 and 10 nM) induced a significant concentration-dependent increase

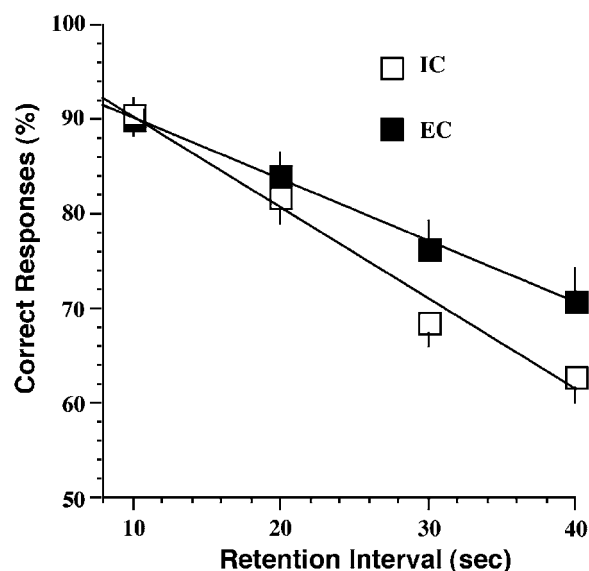


Figure 4 IC rats demonstrate impaired performance in spatial working memory compared to EC subjects. Once rats were trained to a forced choice delayed alternation task in a T-maze using a 10 s intertrial delay (retention interval), the intertrial delay was changed for 7-day periods. Each data point is the mean \pm SEM performance of the last 3 days at each intertrial interval ($n=10$ /group). A two-way ANOVA with repeated measures over intertrial interval revealed a significant effect of rearing environment.

above basal extracellular glutamate levels in EC ($n=6$) ($F(13,65) = 3.5$, $p < 0.01$) and NC ($n=5$) ($F(13,52) = 4.3$, $p < 0.01$) rats. In contrast, IC rats ($n=5$) showed no significant increase in extracellular glutamate in response to LY 341495 when compared with baseline values over the concentration range tested ($F(13,52) = 0.7$, $p < 0.7$).

IC Rats Show Working Memory Deficits Relative to EC Rats

Performance in a forced-choice delayed alternation T-maze paradigm that depends on the integrity of the PFC (Aultman and Moghaddam, 2001) was compromised in IC rats relative to EC rats (Figure 4). As delay time was increased from 10 to 40 s, there was a significant reduction in performance in subjects from both rearing environments ($F(1,3) = 29.7$, $p < 0.001$). However, while EC ($n=10$) and IC ($n=10$) subjects demonstrated equivalent task performance when intratrial delay was low (eg 10 s), as the delay was increased performance deficits appeared in the IC group ($F(1,18) = 5.4$, $p = 0.03$). Although the interaction between delay time and rearing environment was not significant, the difference between the IC and EC subjects was positively correlated with increased delay time ($r^2 = 0.919$, $p = 0.04$), indicating that as the difficulty of the task increased the difference in performance deficits between groups became more pronounced.

DISCUSSION

The present study demonstrates that impoverished rearing conditions produce a significant attenuation in mGluR function in the PFC. The capacity of group I and II mGluRs

to regulate the level of extracellular glutamate was reduced in IC rats. Moreover, the reduction in IC rats was paralleled by differences in the content of mGluR1, mGluR2/3, and mGluR5 in the PFC. Finally, these distinctions in mGluR protein and function appear to correlate with differences in T-maze performance, whereas IC rats demonstrated deficits in spatial working memory relative to EC subjects.

Effects of Rearing Environment on Group I mGluRs

The capacity of the group I mGluR agonist DHPG to elevate extracellular glutamate in the PFC was blunted in IC subjects. This functional difference between EC and IC rats was correlated with decreased mGluR1 and mGluR5 dimer and increased mGluR1 monomer content in the dorsal PFC of IC subjects. The dimer form of mGluR1 and mGluR5 is the active form of the receptor capable of binding glutamate and signal transduction through IP3 receptor stimulation (Kunishima *et al*, 2000; Sato *et al*, 2003). Thus, the increased ratio of monomer to dimer in the IC rats implies decreased receptor signaling. This is consistent with the microdialysis data showing that DHPG less effectively increased extracellular glutamate in IC rats. Of note, the distinctions in protein content between IC and EC subjects were greater for mGluR1 than mGluR5, and, although DHPG does not distinguish between mGluR1 and mGluR5 (Schoepp *et al*, 1999), the release of glutamate by DHPG is primarily via mGluR1 receptor stimulation (Cochilla and Alford, 1998; Swanson *et al*, 2001).

Effects of Rearing Environment on Group II mGluRs

Similar to group I mGluRs, mGluR2/3 exists in the brain in both monomer and dimer forms, and the dimer form is predominant in PFC (Xi *et al*, 2002b). IC rats demonstrated reduced monomer content in the ventral PFC relative to EC subjects. Although the role of dimerization in mGluR2/3 ligand binding and signaling is not known, the microdialysis study revealed that apparent *in vivo* tone on mGluR2/3 was reduced in IC animals. Thus, as previously observed in the nucleus accumbens (Xi *et al*, 2002a), inhibition of mGluR2/3 caused a rise in extracellular glutamate in EC rats, presumably by removing glutamatergic tone on mGluR2/3 regulation of glutamate release from synaptic glutamate release and cysteine–glutamate exchange (Gereau and Conn, 1995; Baker *et al*, 2002). The altered mGluR2/3 monomer content and apparent equivalent levels of basal extracellular glutamate between IC and EC rats argue that reduced mGluR2/3 signaling, not altered extracellular glutamate levels, mediates the blunted effect in IC rats. Consistent with this possibility, following chronic cocaine administration, mGluR2/3 monomer content is reduced in the PFC, and this is associated with reduced responsiveness to mGluR2/3 antagonists, as well as reduced mGluR2/3 GTP γ S binding (Xi *et al*, 2002b). Nonetheless, as dialysis probes in the present study were not corrected for recovery of glutamate, it is possible that altered basal glutamate levels may also contribute to differential sensitivity of EC and IC subjects to mGluR2/3 inhibition.

Effects of Rearing Environment on Working Memory

The PFC has a pivotal role in the neural network subserving working memory (Goldman-Rakic, 1996). To our knowledge, four studies employing the EC/IC paradigm have assessed working memory and find that IC subjects show reduced performance (Juraska *et al*, 1984; Escorihuela *et al*, 1995; Winterfeld *et al*, 1998; Martinez-Cue *et al*, 2002). In the present study, working memory performance in IC rats was significantly impaired when assayed with a delayed alternation T-maze task that has been shown to depend on an intact PFC (Aultman and Moghaddam, 2001). Moreover, the distinction between IC and EC subjects was apparent at higher delay intervals, indicating a decreased ability to retain rather than encode information (Aultman and Moghaddam, 2001). Whether decreased mGluR function in the PFC of IC subjects is causal in the working memory deficits is unknown. Supporting the involvement of mGluRs in regulating working memory, stimulating mGluR2/3 directly in the PFC and systemic administration of an mGluR2/3 agonist diminishes working memory performance (Aultman and Moghaddam, 2001; Gregory *et al*, 2003). However, based on these findings, it might be anticipated that reduced mGluR2/3 function in IC subjects would improve the working memory performance. One explanation for this apparent paradox is indicated by the findings that increased extracellular glutamate in the PFC produces working memory deficits (Moghaddam *et al*, 1997). For example, administration of NMDA antagonists disrupt working memory by reducing glutamate with a release in the prefrontal cortex (Moghaddam *et al*, 1997), and this effect is reversed by reducing glutamate with an mGluR2/3 agonist (Moghaddam and Adams, 1998). Thus, decreased sensitivity of mGluR2/3 receptors in IC rats would diminish presynaptic regulation of glutamate release, thereby permitting increases in glutamate that could disrupt working memory.

CONCLUSION

The capacity of group I and II mGluRs to affect extracellular glutamate levels in the PFC was significantly blunted in IC rats relative to EC or NC rats. The reduced efficacy of group I and II mGluRs to regulate glutamate likely arises in part from the altered ratio of dimer to monomer forms of the receptors. Moreover, the distinctions in mGluRs between IC and EC subjects correlated with differences in performance in a T-maze task, which depends on intact PFC function and is modulated by mGluR2/3 receptors. Since rearing rats in an impoverished environment may mimic the effect of stress on brain development (Bremner, 1999; Heim and Nemeroff, 2001), this study poses a potential role for dysfunction of mGluR receptors in the PFC in neuropsychiatric disorders such as schizophrenia and addiction, which are influenced by stress and thought to involve PFC-associated cognitive deficits (Goldstein and Volkow, 2002; Moghaddam, 2002).

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REFERENCES

- Anwyl R (1999). Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res Brain Res Rev* 29: 83–120.
- Aultman JM, Moghaddam B (2001). Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. *Psychopharmacology (Berl)* 153: 353–364.
- Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW (2002). The origin and neuronal function of *in vivo* nonsynaptic glutamate. *J Neurosci* 22: 9134–9141.
- Bardo MT, Klebaur JE, Valone JM, Deaton C (2002). Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. *Psychopharmacology (Berl)* 155: 278–284.
- Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL et al (1997). Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* 386: 284–288.
- Bremner JD (1999). Does stress damage the brain? *Biol Psychiatry* 45: 797–805.
- Cartmell J, Schoepp DD (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem* 75: 889–907.
- Chen J, Backus KH, Deitmer J (1997). Intracellular calcium transients and potassium current oscillations evoked by glutamate in cultured rat astrocytes. *J Neurosci* 17: 7278–7287.
- Cochilla AJ, Alford S (1998). Metabotropic glutamate receptor-mediated control of neurotransmitter release. *Neuron* 20: 1007–1016.
- Escorihuela RM, Tobena A, Fernandez-Teruel A (1995). Environmental enrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (Roman high-avoidance) and hyperemotional (Roman low-avoidance) rats. *Learn Mem* 2: 40–48.
- Fagni L, Chavis P, Ango F, Bockaert J (2000). Complex interactions between mGluRs, intracellular Ca²⁺ stores and ion channels in neurons. *Trends Neurosci* 23: 80–88.
- Gereau IV RW, Conn PJ (1995). Multiple presynaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. *J Neurosci* 15: 6879–6889.
- Goldman-Rakic PS (1996). Regional and cellular fractionation of working memory. *Proc Natl Acad Sci USA* 93: 13473–13480.
- Goldstein RZ, Volkow ND (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry* 159: 1642–1652.
- Gregory ML, Stech NE, Owens RW, Kalivas PW (2003). Prefrontal group II metabotropic glutamate receptor activation decreases performance on a working memory task. *Ann NY Acad Sci* 1003: 405.
- Hall FS (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Crit Rev Neurobiol* 12: 129–162.
- Heim C, Nemeroff CB (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 49: 1023–1039.
- Juraska JM, Henderson C, Muller J (1984). Differential rearing experience, gender, and radial maze performance. *Dev Psychobiol* 17: 209–215.
- Kolb B, Whishaw IQ (1998). Brain plasticity and behavior. *Annu Rev Psychol* 49: 43–64.
- Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T et al (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 407: 971–977.
- Manzoni O, Michel JM, Bockaert J (1997). Metabotropic glutamate receptors in the rat nucleus accumbens. *Eur J Neurosci* 9: 1514–1523.
- Martinez-Cue C, Baamonde C, Lumberras M, Paz J, Davisson MT, Schmidt C et al (2002). Differential effects of environmental enrichment on behavior and learning of male and female Ts65Dn mice, a model for Down syndrome. *Behav Brain Res* 134: 185–200.
- Moghaddam B (2002). Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. *Biol Psychiatry* 51: 775–787.
- Moghaddam B, Adams B, Verma A, Daly D (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 17: 2921–2927.
- Moghaddam B, Adams BW (1998). Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 28: 1349–1352.
- Moroni F, Cozzi A, Lombardi G, Sourtcheva S, Leonardi P, Carfi M et al (1998). Presynaptic mGluI type receptors potentiate transmitter output in the rat cortex. *Eur J Pharmacol* 347: 189–195.
- Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates* 4th edn. Academic Press: New York.
- Renner MJ, Rosenzweig MR (1987). *Enriched and Impoverished Environments: Effects of Brain and Behavior*. Springer-Verlag: New York.
- Robinson TE, Whishaw IQ (1988). Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. *Brain Res* 450: 209–224.
- Romanides AJ, Duffy P, Kalivas PW (1999). Glutamatergic and dopaminergic afferents to the prefrontal cortex regulate spatial working memory in rats. *Neuroscience* 92: 97–106.
- Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW (1995). Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol* 355: 455–469.
- Sato T, Shimada Y, Nagasawa N, Nakanishi S, Jingami H (2003). Amino acid mutagenesis of the ligand binding site and the dimer interface of the metabotropic glutamate receptor 1. Identification of crucial residues for setting the activated state. *J Biol Chem* 278: 4314–4321.
- Schoepp DD, Jane DE, Monn JA (1999). Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* 38: 1431–1476.
- Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW (2001). Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. *J Neurosci* 21: 9043–9052.
- Van Praag H, Kempermann G, Gage FH (2000). Neural consequences of environmental enrichment. *Nat Rev Neurosci* 1: 191–198.
- Winterfeld KT, Teuchert-Noodt G, Dawirs RR (1998). Social environment alters both ontogeny of dopamine innervation of the medial prefrontal cortex and maturation of working memory in gerbils (*Meriones unguiculatus*). *J Neurosci Res* 52: 201–209.
- Wright RA, Arnold MB, Wheeler WJ, Ornstein PL, Schoepp DD (2001). [³H] LY341495 binding to group II metabotropic glutamate receptors in rat brain. *J Pharmacol Exp Ther* 298: 453–460.

Xi Z, Ramamoorthy S, Baker DB, Shen H, Samuvel DJ, Kalivas PW (2002a). Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J Pharmacol Exp Ther* **303**: 608–615.

Xi ZX, Baker DA, Shen H, Carson DS, Kalivas PW (2002b). Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. *J Pharmacol Exp Ther* **300**: 162–171.