

# The Amygdala Modulates Neuronal Activation in the Hippocampus in Response to Spatial Novelty

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**ABSTRACT:** Emerging evidence indicates that the amygdala and the hippocampus play an important role in the pathophysiology of major psychotic disorders. Consistent with this evidence, and with data indicating amygdala modulation of hippocampal activity, animal model investigations have shown that a disruption of amygdala activity induces neurochemical changes in the hippocampus that are similar to those detected in subjects with schizophrenia. With the present study, we used induction of the immediate early gene Fos, to test the hypothesis that the amygdala may affect neuronal activation of the hippocampus in response to different spatial environments (familiar, modified, and novel). Exploratory and anxiety related behaviors were also assessed. In vehicle-treated rats, exposure to a modified version of the familiar environment was associated with an increase of numerical densities of Fos-immunoreactive nuclei in sectors CA1 and CA2, while exposure to a completely novel environment was associated with an increase in sectors CA1, CA4, and DG, compared with the familiar environment. Pharmacological disruption of amygdala activity resulted in a failure to increase Fos induction in the hippocampus in response to these environments. Exploratory behavior in response to the different environments was not altered by manipulation of amygdala activity. These findings support the idea that the amygdala modulates spatial information processing in the hippocampus and may affect encoding of specific environmental features, while complex behavioral responses to environment may be the result of broader neural circuits. These findings also raise the possibility that amygdala abnormalities may contribute to impairments in cognitive information processing in subjects with major psychoses. © 2007 Wiley-Liss, Inc.

**KEY WORDS:** hippocampus; amygdala; spatial processing; FOS; schizophrenia

## INTRODUCTION

Growing evidence from imaging and postmortem studies indicate the presence of structural, neurochemical, and functional abnormalities in the amygdala and the hippocampus of patients with major psychotic dis-

orders (Reynolds, 1983; Bogerts et al., 1985; Korpi et al., 1987; Simpson et al., 1989; Reynolds et al., 1990; Okada et al., 1991; Breier et al., 1992; Lawrie and Abukmeil, 1998; Schneider et al., 1998; Yang et al., 1998; Kosaka et al., 2002; Harrison, 2004; Williams et al., 2004; Aleman and Kahn, 2005; Carletti et al., 2005; Heringa et al., 2006; Holt et al., 2006; Weidenhofer et al., 2006). Abnormalities affecting these medial temporal lobe structures are postulated to contribute to deficits in cognitive and memory processing observed in schizophrenia (Heckers et al., 1998; Lawrie and Abukmeil, 1998; Schneider et al., 1998; Heckers, 2001; Kim et al., 2001; Richardson et al., 2004; Holt et al., 2006). In two recent studies, a partial rodent model was used to test the hypothesis that circuits linking these regions might play a role in the pathophysiology of schizophrenia (Berretta et al., 2001, 2004). Findings from these studies support the hypothesis and suggest that a defect of GABAergic transmission in the amygdala may contribute to the induction of neurochemical abnormalities in the hippocampus, such as those detected in postmortem schizophrenic brains (Reynolds et al., 1990; Harrison, 1999; Eastwood and Harrison, 2000; Berretta et al., 2001, 2004; Heckers and Konradi, 2002; Zhang and Reynolds, 2002). In particular, infusion of the GABA-A antagonist picrotoxin into the basolateral nucleus of the amygdala induces region-specific neuronal abnormalities in the hippocampus 96 h later (Berretta et al., 2004). The present study tested the hypothesis that such neurochemical abnormalities may be accompanied by altered hippocampal functions, specifically the physiological response of the hippocampus to familiar and novel environments.

Several lines of evidence suggest that memory processing in the hippocampus is modulated by the amygdala. The basolateral complex (BLC) sends substantial projections to the hippocampus, both directly and indirectly via the entorhinal cortex (Amaral and Cowan, 1980; Pikkarainen et al., 1999). Electrophysiological studies have shown that the amygdala and the hippocampus are bound by coherent oscillations suggesting that recurrent time windows may facilitate their interactions and may contribute to hippocampal functions such as long-term fear memory (Gotman and Levitova, 1996; Pare and Gaudreau, 1996; Pape et al., 2005). Additionally, neuronal population events

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and abnormal neuronal activity during temporal lobe seizures recorded in the amygdala are followed by corresponding events in the hippocampus (Pare et al., 1995; Gotman and Levtova, 1996). Several hormonal systems, such as epinephrine, norepinephrine, and glucocorticoids have been shown to affect neurotransmitter systems within the basolateral amygdala and, in turn, to modulate hippocampal functions such as inhibitory avoidance learning, contextual fear conditioning, memory for context and spatial water maze training (Hatfield and McGaugh, 1999; Passani et al., 2001; Cangioni et al., 2002; LaLumiere et al., 2003; Huff and Rudy, 2004; Huff et al., 2005, 2006; McIntyre et al., 2005).

The representation of new environments is used in this study as a model of hippocampal memory function. Electrophysiological studies in rats have illustrated that the hippocampus is involved in forming representations of space (O'Keefe, 1978; McNaughton et al., 1996; Muller et al., 1996). Hippocampal place cells fire selectively in response to specific locations (place fields) as the rat navigates through an open-field environment. It has been argued that the spatial firing patterns of hippocampal cells provides the animal with an internal representation of the environment and information about its relative position within that particular environment (O'Keefe and Dostrovsky, 1971; Muller et al., 1996; Best et al., 2001). When a rat enters a new environment, the spatial firing patterns of hippocampal neurons change. Some cells that fired when the animal was in an old environment cease firing, whereas others form new place fields within minutes and these stabilize over time (Bostock et al., 1991; Wilson and McNaughton, 1993; Frank et al., 2004). Immediate-early gene (IEG) expression studies have shown activation of hippocampal neurons in response to spatial novelty (Wan et al., 1999; Vann et al., 2000; Jenkins et al., 2002, 2004; Vazdarjanova and Guzowski, 2004; Wirtshafter, 2005).

Most of these latter studies have focused on changes introduced within the environment (cue changes, removal of barrier, etc.) or outside the environment (different room, extramaze cues). Relatively little is known about hippocampal IEG expression during exploration of familiar versus novel environments (i.e. changes in cues, color, size, and shape). The goal of the current study is twofold: (a) to extend previous findings by exploring the subregional hippocampal neuronal response explicitly to familiar versus partially modified and novel environments and (b) to investigate whether a disruption of amygdala activity might affect hippocampal activity during exploration of these environments. Picrotoxin or saline was infused into the basolateral complex and animals were exposed to the different environments 96 h later. The delay between the infusions and testing was used to mimic the evolving, long-term neurochemical changes shown to take place within the hippocampus after picrotoxin infusion (Berretta et al., 2004). The delay also allowed the animals to recover from surgery and ensured that the surgery did not interfere with the observed changes. Fos expression was used to detect differences in neuronal activation (Dragunow and Faull, 1989; Shirane et al., 1992; Hoffman et al., 1993, 1994; Piechaczyk and Blanchard, 1994; Chaud-

huri, 1997; Pennypacker, 1997) in vehicle- and picrotoxin-treated rats in response to a novel environment and to characterize the distribution of activated neurons within hippocampal sectors. We also tested whether a disruption of amygdala activity may result in observable changes in hippocampal-dependent functions such as exploratory and anxiety-related behavior in the different environments.

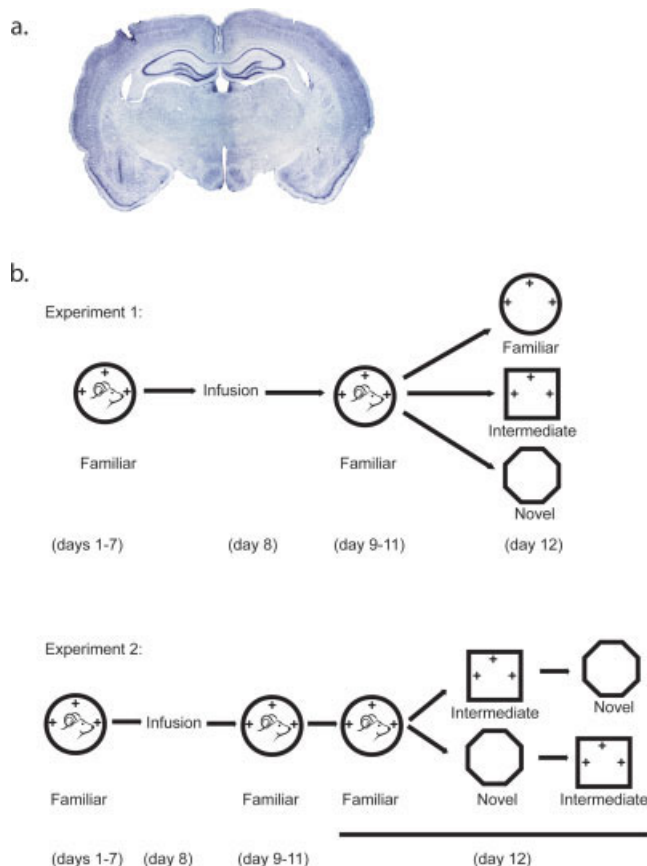
## MATERIALS AND METHODS

### Subjects

Long-Evans male rats (Charles River Laboratories, Wilmington, MA), weighing 200–250 g, were singly housed in polypropylene cages and kept on a 12-h dark/12-h light cycle. They were given limited access to food to ensure extensive exploration of the environments, and had free access to water. Animal care conformed to guidelines of the National Institute of Health Guide for the Care and Use of Animals and guidelines set by the Boston University Animal Care and Usage Committee. Rats were trained to explore a familiar environment ( $n = 64$ ) and used in two distinct experiments. In experiment 1, rats received unilateral injections of picrotoxin ( $n = 18$ ) or saline ( $n = 23$ ) in the left amygdala. On the test day, animals in each treatment group were tested in one of three environments: familiar (picrotoxin = 5, saline = 8), intermediate (picrotoxin = 6, saline = 8) and unfamiliar (picrotoxin = 7, saline = 7). In a separate group of five rats picrotoxin was infused within regions surrounding the amygdala (piriform cortex,  $n = 2$ ; posterior to basolateral complex,  $n = 3$ ) to control for extra-amygdala drug diffusion. In experiment 2, rats received bilateral injections of picrotoxin ( $n = 11$ ) or saline ( $n = 11$ ). On the test day, all rats were tested in each of the three environments.

### Surgical Procedures

Rats were kept under halothane (2%) and nitrous oxide/oxygen (7:3) anesthesia throughout the surgery, and given valium (0.02 ml/100 g; i.m.), butorphenol (0.02 ml/100 g; s.c.) and atropine sulphate (0.15 ml; i.p.). The rat's head was shaved and placed in a stereotaxic instrument (Kopf Instruments); the tooth bar was set to  $-3.3$ . The skull was exposed by a midline incision, and a burr hole was drilled into the skull above the left hemisphere for animals in the first experiment ( $n = 42$ ) and both hemispheres for animals in the second experiment ( $n = 22$ ). A Hamilton syringe (1  $\mu$ l) with a 30-gauge blunt tip needle which was attached to a syringe pump, was used to slowly (0.8  $\mu$ l/h) infuse picrotoxin ( $n = 18$ , 50 ng/0.8  $\mu$ l; RBI, Natick, MA) or vehicle ( $n = 24$ ; 0.8  $\mu$ l saline, 0.9%) into the BLC (anteroposterior (AP) + 2.6, mediolateral (ML) + 5.0 (Experiment 1) and mediolateral (ML)  $\pm$  5.0 (Experiment 2), dorsoventral (DV)  $-7.4$ ). Figure 1 shows the site of infusion. The wound was then medicated and sutured, and rats were returned to their home cages.



**FIGURE 1.** (a) Coronal nissl-stained section showing the needle track terminating at the infusion site (basolateral nucleus of the amygdala) and the hippocampal region assessed for Fos-immunoreactivity. (b) Schematic diagram summarizing the events and time course of experiments 1 and 2. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

### Apparatus and Experimental Design for Environment Training and Testing

#### Apparatus

Three different environments were used for these experiments (Fig. 1). The “familiar” environment was an aluminum cylinder (61 cm diameter × 61 cm height) with a black wall to which three white cues (square, circle, and triangle) were attached. This environment was used during training as well as testing. The second “intermediate” environment involved a modified version of the familiar one, similar to it in every aspect with the exception of its shape. It consisted of a Plexiglas square box (61 cm width × 61 cm length × 61 cm height) with black walls and the same white cues used for the familiar environment. The third “novel” environment was explicitly different from the first two environments, in terms of size and shape and it did not have any cues. It was a larger Plexiglas hexagon (61 cm per side) with five white walls and one black wall. The intermediate and novel environments were only used on the test day, so that the rats received no prior exposure to these beforehand. A circular bright light was placed directly

above the environment and a curtain was drawn around the environment during training and testing to block the rest of the room from the rats’ view, thus ensuring that the rats could not use cues outside the environment. The room in which training and testing took place is acoustically isolated. A digital video camera mounted on the ceiling 5’ above the environments and connected to computer was used for behavioral analysis in the second experiment.

#### Training

Subjects in both experiments were exposed to the familiar environment daily for 7 days, underwent surgical procedures (described above) on the eighth day (96 h prior to testing), and received three more days of exposure to the familiar environment after surgery. Animals were tested 96 h post-surgery to allow for the long-term effects of GABAergic disruption in the amygdala on subpopulations of hippocampal interneurons (Berretta et al., 2004). For Experiment 1, training consisted of exposure to the familiar environment for 20 min a day. For Experiment 2, rats were exposed to the familiar environment twice a day for 10 min with 90 min between the two exposures (Fig. 1). Bits of “fruity pebbles” (General Foods Corporation, White Plains, NY) were scattered on the floor to ensure that the rats explored the entire environment.

#### Testing

In Experiment 1, rats in each treatment group were randomly assigned to one of three subgroups and exposed to the familiar, intermediate or novel environment for 20 min. Ninety minutes following the end of the environment exposure animals were anesthetized using an overdose (100 mg/kg) of sodium pentobarbital and perfused transcardially with 0.9% saline followed by 350 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS).

In Experiment 2, all rats were re-exposed to the familiar environment for 10 min. Then half of the rats were exposed to the intermediate environment for 10 min and then to the novel environment for 10 min, with 90 min between each exposure. The other half of the rats were exposed to the novel environment, and then to the intermediate environment for 10 min each, again with 90 min between each exposure. Exposure to all environments on the test day was recorded with a video camera mounted 5’ above the environments. The position of each rat within the testing enclosure was obtained by using custom software to analyze each frame of high resolution digital video recorded for each session (frame rate = 30 frames per second). The software first applied a brightness threshold such that only very dark parts of the video image survived, including the dark patches on the rat’s fur and shadowy corners of the enclosure. The software then applied a motion threshold such that only parts of the video image that changed from frame to frame survived. On frames in which no motion was detected (i.e., the rat remained still), the rat’s position was recorded as the most recent motion position. This process resulted in a very accurate measurement of each rat’s position and move-

ments. These recordings were analyzed and scored for distance traveled and amount of time spent in the center (2/3 of the total floor surface) of each environment as measures of exploration and anxiety, respectively.

### Immunocytochemical Procedures

Following perfusion, brains were removed, postfixed for 1 h in 4% paraformaldehyde in 0.1 M PBS and cryoprotected overnight in 20% glycerol in 0.1 M phosphate buffer (PB). Twelve identical sets of serial 30  $\mu\text{m}$ -thick coronal sections were cut using a freezing microtome. One series of sections was stained with cresyl violet to aid the identification of the infusion site and the delineation of hippocampal sectors. A second series of sections, adjacent to the first one, was processed for immunocytochemistry using an antibody raised against Fos protein products. Free floating sections were washed in 0.01 M PB containing 0.9% NaCl, 0.2% Triton X-100 (PBS-Tx; pH 7.4) incubated with 0.3%  $\text{H}_2\text{O}_2$  and 10% methanol in PBS-Tx (10 min) and then in 2% bovine albumin serum (BSA; 30min). Sections were placed in the Fos primary antiserum for 48 h at 4°C (raised in rabbit 1:30,000; Oncogene Research Products, Cambridge, MA) and then incubated (2 h) at room temperature with a 1:400 solution containing biotinylated goat anti-rabbit secondary antiserum (Vector laboratories, Burlingame, CA) and then in streptavidin (Zymed, San Francisco, CA) (1:4000; 2 h; room temperature). Finally, sections were incubated in nickel-enhanced diaminobenzidine (DAB; 0.02%; 0.08% nickel sulfate in 0.1 M PB) containing 0.002% hydrogen peroxidase. Sections were rinsed with 0.1 M PB repeatedly and placed overnight in 0.01 M PBS-Tx on a rotator at 4°C. PBS-Tx was used for rinses before and after each step and for all solutions unless mentioned otherwise. Sections from all brains included in the study were processed simultaneously within the same immunocytochemistry session to avoid procedural differences. Care was taken that each 6-well staining dish used for immunocytochemistry contained sections from rats tested in each of the three environments and from both picrotoxin- and vehicle-treated rats and was carried through each immunocytochemistry step (particularly the DAB incubation) for the same duration of time, so to avoid sequence effects.

### Cell Counts

Computer-assisted light microscopy with quantification software (Bioquant Nova Prime v6.0, R&M Biometrics, Nashville, TN) was used for data collection. Slides were coded for blind analysis. For each rat, two 30- $\mu\text{m}$  thick sections labeled for Fos and including the hippocampus (distance between sections  $\sim 360$   $\mu\text{m}$ ) were included in the analysis. Specific anatomical criteria, e.g., the size and shape of dorsal hippocampus and hippocampal subfields, were used to choose the two sections so that their rostrocaudal level was consistent across rats. The hippocampal subfields (CA1, CA2, CA3, CA4, and DG) of interest and the borders of these subfields were traced with a 4 $\times$  objective, using cytoarchitectonic criteria (Swanson, 1992) evident in the adjacent sections stained with cresyl violet. Using a

40 $\times$  objective, all identifiable Fos-immunoreactive (Fos-IR) nuclei across all hippocampal layers of each sector were counted.

### Data and Statistical Analyses

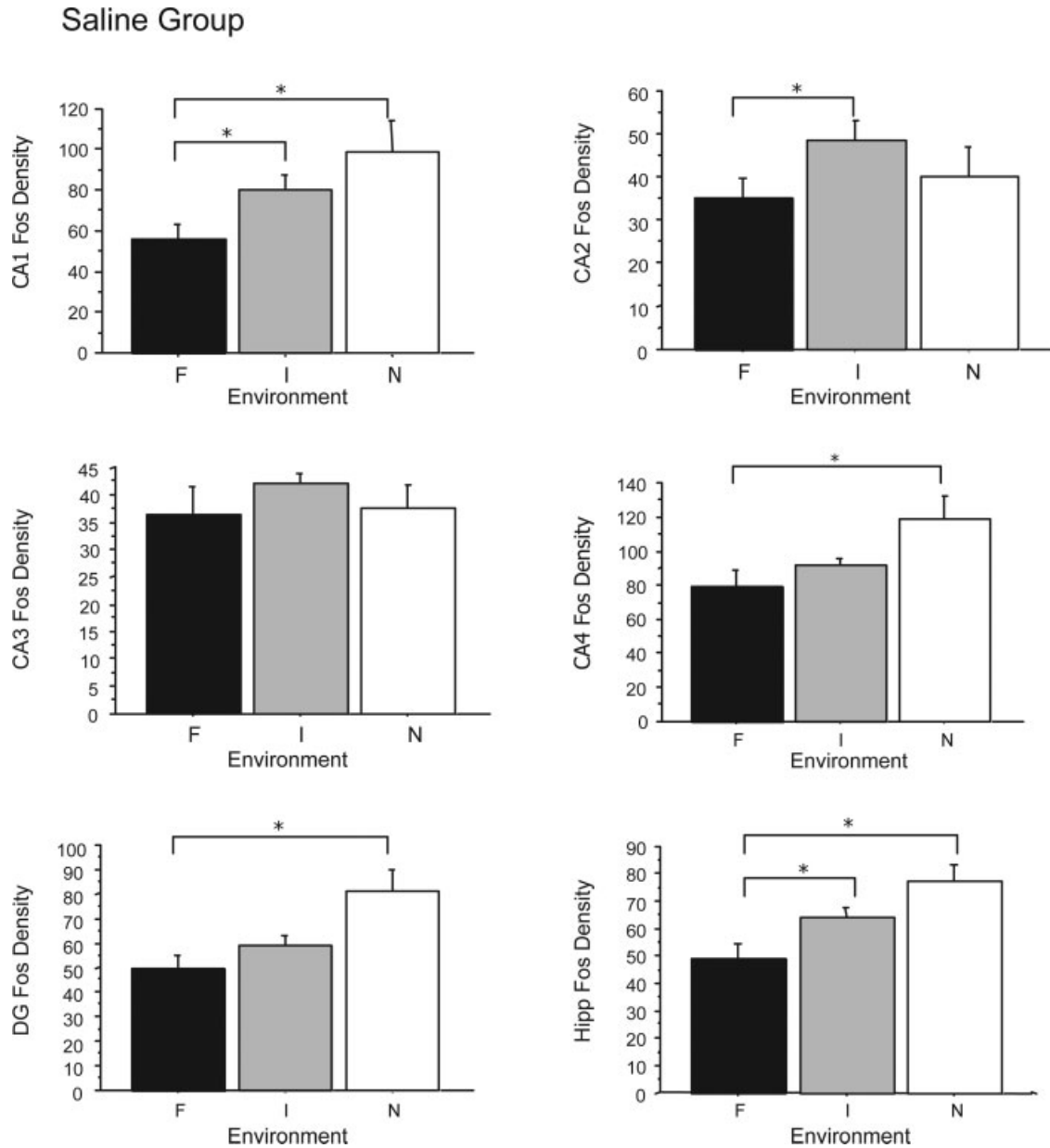
For Experiment 1, numerical densities ( $N_d$ ) of Fos-IR nuclei were calculated for each subfield (CA1, CA2, CA3, CA4, and DG) using the formula  $N_d = T_n/\Sigma a$ , where  $T_n$  is the total number of Fos positive nuclei counted in the two sections and  $\Sigma a$  is the sum of the two areas of the sections studied. Logarithmic transformation was used because these values showed non-Gaussian distributions. Two separate comparisons were made using analyses of variance (ANOVA). The first comparison tested differences in numerical densities of Fos-IR nuclei between the three environments (familiar, intermediate, and novel) in vehicle-treated rats. The second comparison tested for differences between treatment groups (saline versus picrotoxin) for each environment. Finally, a linear model of least squares was fit to determine any treatment–environment interaction effects while including the main effects. For each subregion of the hippocampus, the model internally corrects for multiple comparisons. The linear model in which we consider the whole hippocampus corrects for one region versus another.

For Experiment 2, a custom data analysis program was used to generate spatial coordinates for the rats' position every 1/30th of a second. These coordinates were used to calculate the distance traveled by each rat and the time spent in the central two-thirds of each environment. The data were analyzed with statistical software (StatView; SAS Institute, Cary, NC). Comparisons of exploratory behavior between treatment groups and across environments were performed using repeated measures ANOVAs.

## RESULTS

### Fos Expression in the Hippocampus in Response to Familiar and Novel Environments

Only saline treated rats were considered for these analyses. Counts for ipsilateral and contralateral hemispheres were summed as there were no significant differences in Fos-IR between the two hemispheres in saline-treated rats. The following means and standard deviations are expressed on the  $10^{-5}$  scale. In rats exposed to the familiar environment, Fos-IR nuclei were detected in all hippocampal sectors, i.e., CA1 ( $N_d$ ,  $5.56 \pm 2.095$ ), CA2 ( $N_d$ ,  $3.49 \pm 1.35$ ), CA3 ( $N_d$ ,  $3.66 \pm 1.37$ ), CA4 ( $N_d$ ,  $7.94 \pm 2.72$ ) and DG ( $N_d$ ,  $4.98 \pm 1.54$ ); entire dorsal hippocampus ( $N_d$ ,  $4.94 \pm 1.43$ ). Fos-IR nuclei were predominantly found in the pyramidal cell layer of the CA subfields and the granule cell layer of DG. The stratum oriens, stratum radiatum, stratum lacunosum–moleculare in CA and the molecular layer and polymorph layer in DG contained some scattered IR nuclei.

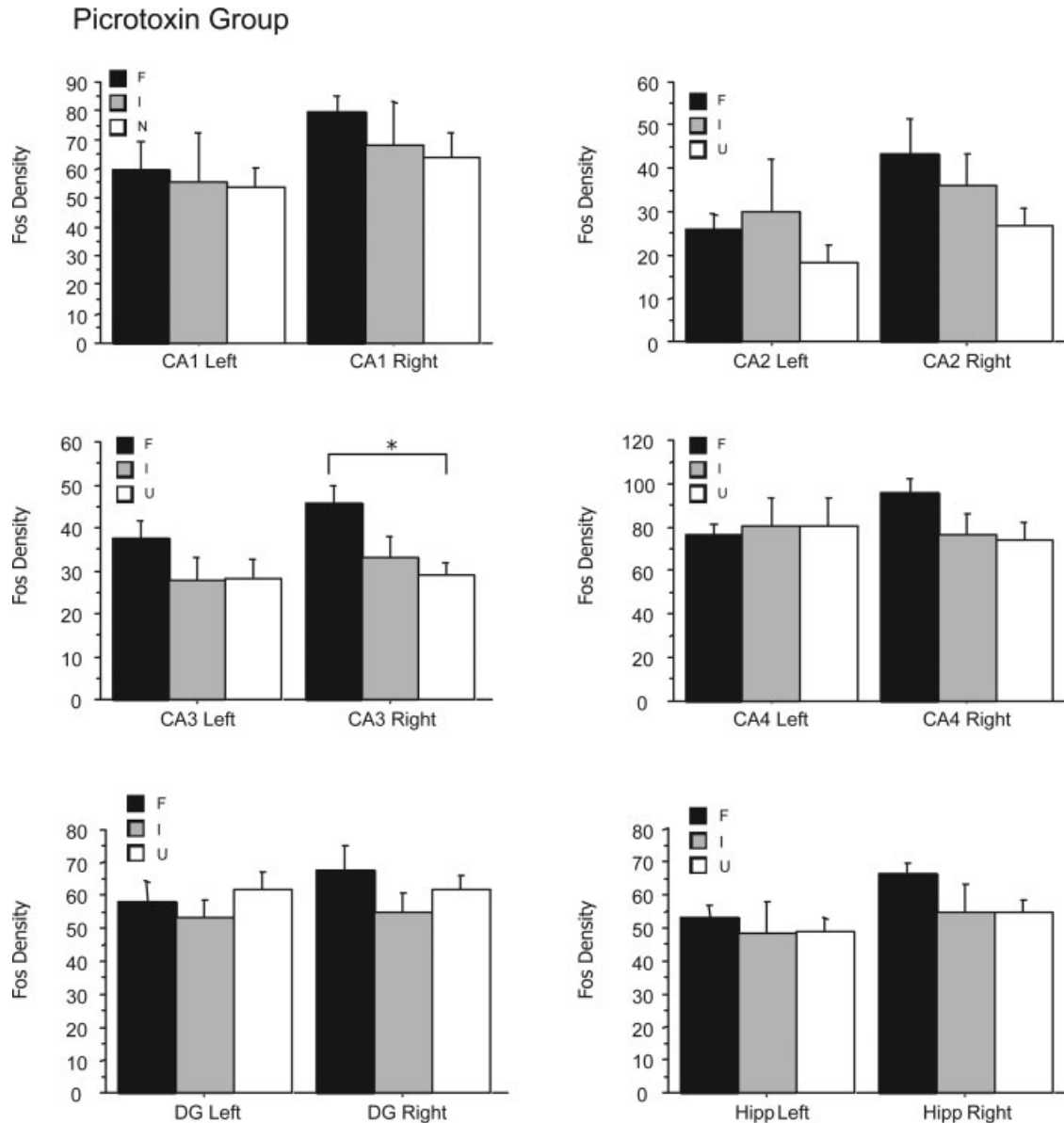


**FIGURE 2.** Numerical densities of Fos-positive nuclei in the subregions of the hippocampus (CA1, CA2, CA3, CA4, and DG) and total hippocampus. Data were summed for left and right hemispheres. Results are given for saline-treated rats exposed to familiar (F), intermediate (I), and novel (N) environments. Exposure to the intermediate environment was associated with a significant

increase of numerical densities of Fos-immunoreactive (IR) nuclei in sectors CA1, CA2, while exposure to the novel environment was associated with significant increases in sectors CA1, CA4, DG. Data are shown as mean ± SE. Significance of group differences in densities: \* $P < 0.05$ . Values on the y-axis were multiplied by  $10^6$ .

Fos expression was increased in the hippocampal subregions following exposure to intermediate and novel environments, when compared with re-exposure to the familiar environment (Fig. 2). Exposure to the intermediate environment was associated with increases of numerical densities of Fos-IR nuclei in sectors CA1 ( $t = -2.461, P = 0.028$ ; 45%) and CA2 ( $t = -2.187, P = 0.046$ ; 39%). These increases were reflected in an overall 20% increase within the dorsal hippocampus ( $t = -2.436, P = 0.029$ ). Sectors CA3, CA4, and DG showed no

significant changes. Exposure to the novel environment was associated with substantial increases of numerical densities of Fos-IR nuclei in sectors CA1 ( $t = -2.699, P = 0.018$ ; 77%), CA4 ( $t = -2.45, P = 0.029$ ; 50%), and in the DG ( $t = -3.022, P = 0.009$ ; 63%). Overall, these changes resulted in a 63% increase within the dorsal hippocampus ( $t = -3.373, P = 0.005$ ). Sectors CA2 and CA3 showed no significant changes with exposure to a novel environment compared with the familiar environment.



**FIGURE 3.** Numerical densities of Fos-positive nuclei in the hippocampal subregions (CA1, CA2, CA3, CA4, DG) and total hippocampus for left (ipsilateral) and right (contralateral) hemispheres. Results are given for picrotoxin-treated rats exposed to familiar (F), intermediate (I), and novel (N) environments. Exposure to intermediate and novel environments was not associated with

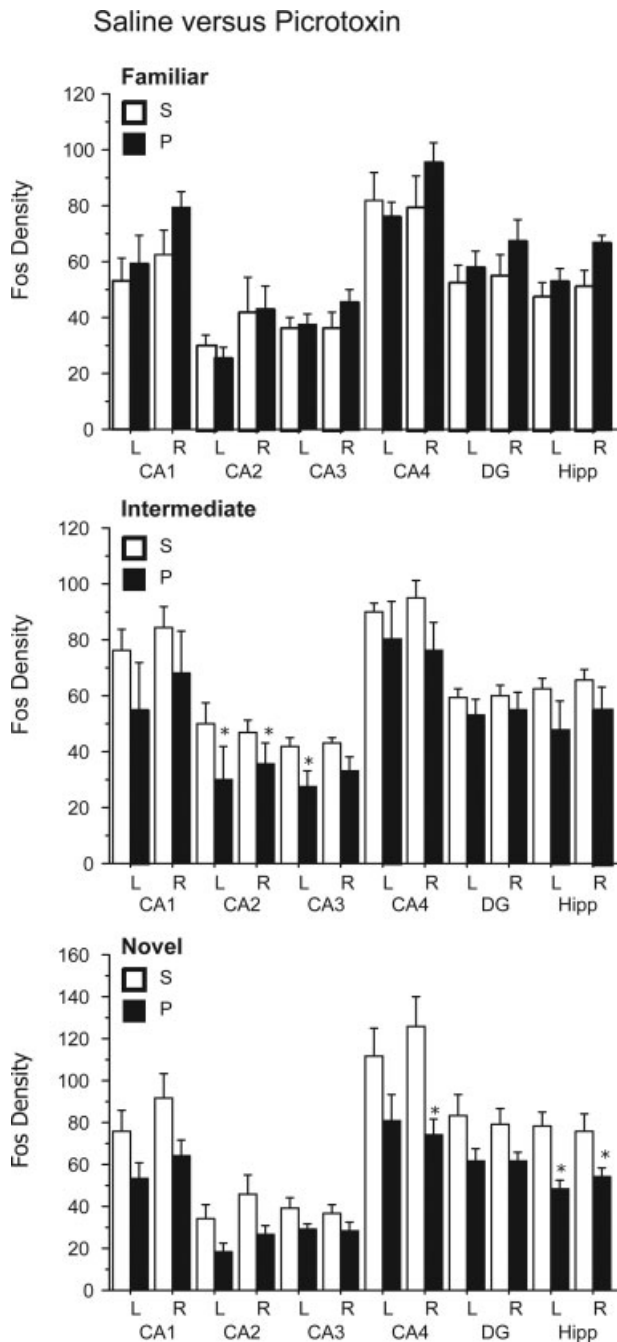
significant changes in numerical densities of Fos-IR in any hippocampal sectors except for contralateral CA3 compared with the familiar environment. Data are shown as mean  $\pm$  SE. Significance of group differences in densities: \* $P < 0.05$ . Values on the  $y$ -axis were multiplied by  $10^6$ .

### Effect of Intra-Amygdala Picrotoxin Infusion on Fos Induction in the Hippocampus

In picrotoxin treated rats, exposure to intermediate and novel environments was not associated with significant changes in numerical densities of Fos-IR in any hippocampal sectors except for contralateral CA3 compared with the familiar environment (Fig. 3). In this region, exposure to a novel environment was associated with a decrease in numerical densities of Fos-IR nuclei ( $t = 2.131$ ,  $P < 0.05$ ), as compared to the familiar environment (Fig. 3). No changes in Fos expression were

detected in comparison with controls when animals were infused with picrotoxin outside the amygdalar borders (piriform cortex,  $n = 2$ ; posterior to basolateral complex,  $n = 3$ ).

Infusion of picrotoxin in the amygdala compared with saline infusion did not affect Fos induction associated with exposure to the familiar environment in any of the hippocampal sectors (Fig. 4). In rats exposed to the intermediate environment, numerical densities of Fos-IR nuclei were significantly lower in ipsilateral CA2 ( $t = 2.086$ ,  $P < 0.05$ ) and bilaterally in CA3 ( $t = 2.086$ ,  $P < 0.05$ ) of picrotoxin-treated rats as compared with vehicle-treated rats (Fig. 4). Sectors CA1, CA4, DG, and



**FIGURE 4.** Numerical densities of Fos-positive nuclei in the hippocampal subregions (CA1, CA2, CA3, CA4, DG) and total hippocampus for left (ipsilateral) and right (contralateral) hemispheres. Results are given for saline- and picrotoxin-treated rats exposed to familiar, intermediate, and novel environments. Infusion of picrotoxin in the amygdala compared with saline infusion did not affect Fos induction associated with exposure to the familiar environment in any of the hippocampal sectors. Exposure to the intermediate environment resulted in lower numerical densities of Fos-IR nuclei in ipsilateral CA2 and bilaterally in CA3 of picrotoxin-treated rats when compared with vehicle-treated rats. Exposure to the novel environment resulted in lower numerical densities of Fos-IR nuclei in contralateral CA4 and bilaterally in the entire dorsal hippocampus in picrotoxin-treated rats compared with saline-treated rats. Data are shown as mean  $\pm$  SE. Significance of group differences in densities: \* $P < 0.05$ . Values on the y-axis were multiplied by  $10^6$ .

contralateral CA2 showed no significant changes (Fig. 4). In rats exposed to the novel environment, numerical densities of Fos-IR nuclei were significantly lower in contralateral CA4 ( $t = 2.086, P < 0.05$ ) and bilaterally in the entire dorsal hippocampus ( $t = 2.086, P < 0.05$ ) in picrotoxin-treated rats compared with saline-treated rats (Fig. 4). CA1, CA2, CA3, ipsilateral CA4, and DG did not show significant changes (Fig. 4).

### Interactions Between Environment and Amygdala Activation on Fos Induction in the Hippocampus

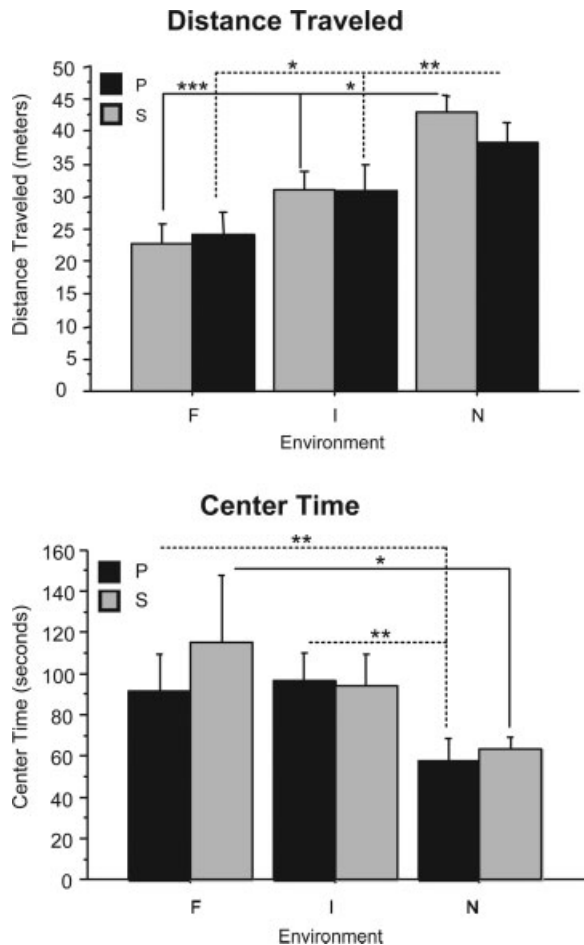
A linear model of least squares was fit to determine any treatment–environment interaction effects while including the main effects to test the relationship between exposure to familiar/novel environments and picrotoxin treatment on Fos expression in the hippocampus. Although many main effects were not significant, the results showed significant interactions within the hippocampus contralateral to the injection site: CA1 ( $t = -2.21, P = 0.03$ ), CA3 ( $t = -2.20, P = 0.03$ ), CA4 ( $t = -3.43, P = 0.0015$ ), and DG ( $t = -2.13, P = 0.04$ ). No significant effects were observed in the contralateral CA2 or in any of the ipsilateral hippocampal sectors.

### Behavior: Effects of Picrotoxin Infusion in the Amygdala

Exposure to the intermediate and novel environments resulted in a graded increase in distance traveled compared with the familiar environment in both saline- and picrotoxin-treated groups (Fig. 5). A repeated measures ANOVA performed on the distance traveled data for saline- and picrotoxin-treated rats did not indicate a significant effect of treatment ( $F_{(1,20)} = 0.18; P = 0.68$ ) or a significant treatment group by environment interaction ( $F_{(2,40)} = 0.30; P = 0.74$ ). However, the repeated measures ANOVA did reveal a significant effect of environment ( $F_{(2,40)} = 14.36; P < 0.0001$ ) (Fig. 5). Exposure to the novel environment resulted in a decrease in the amount of time spent in the center of the environment compared with the familiar and intermediate environments in both saline- and picrotoxin-treated groups (Fig. 5). A repeated measures ANOVA performed on the center time data for saline- and picrotoxin-treated rats did not indicate a significant effect of treatment ( $F_{(1,20)} = 0.39; P = 0.54$ ) or a significant treatment group by environment interaction ( $F_{(2,40)} = 0.55; P = 0.58$ ). However, the repeated measures ANOVA did reveal a significant effect of environment ( $F_{(2,40)} = 8.57; P = 0.0008$ ) (Fig. 5).

## DISCUSSION

The results of this study demonstrate that exploration of a novel environment activates neurons within the pyramidal layer of specific hippocampal sectors (Fig. 2). Pharmacological



**FIGURE 5.** Exploratory behavior during exposure to environments: distance traveled and time spent in the center. Results are given for saline- and picrotoxin-treated rats exposed to familiar (F), intermediate (I), and novel environments (N). Exposure to the intermediate and novel environments resulted in a graded increase in distance traveled compared with the familiar environment in both saline- and picrotoxin-treated groups (top). Exposure to the novel environment resulted in a decrease in the amount of time spent in the center of the environment compared with the familiar and intermediate environments in both saline- and picrotoxin-treated groups (bottom). Data are shown as mean  $\pm$  SE. Significance of group differences in densities: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

disruption of amygdala activity is associated with a failure to increase neuronal activation in the hippocampus in response to intermediate and novel environments (Fig. 3). Manipulation of amygdala activity did not alter exploratory behavior or changes in exploration patterns in the partially altered or novel environments (Fig. 5).

### Technical Considerations

In the present study, Fos expression is used as a marker of neuronal activity (Dragunow and Faull, 1989; Shirane et al., 1992; Hoffman et al., 1993, 1994; Piechaczyk and Blanchard, 1994; Chaudhuri, 1997; Pennypacker, 1997). *c-fos* induction in the hippocampus has been demonstrated as a consequence

of specific forms of learning and memory (Hughes and Dragunow, 1995; Herrera and Robertson, 1996; Campeau et al., 1997; Grimm et al., 1997; Tischmeyer and Grimm, 1999; Guzowski, 2002; He et al., 2002; Montag-Sallaz and Buonviso, 2002). One of the main advantages of the use of immediate early gene (IEG) expression to monitor neuronal activation is the ability to detect activation of neuronal ensembles and to investigate their anatomical distribution (Sagar et al., 1988; Hughes and Dragunow, 1995). The role of IEGs, such as *c-fos*, in the regulation of protein synthesis implies that their induction affects cellular functions for extended periods of time (Morgan et al., 1987; Morgan and Curran, 1991). A limitation of this method is that failure to detect Fos induction does not rule out neuronal activation. Furthermore, Fos is generally not constitutively expressed, so that only increases, but not decreases, of neuronal activation can be detected (Labiner et al., 1993; Chaudhuri, 1997). In our experiments, changes of Fos induction are consistent with expected patterns of neuronal activation, i.e., increased induction was associated with spatial novelty (Hess et al., 1995; Zhu et al., 1997; Vann et al., 2000; Jenkins et al., 2002; Vazdarjanova and Guzowski, 2004). These patterns support the usefulness of this method.

For these experiments we used local, intra-amygdala, blockade of GABA-A receptors as a tool for altering amygdalar activity. This procedure offers several experimental advantages for studying amygdalo-hippocampal interactions. For example, this paradigm elicits an increase of neuronal discharge activity in local neurons without activation of en passant fibers (Chagnac-Amitai and Connors, 1989). By blocking an intrinsic inhibition, picrotoxin infusion activates neurons releasing endogenous excitatory activity. Additionally, because blockade of GABA-A receptors with picrotoxin can potentially mimic a decrease of inhibitory tone, it provides a relevant model for the study of a GABA defect in the amygdala, like that postulated to occur in SZ (Reynolds et al., 1990; Benes and Berretta, 2001; Berretta et al., 2001, 2004).

A potential concern is the possibility of diffusion of picrotoxin to other regions surrounding the deep nuclei of the amygdala. However, control picrotoxin infusions outside the amygdala did not result in effects similar to those described here. Another concern is the possibility that some of the picrotoxin effects described in this study may be mediated by chloride ionophores other than those forming the GABA receptor supramolecular complex. However, a number of previous studies and considerations contradict these possibilities (see Berretta et al., 2004 for a more detailed discussion). Hence it is assumed that, in the present experiments, GABA-A receptor blockade mediated most of the effects induced by picrotoxin infusion in the amygdala. Another potential issue is whether picrotoxin infusion in the amygdala may have impaired the ability of hippocampus neurons to express Fos protein products. The specificity of the effects of picrotoxin infusion with respect to hippocampal sectors and distinct environment does not support this possibility. In fact, the hippocampus was normally activated in response to familiar but not in response to novel environments. Finally, no seizure activity or changes in

spontaneous behavior were observed in rats receiving picrotoxin infusions. This could be ascribed to the low doses of picrotoxin used, the low rate of infusion or both. Evidence from other laboratories also supports these findings (Cain, 1987).

Because the familiar and novel environments used in the current experiments differ in terms of a number of features (i.e., cues, color, shape, and size), the present results do not allow to assess explicitly the contribution of each of these features to the hippocampal response to novelty (see also Wilson and McNaughton, 1993; Wilson et al., 2004).

### Effects of Environment on Fos Expression in the Hippocampus

In vehicle-treated rats, Fos immunoreactivity revealed increased neuronal activation in response to the intermediate and novel environments when compared with the familiar one. These results are consistent with previous findings showing increased activation of hippocampal neurons in response to spatial novelty, e.g., novel room, environment, and rearrangement of familiar cues within a familiar environment (Wan et al., 1999; Vann et al., 2000; Jenkins et al., 2002, 2004; Vazdarjanova and Guzowski, 2004; Wirtshafter, 2005). Together with these studies, our current findings indicate that the hippocampus is involved in recognizing spatial novelty and suggest that changes induced by exposure to novel environments are related to the formation of new spatial representations within the hippocampus.

The current study explicitly investigates distribution patterns of hippocampal neuronal response within distinct sectors and layers in response to familiar versus novel environments. The predominant localization of Fos-IR nuclei in the pyramidal cell layer of the CA subfields and the granule cell layer of DG suggests that projection neurons may play a specific role in processing information about spatial novelty.

Differences relative to Fos induction in hippocampal sectors suggest specialized roles in processing spatial novelty. In CA1, substantial Fos induction was detected following exposure to both the intermediate and novel environments. This hippocampal sector has been proposed to play a role in comparing sensory information about the environment conveyed by the entorhinal cortex with internal representations of the environment stored in CA3 (McClelland et al., 1995; Lisman and Otmakhova, 2001). It is conceivable that a mismatch between the actual environment (intermediate and novel environments) and the internal representation (familiar environment) results in increased activation in CA1 neurons in response to both the intermediate and novel environments. In CA2, Fos expression was induced by exposure to the intermediate (but not the novel) environment. Such specificity suggests that this sector may play an important role in processing differences between similar environments. Sectors CA4 and DG of the hippocampus showed Fos induction in response to the novel environment, whereas they did not appear to respond to the intermediate one. We speculate that this selectivity could be attributed to the suggested role of the dentate gyrus in pattern

separation (McNaughton and Morris, 1987; Treves and Rolls, 1994; McClelland et al., 1995; Morris, 2001). Relevant to our current findings, Lee et al. (2005) used selective lesions of hippocampal subregions to show that a pattern separator function attributed to the DG may contribute to processing match-mismatch information within the hippocampus.

### Effect of GABA-A Receptor Blockade in the Amygdala on Fos Induction in the Hippocampus

Intra-amygdala infusion of picrotoxin was associated with a failure to increase Fos induction in the hippocampus in response to intermediate and novel environments. This effect included all hippocampal sectors ipsilateral and contralateral to the treated amygdala, with the exception of the contralateral CA3 sector where exposure to a novel environment was associated with a decrease in numerical densities of Fos-IR nuclei compared with the familiar environment. A failure to increase Fos-IR neurons in the hippocampus in response to the novel environments following picrotoxin infusion in the amygdala is consistent with the hypothesis that the amygdala may preferentially contact hippocampal inhibitory neurons, which in turn may suppress activation of pyramidal neurons. This hypothesis is supported by results showing that picrotoxin infusion in the amygdala induces increases of GAD67- and GAD65-IR terminals clustered around pyramidal cell somata at 2 h survival time (Berretta et al., 2001) and profound changes of markers expressed in inhibitory intrinsic neurons as well as tonic GABA-mediated inhibition at 96-h survival time (Berretta et al., 2004; Gisabella et al., 2005).

Together, these results suggest that, under normal conditions, the amygdala modulates aspects of hippocampal information processing relative to a novel environment and that a disruption of amygdalar activity may interfere with this hippocampal function. Given the role played by IEGs in the regulation of protein synthesis, these results also indicate that amygdalar modulation may induce medium- and long-term plastic changes in hippocampal neurons.

A particularly intriguing aspect of the present results is that effects of the amygdala on Fos induction in the hippocampus were detected both ipsilaterally and contralaterally. In fact, significant interactions between treatment and environment were detected specifically in the contralateral hippocampus, in sectors CA1, CA3, CA4, and DG. These observations, together with the fact that the amygdala is known to project only ipsilaterally to the hippocampus (Pikkarainen et al., 1999), raise the possibility that some of the contralateral amygdala effects may be mediated by a third brain region and/or inter-amygdala or inter-hippocampal connections. Substantial projections from the basal and accessory basal nuclei to the equivalent contralateral nuclei, and interhippocampal connections support this possibility (Van Groen, 1990; Pitkanen et al., 1995; Savander et al., 1997a,b; Akirav and Richter-Levin, 1999). A third region that may mediate the contralateral effects of the amygdala on the hippocampus is the medial prefrontal cortex which projects to the amygdala bilaterally (McDonald et al., 1996;

McDonald, 1998) while receiving only ipsilateral projections from this region (Sesack et al., 1989; McDonald, 1991).

### Effect of GABA-A Receptor Blockade in the Amygdala on the Rat's Behavior in Response to a Novel Environment

Experimental evidence suggests that the amygdala modulation of hippocampal functions may be mediated, at least in part by IEG induction (McIntyre et al., 2005; Huff et al., 2006). McIntyre et al. (2005) reported that the amygdala affects both inhibitory avoidance learning and the expression of the IEG protein product, Arc, in the hippocampus. Two studies reported that inactivation of the amygdala with muscimol, a GABA-A receptor agonist, impairs the consolidation of both contextual fear conditioning and memory for context (Huff and Rudy, 2004; Huff et al., 2005), both of which depend on the hippocampus (Barrientos et al., 2002; Rudy et al., 2002; Matus-Amat et al., 2004). Increased *Arc* and *c-fos* mRNA expression in the hippocampus after exposure to context plus shock experience was attenuated by amygdala inactivation with muscimol (Huff et al., 2006). However, Arc expression in the hippocampus associated with context exploration was not altered by amygdala inactivation (Huff et al., 2006). The apparent discrepancy between these results and ours may be due to a number of differences in the experimental paradigm used, including (1) the use of a GABA-A receptor agonist, muscimol, as opposed to a non-competitive antagonist, picrotoxin, is likely to result in fundamentally different effects on amygdala activity; (2) in our experiments, rats were killed 90 min following test-exposure to environment, but 96 h following acute drug treatment while in Huff et al. (2006) rats were killed 30–60 min following both drug treatment and behavioral testing; (3) different IEGs, Fos versus Arc, may result in distinct activation patterns; (4) comparing IEG expression after environmental exposure with home cage controls versus comparing IEG expression after exposure with familiar and novel environments is also likely to account for different neural responses.

In the present study, we tested the hypothesis that amygdala modulation of Fos induction in the hippocampus in response to a novel environment may also affect exploratory- and anxiety-related behaviors in response to the same stimuli. We chose distance traveled within the environment and tendency to avoid the center of the environment, as commonly used measures to assess exploratory- and anxiety-related behaviors, respectively (Archer, 1973; Treit and Fundytus, 1988; Simon et al., 1994). As expected, vehicle-treated rats traveled greater distances and spent less time in the center of the novel environment when compared with the familiar one. Disruption of amygdala activity did not significantly affect these behaviors, indicating intact ability to recognize a familiar environment and distinguish it from a novel one. Together, these findings suggest that the amygdala may affect aspects of spatial novelty encoding in the hippocampus without altering the overall ability of the animal to recognize distinct environments. A possible explanation for the apparent discrepancy between the neuronal data and behav-

ior could be the involvement of a broader neural circuit (i.e., perirhinal cortex) that may be compensating for a disruption of hippocampal processing thus allowing some measure of spatial recognition. It is possible that with higher doses, more chronic conditions of picrotoxin infusion, or a behavioral measure that is more selectively sensitive to hippocampal dysfunction there may have been observable changes in exploratory behavior. However, the experimental conditions were kept mild and least invasive to investigate the neuronal changes in the hippocampus associated with a disruption of GABAergic neurotransmission in the amygdala. Importantly, the observation that exploratory behavior was not affected by the treatment eliminates the possibility that the changes in IEG expression were secondary to differences in sensory stimulation or motor activity.

### Relevance for Major Psychoses

The present results indicate that a disruption of amygdala activity affects the hippocampal neuronal response to a novel stimulus. Growing evidence supports the idea that these circuits are involved in the pathogenesis of major psychoses (Simpson et al., 1989; Reynolds et al., 1990; Benes et al., 1996, 1997, 1998, 2001, 2004; Arnold, 1997; Harrison, 1999; Benes, 2000; Benes and Berretta, 2000, 2001; Lipska and Weinberger, 2000; Berretta et al., 2001; Lipska et al., 2002; Zhang and Reynolds, 2002). In recent experiments, picrotoxin infusion in the amygdala has been used to mimic changes observed in the hippocampus of schizophrenic subjects. These experiments showed that this manipulation induces neurochemical changes in the hippocampus that are strikingly similar to those detected in subjects with major psychoses (Berretta et al., 2001, 2004). The present experiments add to this evidence suggesting that the amygdala may be involved in modulating the hippocampal neuronal response to novel environments. Thus, a GABA defect in the amygdala of subjects with major psychoses (Roberts et al., 1983; Korpi et al., 1987; Simpson et al., 1989; Reynolds et al., 1990; Berretta et al., 2006), may contribute to impairments in cognitive information processing and memory consolidation along these circuits (Heckers et al., 1998, 2004; Weiss et al., 2003, 2004, 2006; Barnett et al., 2005; Ongur et al., 2005).

## CONCLUSIONS

We found that a disruption of amygdala activity is associated with a failure to increase Fos induction in the hippocampus in response to a novel environment. These findings support the idea that the amygdala modulates spatial information processing in the hippocampus and may affect encoding of specific environmental features. Given the role of IEGs in regulating protein synthesis, the present results suggest that amygdala modulation of hippocampal functions may be long lasting and involve newly synthesized molecules. These findings raise the possibility that a disruption of amygdala–hippocampal circuits

in the major psychoses, as suggested by current literature, may contribute to an impairment of cognitive processing and memory functions in these diseases.

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