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Hippocampal GluR1 Associates with Behavior in the Elevated Plus Maze and Shows Sex Differences

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Abstract

The hippocampus is involved in anxiety as well as spatial memory formation and is sexually dimorphic. Female rats typically show less anxiety in elevated plus maze procedure (EPM), a standard animal model of anxiety. Many intracellular proteins, including α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluR1 and the cyclic AMP response element-binding protein (CREB), in hippocampus contribute to memory formation. However, less is known about the roles for hippocampal GluR1 and CREB in anxiety. We examined behavior in EPM in male and female rats and obtained hippocampal tissue samples to determine levels of GluR1 and CREB with western blots. EPM results showed that female rats exhibited less anxiety-like behaviors than male rats. Further, behaviors in EPM were significantly correlated with hippocampal GluR1 levels, but not with CREB. Yet, both proteins showed sex differences with lower levels in female rats. These data not only suggest some potential bases for sex differences in behaviors to which the hippocampus contributes but demonstrate that there is a strong association between hippocampal GluR1 levels and anxiety as assessed with EPM.

Keywords

CREB; AMPA; anxiety; female; rat

1. Introduction¹

The hippocampus is widely known for its importance in the formation and consolidation of spatial/relational memories and for its high degree of synaptic plasticity (McGaugh, 2000; Scoville and Milner, 1957). Hippocampal synaptic plasticity involves, in part, the calcium-calmodulin-dependent protein kinase II (CaMKII) intracellular pathway and the signal-transducing enzyme protein kinase A (PKA). CaMKII phosphorylates α -amino-3-

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Disclosures/Conflicts of Interest

None.

¹AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaMKII, calcium-calmodulin-dependent protein kinase II; CaMKIV, calcium-calmodulin-dependent protein kinase IV; CREB, cyclic-AMP response element binding protein; EPM, elevated plus maze; NMDA, N-methyl-D-aspartate; pCREB, phosphorylated CREB; PDE4, phosphodiesterase-4; PKA, protein kinase A

hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluR1. CaMKII and PKA regulate and phosphorylate the transcription factor cyclic-AMP response element binding protein (CREB) that is linked to synaptic plasticity in hippocampus (Guzowski and McGaugh, 1997; Kudo *et al*, 2004; Lonze and Ginty, 2002; Restivo *et al*, 2009; Silva *et al*, 1998; Viosca *et al*, 2009).

The hippocampus also contributes to anxiety (Barkus *et al*, 2010; Gray, 1982; McHugh *et al*, 2004). Anxiety-like behavior in rodents is assessed with elevated plus maze (EPM) (Pellow *et al*, 1985; Walf and Frye, 2007). Animals explore open and closed arms of a raised maze; increased time in open arms reflects decreased anxiety and *vice versa*. Although an extensive literature on anxiety and glutamate exists, it focuses on N-methyl-D-aspartate (NMDA) or metabotropic receptors (Barkus *et al*, 2010). Involvement of AMPA receptors, specifically GluR1 subunits, has been studied less and the data are contradictory. For example, GluR1 subunit deletion enhances anxiety (Bannerman *et al*, 2004; Mead *et al*, 2006) but other studies suggest the opposite (Alt *et al*, 2006; Das *et al*, 2008; Kapus *et al*, 2008). The relationship of CREB activation in hippocampus to anxiety is also unclear. CREB deletion enhances (Graves *et al*, 2002; Hebda-Bauer *et al*, 2004; Valverde *et al*, 2004) but CaMKIV deletion reduces anxiety (Shum *et al*, 2005). Increasing CREB reduces (Li *et al*, 2009), enhances (Rubino *et al*, 2008), or has no effect on anxiety (Restivo *et al*, 2009). To directly test the relationships of hippocampal GluR1 and CREB levels to anxiety, we examined EPM behavior and levels of these proteins in the same animals.

Sex differences are seen in anxiety. Females spend more time in open arms of EPM than male rats indicating they are less anxious (Ferguson and Gray, 2005; Johnston and File, 1990; Zimmerberg and Farley, 1993). However, no sex differences in EPM have been reported as well (Vaglenova *et al*, 2008). Ovarian hormones interact with illumination and affect EPM performance (Marcondes *et al*, 2001; Mora *et al*, 1996; Zimmerberg *et al*, 1993). Females show lower anxiety-like behavior during proestrus or metestrus (Marcondes *et al*, 2001; Mora *et al*, 1996) although this finding is not consistent across studies (Nomikos and Spyraiki, 1988). Estrogen enhances hippocampal pCREB levels in rats of both sexes (Abraham and Herbison, 2005) but only males show CREB activation after context conditioning (Kudo *et al*, 2004). AMPA receptor density is lower in female vs. male hippocampus (Palomero-Gallagher *et al*, 2003). Estrogen increases hippocampal GluR1 levels in females (Waters *et al*, 2009) but does not affect AMPA receptors (Cyr *et al*, 2000; Weiland, 1992).

Previously, we found a trend for female vs male rats to show lower GluR1 protein levels in hippocampal dendritic fields although we saw no sex differences in inhibitory avoidance or object recognition memory (Kosten *et al*, 2007). The purpose of the present study is to test whether hippocampal levels of GluR1 and CREB are lower in female vs. male rats and to extend our prior behavioral tests to EPM. We examined EPM under low illumination during diestrus in females to minimize potential circulating ovarian hormone effects.

2. Materials and Methods

2.1 Subjects and setting

Adult (70–90 days) male (n=11) and female (n=10) rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) were used in the elevated plus maze (EPM) experiment. Brain tissue samples were obtained from 9 male and 8 female rats of the original group of 21 rats. Rats were group-housed (2–3 per cage) in polypropylene cages in a temperature- and humidity-controlled room maintained on a 12:12 light/dark cycle (lights on at 7:00). Food and water were available *ad libitum*. Protocols were approved by the Baylor College of Medicine

Institutional Animal Care and Use Committee and followed the “Principles of Laboratory Animal Care” (NIH publication No.85-23, revised 1996).

2.2 Vaginal cytology characterization

Estrous cycle phase was determined by vaginal cytology performed in the early afternoon as described previously (Kosten *et al.*, 2005). Once the female exhibited at least two regular 4-day cycles, she was monitored for the diestrus stage, the stage during which testing was conducted. We chose the diestrus stage of the estrous cycle to standardize the effects of ovarian hormones on behaviors and protein levels.

2.3 Elevated plus maze apparatus and testing procedure

The elevated plus maze (EPM) consisted of two open arms (45-cm long $\times$ 10-cm wide), two closed arms (45-cm long $\times$ 10-cm wide $\times$ 30-cm high), and a middle compartment (4-cm long $\times$ 4-cm wide) forming the shape of a plus. The EPM was elevated 50-cm above the ground. The floor of the EPM and the walls of the enclosed were made of black acrylic. Rats were habituated to the testing room for 30-min before testing and the room was under dim light, with no experimenter present in the room. Each rat was placed in the middle compartment (head facing an open arm) and allowed to freely explore the apparatus for 5-min. The apparatus was cleaned with disinfectant after each 5-min run. The movements of each rat were recorded by a digital video camera mounted at a height of 130-cm and connected to a computer. Data were analyzed by an automated software program (ANY-maze; Stoelting Co.; Wood Dale, IL) installed on the computer. We measured times spent (sec) in the open and closed arms of the EPM and as well as time spent in the center zone and total distance traveled.

2.4 Tissue preparation and procedure

Approximately 30-min after EPM testing, rats were decapitated and the left and right hippocampi were rapidly dissected in slices of 1.5-mm using a Rodent Brain Matrix (RBM-4000C, ASI Instruments, Warren, MI) then immediately placed on dry ice. Samples were stored at -80°C until they were processed for western blot analysis. Tissues were homogenized in ice-cold equal EMSA buffer consisting of 20 mM HEPE, 0.5 mM EDTA, 0.1 mM EGTA, and the appropriate concentration of the following protease inhibitors: 10- $\mu\text{g}/\text{ml}$ leupeptin, 0.1 M P-aminobenzamidine, 1- $\mu\text{g}/\text{ml}$ pepstatin, 1 mM PMSF and 5 mM DTT. Samples were then centrifuged at 15,000 $\times$ g for 30-min at 4°C . The supernatant was aliquoted and stored at -80°C . Total protein was determined with BCA protein assay kit (BCA, Pierce Protein, Rockford, IL). Samples were diluted to equal protein concentration in sample loading buffer, boiled for 5-min at 98°C and loaded onto 7% Tris-HCL SDS-polyacrylamide gel (50% Glycerol 2.8=ml, H_2O 2.85ml, 1.5 M Tris pH 8.8 2.55=ml, 10% SDS 50- μl , 40% A/B 1.75=ml, 10% APS 37.5- μl , TEMED 3.75- μl). Thirty and 20- μg of total protein were loaded for GluR1 and CREB, respectively and gels run at 100 V. Proteins were then transferred to nitrocellulose membranes (Amersham Bioscience, Sweden). Membranes were blocked in 5% TBS-T milk for 1-hr (TBS, Bio-Rad; Copenhagen, Denmark). Primary antibodies were diluted in 5% w/v nonfat dry milk, 1 $\times$ TBS, 0.1% Tween 20 as follows: Anti-GluR1-NT (N-terminus), clone RH95 mouse mono-clone (Mill-pore, Temecula, CA) 1:500; anti-CREB (86B10) (Cell Signaling Technology, Danvers, MA) 1:1000; anti- β -actin (Sigma Chemical, St. Louis, MO) 1:10000. Primary antibodies were incubated overnight at 4°C with gentle shaking. After three 5-min washes in TBS with 0.1% Tween 20, the blots were then incubated for 2-hrs at room temperature with goat anti-mouse (for GluR1 1:10000, CREB 1:2000; or β -actin 1:10000) secondary antibodies (Pierce Protein, Rockford, IL) in 1 $\times$ TBS with 5% w/v nonfat dry milk and 0.1% Tween 20. Blots were washed again four times for 20-min in TBS-T. Immunodetection was accomplished by enhanced chemiluminescence using SuperSignal west Pico enhanced chemiluminescence kit (Pierce).

Membranes were then imaged using 100 Plus Film Processor (All-Pro Imaging Corp., Melville, NY). Quantification of the bands was performed with UVP Vision Works LS image Acquisition and Analysis software (version 6.8). Background was subtracted from each band density reading then normalized with that of β -actin.

2.5 Data analysis

Data on times spent in the open and closed arms as well as in the center of the elevated plus maze (EPM) and total distance (m) traveled across the entire maze were analyzed using t-tests for independent samples to test for sex differences. Significance was set at $P < 0.05$.

Data on densitometry units representing levels of GluR1 and CREB were analyzed using a 2 X 2 ANOVA representing the between group factor of Sex with repeated measures on Side (right vs. left hippocampus). Data obtained from the subset of rats tested in the EPM and examined for protein levels in hippocampus were analyzed with multiple regression in order to determine if the behavioral measures could be predicted by levels of GluR1 or CREB. Significant multiple regressions ($P < 0.05$) were followed by tests on single correlations with these significance levels set at $P < 0.01$ in order to accommodate the use of multiple comparisons.

3. Results

3.1 Behavior in Elevated Plus-Maze

The amounts of time (sec) spent in the open and closed arms as well as in the center of the elevated plus maze (EPM) were determined for female and male rats. Both female and male rats spend more time in the closed arms than in the open arms of the maze as shown in Fig. 1. Female rats spend less time in the closed arms, $t(19) = 2.18$; $P < 0.05$, and more time in the open arms, $t(19) = 2.39$; $P < 0.05$, than male rats. Times spent in the center are low and do not show Sex differences ($P > 0.10$). Total distance traveled (m) was greater in female rats (10.0 ± 0.6 ; Mean $\pm$ S.E.M.) compared to male rats (7.9 ± 0.7). This statement is supported by the significant Sex difference, $t(15) = 2.28$; $P < 0.05$.

3.2 Hippocampal GluR1 and CREB levels

Total GluR1 levels in hippocampus are lower in female rats compared to male rats as supported by the significant main effect of Sex, $F(1,15) = 7.39$; $P < 0.02$. These data are shown in the left panel of Fig. 2. GluR1 levels are higher in left hippocampus compared to right hippocampus as supported by the significant main effect of Side, $F(1,15) = 12.08$; $P < 0.005$. The differential levels by side tend to be stronger for male rats as supported by the trend towards significance of the interaction of Sex X Side, $F(1,15) = P < 0.10$. Post-hoc comparisons of sex differences in GluR1 levels by region show that GluR1 levels are lower in female vs. male rats in left hippocampus and in the total amounts for this brain region (P 's < 0.05).

Total CREB levels in hippocampus are also lower in female rats compared to male rats as supported by the significant main effect of Sex, $F(1,15) = 7.37$; $P < 0.05$. These data are shown in the right panel of Fig. 2. Unlike GluR1 levels, there is no difference in CREB levels between left and right hippocampus ($P > 0.10$). Post-hoc comparisons of sex differences in CREB levels by region show that CREB levels are lower in female vs. male rats in right hippocampus and in the total amounts for this brain region, P 's < 0.05 . As determined by two-way ANOVA, there were significant main effects of the factors Sex, $F(1, 68) = 9.50$, $P < 0.01$ and Side, $F(1,68) = 11.74$, $P < 0.01$. Representative blots for CREB and GluR1 as well as β -actin from right and left hippocampi of male and female rats are shown in Fig. 3.

3.3 Correlations of behavior in EPM and hippocampal protein levels

Some female (n=8) and male (n=9) rats run in the EPM procedure were sacrificed to remove hippocampal tissue samples. For these rats, we examined the correlations (Pearson r) between times spent in the open and closed arms as well as in the center of the EPM with hippocampal protein levels and show these values in Table 1. The corresponding R^2 values reflect the proportion of variability accounted for in the model and these are seen in Table 1. There are significant negative correlations between times spent in the open arms of the EPM with GluR1 levels in left hippocampus and with total GluR1 hippocampal levels. That is, lower GluR1 levels are associated with a greater amount of time spent in the open arms. Significant positive correlations are shown for times spent in the closed arms of the maze with GluR1 levels in left hippocampus and total hippocampal GluR1 levels. In these cases, lower GluR1 levels are associated with less time spent in the open arms. There are also significant negative correlations between GluR1 levels in left hippocampus and total hippocampal GluR1 levels with times spent in the center of the maze. These indicate lower GluR1 levels associate with greater times spent in the center. Multiple regression analysis with times spent in all three areas of the EPM and with total distance traveled were run and demonstrate that GluR1 levels in left hippocampus and total hippocampus are significantly associated with anxiety measures obtained from the EPM procedure as seen in Table 1. Overall, lower hippocampal GluR1 levels are significantly correlated with measures that reflect lower anxiety (i.e., less time in closed arms and greater time in open arms).

The relationship of total GluR1 levels to time spent in the open arms of the EPM is shown on the left side of Fig. 4 and to distance traveled on the right side of Fig. 4. The R^2 values on the figure indicate that 49% of the variability in open arm is accounted for by a model of total GluR1 levels whereas a little over 25% of the variability in distance traveled is accounted for by total GluR1 levels. Note that these scatterplots indicate individual female and male rats. Correlations (and R^2 values) between total distance traveled and hippocampal GluR1 levels are not significant for left or right hippocampus (P 's > 0.05) but showed a significant negative correlation with total hippocampal GluR1 levels ($r = -0.50$; $p < 0.05$). None of the EPM measures correlate significantly with CREB levels (P 's > 0.10) as shown in Table 2.

Multiple regression for total hippocampal GluR1 levels with the variables of total distance traveled and times spent in the open and closed arms shows an adjusted R^2 of 0.65. This model is significant, $F(3,13) = 10.90$; $P < 0.001$, and indicates that 65% of the variability in total GluR1 levels is accounted for by this model. Multiple regression for GluR1 levels in left hippocampus with the same EPM variables shows an adjusted R^2 of 0.57 which is significant, $F(3,13) = 5.66$; $P < 0.05$.

Separate analyses by sex demonstrate that GluR1 levels in left hippocampus are significantly associated with times in the closed arm and center zone for male rats (r 's = 0.69 and -0.68 respectively; P 's < 0.05) and that total hippocampal GluR1 levels are significantly associated with times in the closed arm for both male and female rats (r 's = 0.78 and 0.72 respectively; P 's < 0.05). Total distance traveled is not significantly associated with GluR1 levels for either sex (P 's > 0.10).

4. Discussion

Results support our hypothesis that female rats had lower hippocampal GluR1 and CREB levels than male rats. Female rats also showed less anxiety-like behavior than male rats; they spent more time in open arms and less time in closed arms of the EPM compared to male rats. We now add to the limited literature linking GluR1 or CREB to anxiety by demonstrating significant associations between hippocampal GluR1 levels and behavior in

the EPM assay; lower hippocampal GluR1 levels significantly associated with greater times in open arms and lower times in closed arms as well as with greater distance traveled. It is unlikely that these significant correlations are simply a reflection of significant sex differences in the behaviors and protein levels. First of all, separate analyses by sex were still able to uncover significant associations with GluR1 levels and time in the closed arm despite the reduction in sample size and power. Notably, total distance traveled, a measure that shows sex differences, did not correlate significantly with GluR1 levels in the separate analyses by sex. Finally, no such associations were seen with CREB levels and any of the EPM measures.

Despite the lack of significant associations between hippocampal CREB levels and anxiety-like behaviors, these measures all showed sex differences. Estrogen phosphorylates CREB in CA1 region in both female and male mice (Abraham *et al*, 2005). Yet, training in hippocampal-dependent tasks phosphorylated CREB in CA1 of male rats but had no such effect in female rats (Kudo *et al*, 2004). Our finding of lower GluR1 levels in female rats is consistent with our prior study using immunohistochemistry (Kosten *et al*, 2007) and with a prior autoradiography study of AMPA receptors in various hippocampal regions of female vs. male rats (Palomero-Gallagher *et al*, 2003). Further, AMPA receptor levels are greater in female rats in diestrus vs. estrus stage. In one study, estrogen receptor agonists increased GluR1 levels in dorsal hippocampus (Waters *et al*, 2009) although other studies report no effect of estradiol or estradiol plus progesterone treatment on hippocampal AMPA receptor density. The former study employed immunohistochemistry techniques and examined the specific subunit type whereas the latter study used autoradiography (Cyr *et al*, 2000; Weiland, 1992).

The hippocampus is implicated in anxiety (Barkus *et al*, 2010; Gray, 1982). Ventral hippocampal lesions reduce anxiety as measured in EPM (McHugh *et al*, 2004). Anxiety levels relate to CREB activation in hippocampus but the data are inconsistent. Various forms of CREB deletion in mice associate with enhanced anxiety (Graves *et al*, 2002; Hebda-Bauer *et al*, 2004; Valverde *et al*, 2004) and increasing CREB via administration of a phosphodiesterase-4 (PDE4) inhibitor reduces anxiety (Li *et al*, 2009). However, overexpressing CREB in hippocampus with a viral vector has no effect on anxiety (Restivo *et al*, 2009). On the other hand, reduced anxiety occurs with deletion of CaMKIV, an upstream regulator of CREB, (Shum *et al*, 2005) and increased CREB activation induced by THC enhances the anxiolytic effects of THC (Rubino *et al*, 2008). These latter effects are more in line with the present study; female rats have both lower hippocampal CREB levels and less anxiety compared to male rats. Yet, CREB levels do not correlate with anxiety levels. These results suggest that the relationship between anxiety and hippocampal CREB activation is not linear as proposed for spatial memory (Viosca *et al*, 2009).

In contrast to the lack of relationship between hippocampal CREB and anxiety, anxiety-like behaviors are highly correlated with hippocampal GluR1 expression. That is, lower hippocampal GluR1 levels predict greater times in open arms of the EPM and lower times in closed arms indicating that higher GluR1 levels are associated with decreased anxiety. Together, these associations explain about 60% of the variability the data. Our findings are consistent with prior studies that show that GluR1 antagonists have anxiolytic effects in EPM (Kapus *et al*, 2008) or on punished responding (Alt *et al*, 2006). Further, anxiety produced by withdrawal from chronic benzodiazepine heightens GluR1 subunit incorporation into hippocampal CA1 synapses (Das *et al*, 2008). Data from knockout mice (gria1 or GluR-A subunit deletion) suggest a modest increased anxiety in EPM; results with another procedure (e.g., light-dark box) show no differences in the same mice (Bannerman *et al*, 2004; Mead *et al*, 2006). Since there are a limited number of studies that have investigated the role of GluR1 subunit receptor activation in anxiety models, it is difficult to

explain these discrepancies except that deletion of the gene likely leads to compensation in other systems or brain regions or in another part of the GluR1 pathway. Nevertheless, data from the present study show a highly significant association between hippocampal GluR1 levels and anxiety-like behavior.

Consistent with prior studies (Ferguson *et al*, 2005; Johnston *et al*, 1990; Zimmerberg *et al*, 1993), female rats spend more time in open arms and less time in closed arms of the EPM compared to male rats indicating less anxiety. Female rats in the present study were tested during diestrus stage of the estrous cycle when estrogen levels are low. We chose this stage to minimize potential differences due to circulating ovarian hormone levels. Indeed, previous studies show times spent in open arms of EPM are lowest during diestrus compared to other estrous stages (Marcondes *et al*, 2001; Mora *et al*, 1996). Further, estrogen administered subcutaneously or into hippocampus has anxiolytic effects (Walf and Frye, 2006). That diestrus stage female rats demonstrate lower anxiety in EPM compared to male rats suggest that factors other than circulating estrogen levels contribute to this sex difference. However, the sex difference may reflect differences in motor activity not anxiety levels per se (e.g., see (Walf *et al*, 2006). In fact, female rats show greater locomotor activity (i.e., distance traveled) in EPM.

Sex differences in laterality of hippocampal GluR1 levels are seen in the present study. Male rats have higher GluR1 levels in left compared to right hippocampus whereas no side difference occurs in female rats. Laterality of GluR1 mRNA levels in CA1 and dentate gyrus is observed in rats exposed to enriched environment during periadolescence or in offspring of high licking-grooming dams relative to their comparison groups (Bredy *et al*, 2004). Sexual dimorphism in the dentate granule layer volume of rats and mice has been shown. This sexual dimorphism is altered in females by testosterone, correlates significantly with water maze performance (Roof and Havens, 1992), and relates to presence of androgen receptors (Tabibnia *et al*, 1999). Androgen receptor immunoreactivity in CA1 region shows laterality in intact male rats and in female rats treated with testosterone but not in castrated male rats (Xiao and Jordan, 2002). The functional relevance of this laterality effect can be seen by effects of TTX inactivation of right hippocampus on spatial memory. Impairment is greater in female vs. male rats (Cimadevilla and Arias, 2008). According to our data, we predict that male rats would be less impaired because they have higher levels of GluR1 in the intact (left) hippocampus compared to the inactivated (right) hippocampus and relative to female rats.

In summary, we demonstrate lower GluR1 and CREB levels in hippocampus in female vs. male rats. Further, GluR1, but not CREB levels, in hippocampus directly associate with anxiety-like behavior in EPM such that lower GluR1 levels are associated with less anxiety-like behaviors. These data suggest that agents acting at GluR1 receptors may be potential treatments for anxiety disorders and that the focus on NMDA and metabotropic glutamate receptor agents (Barkus *et al*, 2010) be expanded to include agents that act on AMPA receptors.

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Figure 1.

Mean ($\pm$ S.E.M.) times (sec) spent in the open and closed arms as well as center zone of the elevated plus maze are shown for female (closed bars) and male (open bars) rats in the figure. Female rats spend more time in the open arm and less time in the closed arm than male rats but do not differ in time spent in the center zone. (see text for statistics). * represents significant sex differences.

Figure 2.

Mean ($\pm$ S.E.M.) arbitrary densitometry units reflecting GluR1 levels for left, right, and total hippocampus are shown for female (closed symbols) and male (open symbols) rats in the left figure. Mean ($\pm$ S.E.M.) arbitrary densitometry units reflecting CREB levels for left, right, and total hippocampus are shown in the right figure. Female rats show lower GluR1 levels than male rats in the left hippocampus and in total hippocampus but do not differ in GluR1 levels in right hippocampus. There is also a trend for male, but not female rats, to have higher GluR1 levels in left vs. right hippocampus. Female rats show lower CREB levels than male rats in the right hippocampus and in total hippocampus but do not differ in CREB levels in left hippocampus (see text for statistics). * represents significant sex differences and # represents significant side differences for male rats.

Figure 3. Representative bands of GluR1 and CREB from right and left hippocampi of male and female rats are shown.

Total GluR1 Levels and Behavior in EPM

Figure 4.

The correlation of time (min) spent in the open arm with total hippocampal levels of GluR1 is plotted in the left figure. The data show a significant negative association with an R^2 greater than 0.49. The correlation of total distance traveled (m) with total hippocampal levels of GluR1 is plotted in the right figure. The data show a significant positive association with an R^2 greater than 0.25. Closed symbols represent individual female rats and open symbols represent individual male rats.

Table 1

Correlations, (R^2 values), and P values between GluR1 levels in left, right, and total hippocampus with times (sec) spent in zones of elevated plus maze.

Side	Open Arm	Closed Arm	Center	Multiple Regression
Left	-0.62 (0.38); $p<0.01$	0.70 (0.49); $p<0.005$	-0.48 (0.23); $p<0.05$	0.71 (0.50); $p<0.01$
Right	-0.28 (0.08); n.s.	0.25 (0.06); n.s.	0.12 (0.01); n.s.	0.28 (0.08); n.s.
Total	-0.70 (0.49); $p<0.005$	0.76 (0.58); $p<0.0005$	-0.51 (0.26); $p<0.05$	0.78 (0.61); $p<0.005$

Table 2

Correlations and (R^2 values) between levels of CREB in left, right, and total hippocampus with times (sec) spent in zones of elevated plus maze. None of these correlations was significant (P 's>0.10).

Side	Open Arm	Closed Arm	Center
Left	-0.24 (0.06)	0.07 (0.00)	0.11 (0.01)
Right	-0.14 (0.02)	0.24 (0.06)	-0.24 (0.06)
Total	-0.23 (0.05)	0.16 (0.03)	-0.03 (0.00)