

Research report

Differential environmental exposure alters NMDA but not AMPA receptor subunit expression in nucleus accumbens core and shell

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Accepted 12 February 2005
Available online 24 March 2005

Abstract

Environmentally enriched (EE) rats show neurobehavioral differences relative to less stimulated, socially isolated (SI) littermates. Although experience-dependent cortical changes are presumed to underlie learning differences in these differentially housed animals, EE rats show reduced reward-seeking behavior and altered cytoarchitecture and dopaminergic function in the nucleus accumbens (NAcc), a brain area involved in adaptive, goal-directed activity. Given that glutamate and its interaction with dopamine regulate motivational and associative processing in this brain region, we assessed expression of the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor and the GluR1 subunit of the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor in the NAcc core and shell of EE and SI rats. Our results indicate fewer intensely stained NR1 immunopositive neurons in both core and shell of EE relative to SI rats. No such differences were observed in GluR1 staining. These results suggest that environmental experience alters NMDA but not AMPA receptor expression in NAcc. Increased expression of the NR1 subunit in the NAcc of SI rats may augment impulsivity and reward-seeking behavior relative to EE rats.

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Theme: Neural basis of behavior

Topic: Neural plasticity

Keywords: NMDA; Nucleus accumbens; Environmentally enriched rats; Medial prefrontal cortex; Glutamate; Behavioral sensitization; Immunocytochemistry

1. Introduction

Environmentally enriched (EE) rats show neural and behavioral differences relative to littermates placed in less stimulating socially isolated (SI) conditions (for review, see [52]). This manipulation, typically referred to as differential environmental experience, was one of the first procedures used to examine experience-dependent plasticity in the central nervous system. Many early investigations using this technique focused on cortical plasticity, demonstrating a variety of experience-related cortical effects including enhanced gliogenesis, synaptogenesis, and increased cortical weight and size in EE relative to SI animals [1,56,65] and parallel group learning differences [57]. Generally, learning

is presumed to improve in EE relative to SI animals [52], although many studies report conflicting results [7,16,24,31,40,48,59,60]. More recent work reveals emotional [41] and motivational [54] effects of differential environmental experience. Consistent with a motivational change, EE rats show reduced reward-seeking behavior [5,26,63] and altered structure [19] and function [4,25] of the nucleus accumbens (NAcc), a forebrain nucleus known for its involvement in natural and drug reinforcement [14,37].

The NAcc integrates dopaminergic and glutamatergic limbic-related inputs with the extrapyramidal motor system via projections to downstream basal ganglia output structures [49], suggesting that emotional–motivational influences gain access to behavior through this brain region [43]. Although alterations in dopaminergic transmission and metabolism have been observed in the NAcc of EE rats relative to SI and socially housed rats

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[4,11], it is unknown what effect differential environmental experience has on glutamatergic transmission. Given the established function of glutamate in neural plasticity [10] and its interaction with dopamine in motivational and associative processing in this brain region [37], we assessed the effect of EE exposure on glutamate receptor subunit expression in the core and shell subregions of the NAcc. We focused on NR1 subunits because all *N*-methyl-D-aspartate (NMDA) receptors require at least one of these proteins for channel activity [42,44]. In addition, NR1 subunits are involved in the regulation of dendritic architecture in development [32]. We also assessed GluR-1 subunits of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors because they are abundant in the NAcc [8,15] and, like NR1 subunits, are involved in regulating dendritic morphology during development [33].

2. Methods and materials

2.1. Animal care

Male, Sprague–Dawley pups ($n = 12$) bred in our colony from stock rats (Harlan Industries, Indianapolis, IN) were cross-fostered from two litters on postnatal day (p) 2. After weaning at p28, these rats were randomly assigned to either the EE ($n = 6$) or SI ($n = 6$) condition, respectively. SI animals (20 cm \times 23 cm \times 18 cm) were housed individually and EE rats (89 cm \times 69 cm \times 39 cm) were housed together in stainless-steel wire mesh cages. The EE cage contained toys, tunnels, and ladders, which were rearranged 3 times/week. All animals from both conditions were maintained under a 12-h light/dark cycle with ad libitum access to food and water in a quiet, windowless room held at constant room temperature. Animal care met the guidelines of the National Institutes of Health, and our experimental protocol was approved by the Indiana University Institutional Animal Care and Use Committee.

2.2. Immunohistochemistry

Immunohistochemical labeling of either the NR1 subunit of the NMDA receptor or the GluR1 subunit of the AMPA receptor was performed using a procedure similar to that of Kondo et al. [39]. On p58, all rats were deeply anesthetized with urethane and transcardially perfused with cold phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed, post-fixed by immersion in 4% paraformaldehyde for 2 h, and cryoprotected in 20% sucrose in 0.1 M phosphate buffer (pH 7.4). Frozen sections were cut coronally at 30 μ m on a sliding microtome. Equally spaced sections ($n = 5-7$) through the NAcc from each brain were saved and processed free-floating for each antibody. After rinsing in 0.1 M phosphate buffer (pH 7.4) containing 1% bovine

serum albumin and 0.1% Triton X-100 (IPB), sections were incubated in IPB containing 4% normal goat serum to block nonspecific binding and 0.5% H₂O₂ to block endogenous peroxidase activity. Sections were then incubated overnight at 4 °C in IPB containing 1% normal goat serum plus a polyclonal antibody to either NR1 (1:500; Chemicon International, Temecula, CA) or GluR1 (1:500; Chemicon International, Temecula, CA). After rinsing in IPB, sections were incubated 1 h in IPB containing 4% normal goat serum and biotinylated goat anti-rabbit IgG (1:200; Vector, Burlingame, CA). After rinsing in 0.1 M phosphate buffer (pH 7.4), sections were incubated in phosphate buffer with ABC Complex (Vector, Burlingame, CA) for 1 h. Staining was visualized using a nickel-intensified DAB reaction (Fig. 1). After a final rinse, sections were mounted on chrome-alum subbed slides, dehydrated, cleared, and coverslipped. Control sections incubated without the primary antibody were generated and demonstrated virtually no staining.

2.3. Immunopositive neuron counts

NR1 and GluR1 subunit protein expression was assessed in the core and shell regions of NAcc using methods similar

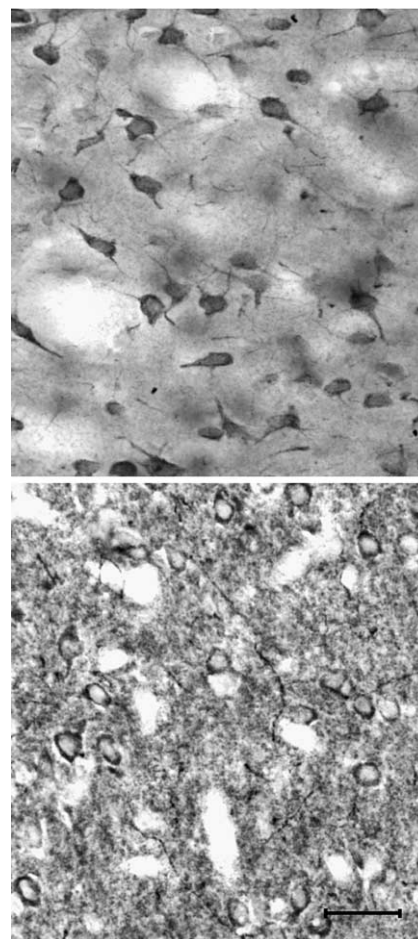


Fig. 1. Digital light micrographs of NR1- (above) and GluR1-immunopositive cells (below) in the core subregion of NAcc. Scale bar = 25 μ m.

to those of Kim et al. [38]. To quantify differences in protein expression, an unbiased stereological technique was used to estimate densities of labeled neurons. For each section, the average optical density of the white matter in a $75 \mu\text{m} \times 75 \mu\text{m}$ sampling area was determined. Optical density of each sample was expressed as average luminosity per pixel within that sample, with luminosity ranging from 0 (black) to 255 (white). For each section, neurons with optical densities up to 1.5 times, between 1.5 and 2 times, between 2 and 3 times, or more than 3 times darker than this mean were then identified using the thresholding function of the image analysis system (MCID; St. Catharines, Ontario) and counted as described below, with the experimenter blind to condition.

The numerical densities of NR1- and GluR1-immunopositive neurons were obtained using an optical dissector procedure similar to that of Srivastava et al. [61]. Shrinkage of sections due to immunohistochemical processing was measured by focusing through each section with a $100\times$ oil-immersion objective and measuring the distance traveled using a stage-mounted micrometer calibrated to a known standard. Shrinkage averaged $57 \pm 0.01\%$; thus, the length of the dissector was $12 \mu\text{m}$. This length was adequate for visualizing neurons in multiple focal planes (see [38]). For each section, counts were made at a final magnification of $1827\times$ in both core and shell regions within a $75 \mu\text{m} \times 75 \mu\text{m}$ grid and unbiased counting frame (i.e., neuronal somata touching the lower and left edge of the frame were not counted) whose medial–lateral position within the region of interest was randomly selected. Cells were identified as neurons based on standard morphological criteria (large, multipolar cell body); small, oval, homogeneously labeled cells were considered glia and therefore excluded. Neurons in the first focal plane (“tops”) were not counted [17,66]. Neuronal counts were divided by the volume of the counting frame ($75 \mu\text{m} \times 75 \mu\text{m} \times 12 \mu\text{m}$) and densities expressed as neurons per mm^3 . These estimates were averaged across the five samples within animals. Effects of differential rearing were assessed by comparing the densities of NR1- or GluR1-immunopositive neurons at each intensity range using 3-way ANOVAs (group \times region \times intensity range). For all analyses, planned comparisons consisted of F tests done within the context of the overall ANOVA [30], comparing SI versus EE groups within each intensity range.

To control for differences in neuronal density due to potential differences in neuron size across groups, the average cross-sectional area of NR1-immunopositive neuronal somata was measured. For each rat, the somata of 20 neurons in each region of interest (core and shell) were randomly selected and measured in sections matched for position along the anterior–posterior axis of the brain. Cells were identified as neurons using the standard morphological criteria described above. All somata were measured using a computerized image analysis system (MCID; Imaging Research, St. Catharines, Ontario) interfaced with a microscope (Nikon

E600) at a final magnification of $1827\times$. Average soma area was computed for each region and compared with a two-way ANOVA (group \times region).

3. Results

3.1. Stereological information

Mean (\pm SEM) optical density of the white matter samples was 184.94 ± 4.62 for NR1-stained sections and 203.75 ± 7.12 for GluR1-stained sections. The mean number of objects counted per frame was 27.24 ± 1.25 and 27.39 ± 1.31 for NR1-positive neurons in core and shell, respectively, and 18.79 ± 0.88 and 19.05 ± 0.99 for GluR1-positive neurons. For NR1-positive neurons, the total number of objects counted across the five frames averaged 129 ± 9.7 and 124 ± 8.3 in core and shell, respectively; for GluR1-positive neurons, total number of objects counted across the five frames averaged 85 ± 3.3 and 124 ± 2.9 in core and shell, respectively. Within-subjects error for NR1-positive neuronal densities averaged $6\% \pm 1\%$ and $4\% \pm 1\%$ for core and shell, respectively, while within-subjects error for GluR1-positive neuronal densities averaged $7\% \pm 1\%$ and $6\% \pm 1\%$. Cross-sectional soma area did not differ significantly across groups in either core or shell [for main effect of group, $F(1,10) = 3.16$, ns; for group \times region interaction, $F(1,10) = 0.005$, ns]. In core, average soma area was $32.97 \pm 2.19 \mu\text{m}^2$ for EE rats and $36.79 \pm 1.99 \mu\text{m}^2$ for SI rats; in shell, soma area averaged $31.72 \pm 0.74 \mu\text{m}^2$ for EE rats and $35.36 \pm 1.75 \mu\text{m}^2$ for SI rats.

3.2. Immunopositive neuron counts

To assess whether expression of NR1 subunit protein changed with differential environmental exposure, the mean density of neurons stained at varying intensities in core and shell was compared in EE and SI rats. A two-way ANOVA (group \times region) indicated that the overall density of NR1-positive neurons (i.e., the mean number of labeled neurons per mm^3) summed over the various intensities (i.e., amount of protein expression per neuron) did not vary across groups in either core or shell [for main effect of group, $F(1,10) = 0.02$, ns; for group \times region interaction, $F(1,10) = 0.55$, ns; Fig. 2]. A three-way ANOVA (group \times region \times intensity range), however, indicated a significant effect of environmental exposure on the distribution of staining intensities in both core and shell. Although the main effect of group was not significant [$F(1,30) = 0.01$, ns], significant two- and three-way interactions were present [group \times intensity range, $F(3,30) = 6.13$, $P < 0.01$; group \times region \times intensity range, $F(3,30) = 3.18$, $P < 0.04$; Fig. 3]. Planned comparisons indicated that the density of lightly stained neurons (<1.5 times background) was markedly higher in

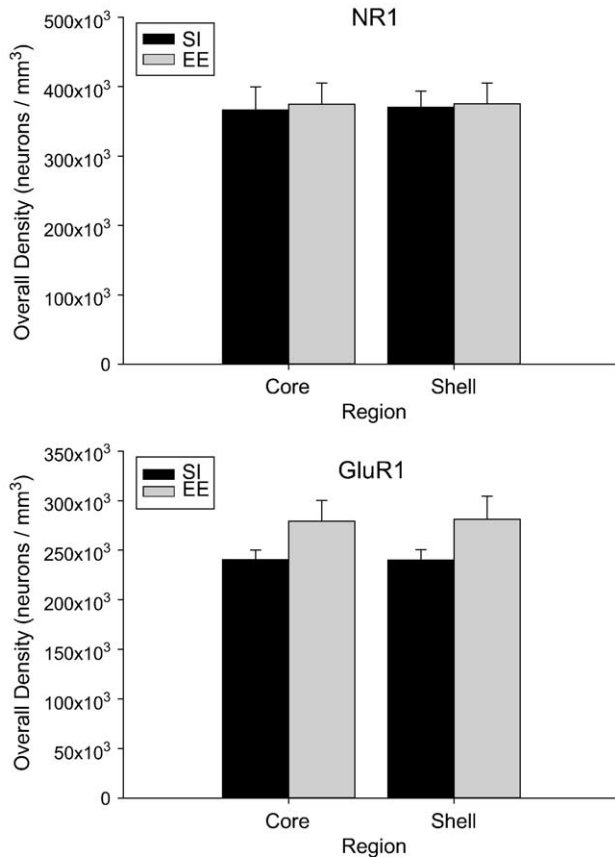


Fig. 2. Mean density of NR1- (above) and GluR1-immunopositive neurons (below) in NAcc core and shell in SI and EE rats. Error bars indicate SEM. Overall, no significant differences in the number of NR1- or GluR1-immunopositive neurons were observed between groups.

both core and shell of EE rats relative to SI rats [in core, $F(1,10) = 7.24$, $P < 0.02$; in shell, $F(1,10) = 10.97$, $P < 0.01$], while the density of more intensely stained neurons was markedly lower in both core and shell of EE rats relative to SI rats [in core, for intensities 2–3 times background and >3 times background, $F_s(1,10) = 5.62$ and 5.68 , $P_s < 0.04$; in shell, for intensity > 3 times background, $F(1,10) = 5.13$, $P < 0.05$]. Thus, while the overall density of neurons did not vary with rearing condition, the distribution of staining intensities differed markedly, with EE rats having fewer intensely NR1-positive neurons.

In contrast, differential environmental exposure failed to significantly alter GluR1 expression in NAcc neurons. A two-way ANOVA (group \times region) indicated that the overall density of GluR1-positive neurons summed over the various intensities did not vary across groups in either core or shell [for main effect of group, $F(1,10) = 2.40$, ns; for group \times region interaction, $F(1,10) = 2.89$, ns; Fig. 2]. Moreover, a three-way ANOVA demonstrated that differential environmental exposure failed to alter the distribution of GluR1 staining intensities in either core or shell [for main effect of group, $F(1,30) = 1.09$, ns; for group \times region

interaction, $F(1,30) = 0.82$, ns; for group \times region \times intensity range, $F(3,30) = 0.46$, ns; Fig. 4].

4. Discussion

Overall, our results indicate that exposure to different housing environments alters expression of NR1 but not GluR1 subunits in neurons in both NAcc core and shell. Specifically, the density of intensely stained NR1-positive neurons in both core and shell was lower in EE relative to SI rats, while the density of lightly stained NR1-positive neurons was higher in both NAcc subregions of EE relative to SI rats. GluR1 immunohistochemistry revealed no such differences. Importantly, neither overall density of neurons (total number per mm³) nor soma size varied across groups, suggesting that these EE–SI differences are due to changes in the expression of NR1 subunit protein, rather than artifactual changes in neuronal densities due to, for instance, neuronal atrophy.

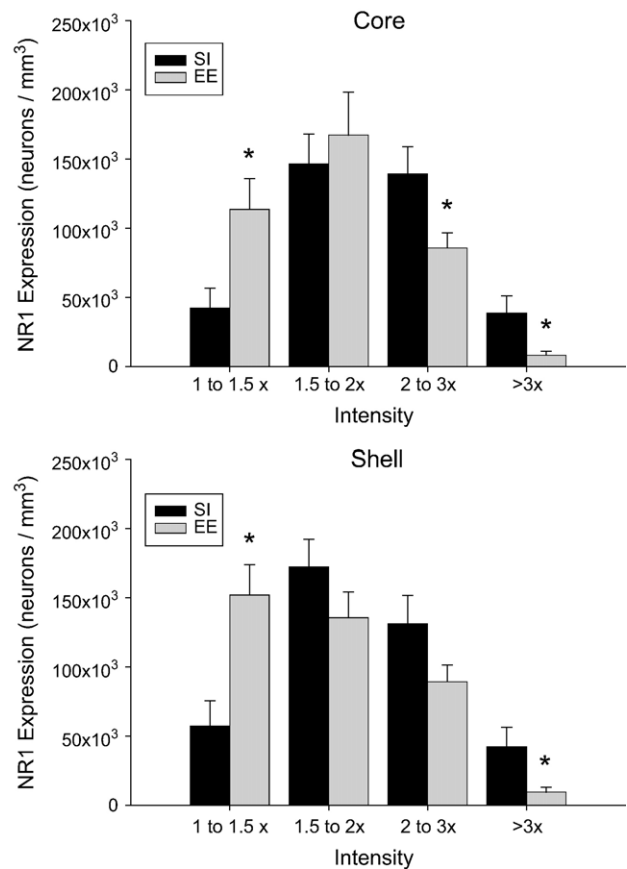


Fig. 3. Density of NR1-immunopositive neurons either 1.0–1.5, 1.5–2.0, 2.0–3.0, or more than 3.0 times darker than background staining in NAcc core (above) and shell (below) for SI and EE rats. Error bars indicate SEM; asterisks (*) indicate significant differences. In both core and shell, EE rats had fewer intensely NR1-immunopositive neurons and more weakly NR1-immunopositive neurons relative to SI rats. Thus, while overall density of neurons did not vary with rearing condition, the distribution of staining intensities differed markedly.

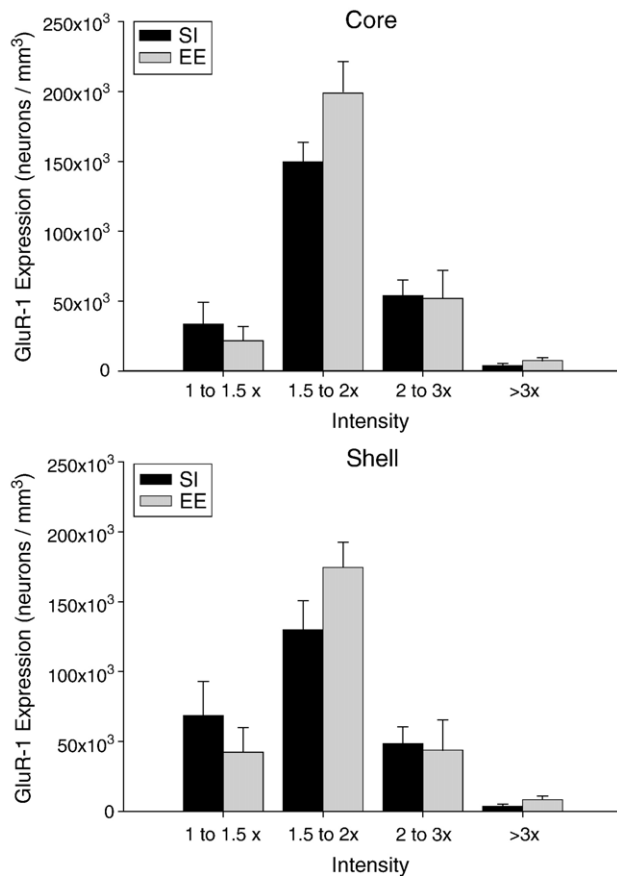


Fig. 4. Density of GluR1-immunopositive neurons either 1.0–1.5, 1.5–2.0, 2.0–3.0, or more than 3.0 times darker than background staining in NAcc core (above) and shell (below) for SI and EE rats. Error bars indicate SEM. The distribution of staining intensities of GluR1-immunopositive neurons did not vary across SI and EE rats in either NAcc subregion.

Although multiple factors may account for these changes in NR1 expression, experience-dependent differences in glutamate transmission may contribute to this effect. While few relevant studies have been conducted, EE rats are reported to have significantly higher levels of glutamate in entorhinal cortex than SI animals [47]. Since EE animals are behaviorally more active than SI animals [64] and glutamate release in cortex and striatum is increased during behavior [9], this behavioral difference may reflect increased glutamate release and transmission in the NAcc of EE rats [28,50,58]. If so, activation of NMDA receptors should be elevated in EE rats, resulting in compensatory down-regulation and reduced NR1 staining in the NAcc of these animals.

Because NMDA receptors play an important role in synapse maturation and stabilization [22,23], preferential activation of these receptors may allow for rapid maturation of neuronal cytoarchitecture in EE relative to SI rats. This hypothesis would be consistent with other neurobehavioral investigations indicating EE rats, prior to adulthood, mature more rapidly than SI rats [20,62]. Given that the total number of NMDA receptors decreases over development [10], both activity- and development-dependent factors

would presumably contribute to an overall reduction in NMDA receptors in EE relative to SI rats. If accelerated maturation in EE rats contributes to a reduction in NMDA receptor expression, we might expect this difference to decrease later in development. Although our study does not address this question, many experience-dependent differences observed in EE and SI rats are most prominently present during adolescence and early adulthood, often decreasing later in adulthood [55,57].

Another consideration is that our study compared isolated animals to socially housed animals in an enriched environment. Thus, the differences we report may be due to social housing, environmental enrichment, or both. Numerous investigations demonstrating neurobehavioral differences in EE and SI animals have used socially housed (SH) controls as a source of comparison. For example, SI rats show elevated approach responding to a conditioned stimulus (CS) signaling food reward compared to SH animals [29], and EE relative to SH rats show reduced anticipatory responding to a CS signaling sucrose reward [63]. Although we did not assess the effect of social housing on the expression of the NR1 subunit expression, accumulating evidence indicates that the SH condition may be an “intermediate step” between the EE and SI conditions and that the neurobehavioral changes brought about by exposure to EE circumstances may derive from the interaction of factors within the EE experience [64].

Several factors may explain why GluR1 unlike NR1 receptor subunit expression was not affected by differential environmental experience. For instance, it is well known that cycling, turnover, and cellular insertion of AMPA and NMDA receptors are independently regulated [69]. In addition, AMPA, unlike NMDA, receptors do not generally decrease as a function of development [10], although they can rapidly increase following learning [12]. Thus, increased glutamate levels may attenuate AMPA receptor generative processes, with this effect being masked by a countervailing experience-dependent increase in EE rats.

Since NAcc receives extensive glutamatergic isocortical and allocortical input and widespread experience-dependent cortical changes in EE rats have been documented [52], altered function of these inputs may contribute to reduced NMDA receptor expression. If so, two hypotheses may be proposed: (1) reduced NMDA receptor expression is not localized to particular subregions in NAcc, but occurs throughout this brain area, and (2) other areas receiving glutamatergic input from overlapping cortical regions, such as the caudate putamen and hippocampus, should show similar changes. Although our data support the first hypothesis, they do not address the second. One relevant study [27] shows that at a similar point in development, mean NR1 mRNA levels decreased in EE relative to SI rats in all assessed regions within the hippocampus (CA1, CA2, CA3, CA4, dentate gyrus). It should be noted, however, that these changes in mRNA, unlike our assessment of NR1 protein expression,

were not significant. This similar trend but different effect may be explained by the fact that transcriptional changes are often related to translational changes, although these processes do not mirror each other.

Our data confirm that these experience-dependent changes are occurring within neurons; converging anatomical data may indicate where these neuronal changes are most prominent. For example, Pickel and associates show that NR1 subunit expression in the dorsolateral striatum is present mainly on dendritic spines [53]. Investigations of dendritic morphology in differentially housed rats show that the density of dendritic spines in EE relative to SI rats increases in striatum [18,19]. Together, these findings indicate, assuming the changes we observed are evident in dorsal as well as ventral striatum, that NMDA receptor expression in EE rats may decrease in dendritic spines.

An overall reduction of NMDA receptors may account for the reduced neuronal excitability detected in EE animals. For example, EE animals show a higher convulsion threshold under stroboscopic lighting and lose the righting reflex more quickly after anesthesia than SI littermates [35]. Consistent with these findings, EE exposure results in reduced kainate-induced seizures [68] and delayed kindling epileptogenesis [2] relative to IC animals. Thus, a reduction in NMDA receptors may diminish NAcc excitability in EE rats and thereby contribute to housing-related neurobehavioral differences.

Concomitant with its role in motivation and learning, glutamate transmission in the NAcc is also implicated in behavioral sensitization, which is characterized by increased locomotion to repeated psychostimulant injections and may underlie the escalation of psychostimulant drug craving [67]. For example, blockade of NMDA receptors within the striatal complex prevents the induction and expression of this behavioral effect [6]. In addition, glutamate efflux is enhanced in NAcc after repeated cocaine administration [51]. Interestingly, behavioral sensitization to amphetamine is attenuated in EE relative to SI rats [3]. Consistent with the notion that reduced sensitization in EE animals may indicate decreased craving for drug reward, EE rats show a reduced propensity to self-administer amphetamine relative to SI animals [5]. These results may reflect differences in the incentive motivational properties of reward and/or inhibitory control. In fact, SI animals repeatedly show deficits in response inhibition relative to control animals [21,45,46]. Since dysfunction in NAcc has been implicated in impulsivity [13,34] and drug craving [36], our results may provide insight into the neural plasticity associated with compulsive drug-seeking behavior.

Acknowledgments

This research was supported by U.S. Public Health Service grants from the National Institute on Drug Abuse (DA 02451, DA 12964, DA05312). We also thank Faye

Caylor for administrative support and Paul Langley for construction of the enriched environment housing.

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