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The effects of isolation rearing on glutamate receptor NMDAR1A mRNA expression determined by in situ hybridization in Fawn hooded and Wistar rats

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Abstract

Rats reared in social isolation exhibit a syndrome of behavioral and biochemical effects indicative of enhanced mesolimbic dopamine (DA) function. The precise nature of the neurodevelopmental changes that produce this state are unknown but result in enhanced DA neurotransmission in the nucleus accumbens (NAC). It was hypothesized that this may be the indirect result of chronic changes in glutamate NMDA receptor function. The same prediction has been made for Fawn hooded (FH) rats that exhibit some of the characteristic effects of isolation-reared rats when compared to Wistar rats. Therefore, mRNA levels of the NMDAR1A receptor subunit were determined by in situ hybridization and were quantified in the striatum, hippocampus and prefrontal cortex of FH and Wistar rats. Isolation rearing alone was not found to have an effect on the expression of NMDAR1A, while FH rats had reduced levels across most brain regions examined. In some areas of the striatum and prefrontal cortex, this effect was greater in FH isolates than in FH socials, while in the hippocampus, the opposite was observed.

Keywords: Isolation rearing; Fawn hooded rat; Glutamate receptors; NMDA; In situ hybridization

1. Introduction

Social isolation of rats produces a variety of behavioral and neurochemical changes that appear to be the result of deprivation of particular types of social stimuli at different developmental stages (Hall, 1998). In the case of isolation rearing, these effects have been related to deprivation of social play (Einon and Morgan, 1977; Einon et al., 1978). However, many of the resulting effects of isolation rearing are indicative of enhanced dopamine (DA) function, including spontaneous hyperactivity (Einon and Morgan, 1978; Gentsch et al., 1982; Jones et al., 1990), increased responsiveness to conditioned reinforcers (Jones et al., 1990), enhanced positive contrast effects (Hall et al., 1997b) and increased response to DA agonists (Jones et al., 1990, 1992; Sahakian et al., 1975). These behavioral and pharmacological studies have been supported by experiments finding enhanced extracellular DA levels after amphetamine (Hall et al., 1998d; Jones et al., 1992; Wilkinson et al., 1994) and enhanced basal extracellular DA levels (Hall et al., 1998d) in isolation-reared rats. Perhaps as a consequence of elevated presynaptic DA function, changes in postsynaptic DA receptor function resulted in the down-regulation of the D2-like receptor function (Hall et al., 1998d; Phillips et al., 1994).

A tentative hypothesis has been forwarded that alteration in nucleus accumbens (NAC) DA function in isolation-reared rats may result from decreased glutamate input from the hippocampus or prefrontal cortex (Hall, 1998). Indeed, MK-801 produces deficits in prepulse inhibition of startle (Hoffman et al., 1993) and locomotor hyperactivity (Frantz and Van Hartesveldt, 1999a,b; Willins et al., 1993). Isolation-reared rats have impaired prepulse inhibition of startle that can be reversed by raclopride (Geyer et al., 1993), haloperidol, risperidone and clozapine (Varty and Higgins,

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1995) and also the D4/5-HT2A antagonist LU-111995 (Geyer et al., 1999). Damage to hippocampal afferents to the NAC induced a state of hyperdopaminergic function that is similar in many respects to that seen after isolation rearing (Wilkinson et al., 1993). Glutamate function has yet to be directly assessed in isolation-reared rats, although it was recently found that synaptophysin immunoreactivity is decreased in the dentate gyrus of isolation-reared rats compared to socially reared controls (Varty et al., 1999b).

Therefore, this study investigated the expression of NMDAR1A mRNA levels in the hippocampus, where they might reflect reduced hippocampal input from the entorhinal cortex (as suggested by Varty et al., 1999b) and also in the NAC where they might reflect reduced glutamate input to the NAC. The effects of isolation rearing were examined in two rat strains: Fawn hooded (FH) and Wistar. These strains vary somewhat in their response to isolation rearing; also, FH rats exhibit some characteristics that are similar to isolation-reared rats (Hall et al., 1997a, 1998a,b,c, 2000).

2. Materials and methods

2.1. Subjects

FH (NCI, Frederick, MD) and Wistar (Charles River, Frederick, MD) male rats were received 21 days postnatal and were randomly divided into two rearing conditions: socially reared ($n=10$ FH rats, $n=10$ Wistar rats) and isolation reared ($n=10$ FH rats, $n=10$ Wistar rats). These FH rats are of the FH/Har substrain. Rats were housed with a 12:12 light/dark cycle (lights on at 8 AM) with free access to food and water. Socially reared rats were housed two per $45 \times 20 \times 20$ cm cage and isolation-reared rats were housed singly in $20 \times 20 \times 20$ cm cages. Isolation only prevented physical contact as all subjects could see, hear and smell other rats. At 12 weeks of age, the subjects were removed from the home cage to an adjacent room where they were quickly decapitated, their brains removed and quickly frozen in isopentane on dry ice. The brains were stored at -80 °C until sectioning.

2.2. In situ hybridization

Coronal brain slices (15 μM) were sectioned on a cryostat, mounted on gelatin-coated slides and dried overnight. Separate sections were taken through the prefrontal cortex, striatum and hippocampus.

A 489-nucleotide-long rat NMDAR-1A cDNA was used for cRNA probe generation corresponding to the 5′ coding sequence of NMDAR1A and was prepared by RT–PCR. with a Lignscript kit (Ambion, Austin, TX). The PCR oligonucleotide primers were 5′ GTGAGGACCTCATCTC-TAGCC (501–521) and 3′ CCGTCATGTTCAGCATTGCG

Table 1
NMDAR1A mRNA expression

	WS		WI		FHS		FHI	
Area	R	L	R	L	R	L	R	L
Prefrontal cortex								
Cg1	35.5±7.0	37.9±8.7	50.6±5.3	49.9±6.1	25.3±10.5	29.6±6.0	29.5±6.1*	19.7±2.0*
Cg3	42.9±7.5	43.8±7.9	54.6±5.9	50.8±5.9	30.0±10.1	33.4±6.0	33.6±6.4*	29.2±2.4*
IL	38.2±8.8	39.3±7.2	46.3±6.7	44.6±7.3	27.6±9.0	28.9±5.0	31.1±5.6	23.7±2.8*
A1	32.2±4.4	29.4±4.2	43.4±5.5	37.9±7.9	30.3±12.9	25.8±5.6	30.0±6.9	19.9±3.5
Striatum								
DLS	27.9±3.6	22.0±2.5	26.3±2.5	26.2±3.6	18.0±1.6*	14.1±1.2*	22.0±2.5	19.0±3.1
DMS	23.8±3.0	22.7±2.3	26.6±1.5	25.3±2.9	15.3±1.3*	15.1±0.7*	17.0±1.7*	15.9±1.9*
VLS	30.9±3.0	24.1±3.7	27.2±1.6	26.2±4.1	21.2±2.2*	17.0±1.9	21.9±2.2	20.3±2.7
VMS	21.3±2.8	18.7±2.5	21.9±2.6	20.5±2.6	13.4±1.5*	12.0±1.7*	13.1±1.1*	9.6±1.2*
NAcc	21.5±3.8	16.5±1.8	23.3±1.8	18.3±2.3	15.6±1.5	14.4±2.0	9.4±1.4*,**	5.4±1.9*,**
NAcs	23.6±2.9	24.8±2.3	17.8±3.0	18.8±2.6	17.0±3.2	15.5±2.6	13.7±2.6	17.6±3.4
Hippocampus								
CA1	63.5±4.1	61.6±5.5	67.4±7.3	56.0±5.5	39.0±4.3*,**	36.6±3.4*,**	61.7±4.6	57.2±5.2
CA2	67.2±6.1	65.1±5.7	68.1±5.9	57.6±4.2	46.3±4.4*,**	41.7±3.9*,**	62.7±5.4	57.9±5.4
CA3	62.0±6.1	60.9±4.9	69.2±6.4	63.1±5.0	38.2±5.1*,**	34.1±5.2*,**	61.2±6.0	62.7±6.9
DG	66.4±5.0	67.3±4.6	75.7±6.6	68.0±5.4	41.4±4.9*,**	39.8±5.1*,**	72.2±5.7	69.8±6.0

The levels of NMDAR1A mRNA expression in brain subregions as determined by in situ hybridization. Data are expressed as means±S.E.M. of the mean optical density in arbitrary units with the background subtracted for both the left and right sides of the brain. The regions analyzed were: *prefrontal cortex*: cingulate cortex area 1 (Cg1), cingulate cortex area 3 (Cg3), infralimbic cortex (IL) and agranular insular cortex (AI); *striatum*: dorsolateral striatum (DLS), dorsomedial striatum (DMS), ventrolateral striatum (VLS), ventromedial striatum (VMS), nucleus accumbens core (NACc), and nucleus accumbens shell (NACs); and *hippocampus*: CA1, CA2, CA3 and dentate gyrus (DG).

* Significant post hoc difference between FH and W rats with the same rearing experience, Scheffe's comparison ($P<.05$).

** Significant post hoc difference between isolation-reared and socially reared rats of the same strain, Scheffe's comparison ($P<.05$).

(970–989). The sense and antisense of NMDAR1A cRNA probes were labeled with S^{35}-labeled UTP using a T7 Transcription procedure (Ambion) and were purified with the NucTrap Push Columns (Stratagene). The in situ hybridization procedure was based on a method described previously (Xing et al., 1997).

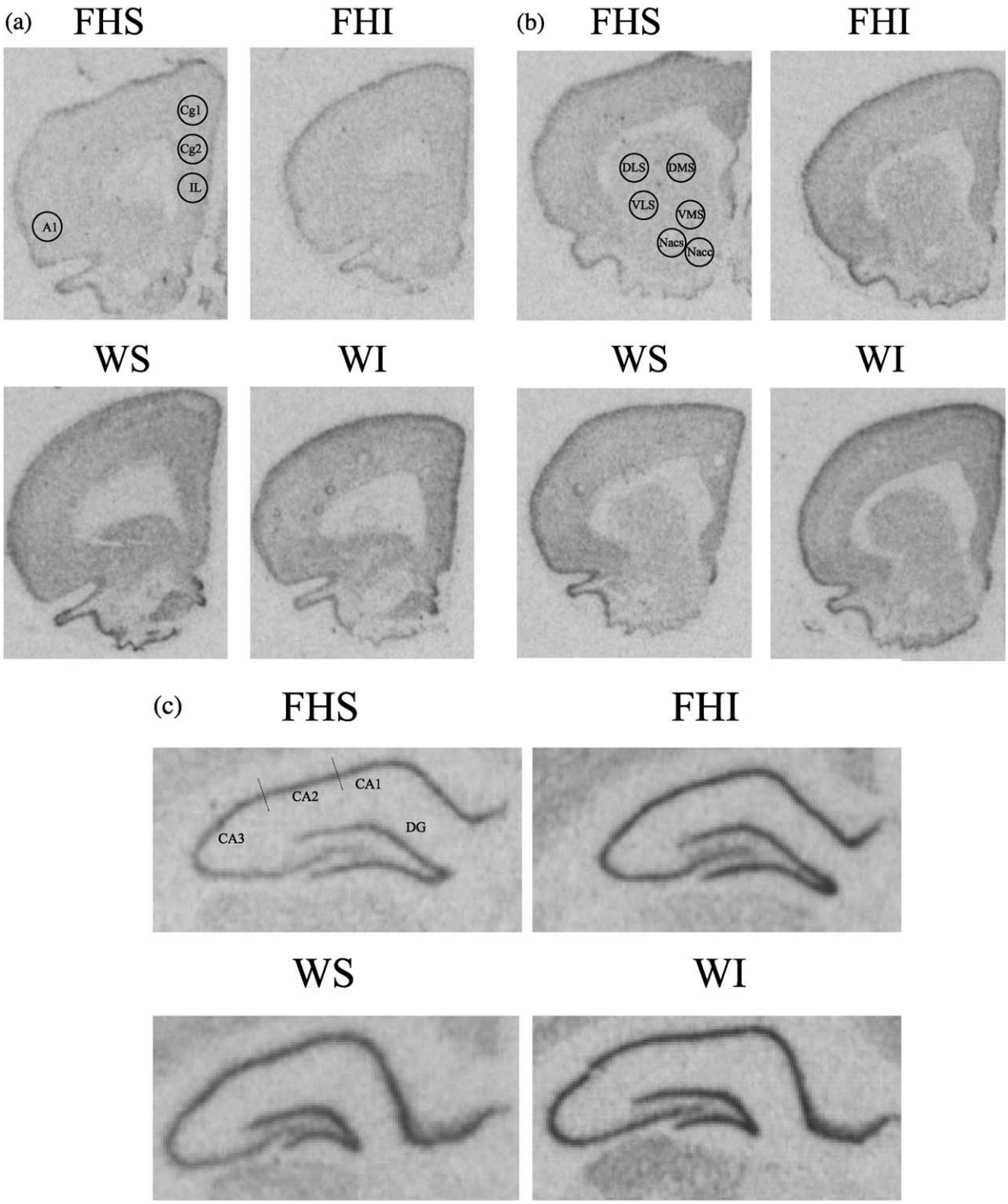

Fig. 1. Examples of autoradiograms of sections labeled for NMDAR1A mRNA by in situ hybridization: prefrontal cortex (a), striatum (b) and dorsal hippocampus (c). Abbreviations of regions analyzed: *prefrontal cortex*: cingulate cortex area 1 (Cg1), cingulate cortex area 3 (Cg3), infralimbic cortex (IL) and agranular insular cortex (AI); *striatum*: dorsolateral striatum (DLS), dorsomedial striatum (DMS), ventrolateral striatum (VLS), ventromedial striatum (VMS), nucleus accumbens core (NACc) and nucleus accumbens shell (NACs); and *hippocampus*: CA1, CA2, CA3 and dentate gyrus (DG).

The autoradiograms of the NMDAR1A mRNA were digitized using a Sierra Scientific CCD video camera, and the data were analyzed with NIH Image software. The following areas of the brain were analyzed from the atlas of Paxinos and Watson (1986): *prefrontal cortex*: cingulate cortex area 1 (Cg1), cingulate cortex area 3 (Cg3), infralimbic cortex (IL) and agranular insular cortex (AI); *striatum*: dorsolateral striatum (DLS), dorsomedial striatum (DMS), ventrolateral striatum (VLS), ventromedial striatum (VMS), nucleus accumbens core (NACc), and nucleus accumbens shell (NACs); and *hippocampus*: CA1, CA2, CA3 and dentate gyrus (DG). The mean optical density was measured for each area (one section for each measurement), and the background (intensity in an adjacent white matter area) was subtracted from this value.

2.3. Statistical methods

Mean optical densities were evaluated by analysis of variance (ANOVA) with two between-subjects factors, Rearing and Strain, and a within-subjects factor, Side (left/right), followed by a post hoc *t* test. Post hoc analysis was done using Scheffe's comparisons.

Table 2
ANOVA results with between-subjects factors, Rearing (isolate vs. social) and Strain (Fawn hooded vs. Wistar), and a within-subjects factor, Side (data not presented)

	Rearing		Strain		Rearing × Strain	
Area	*F*	*P*	*F*	*P*	*F*	*P*
Prefrontal cortex						
Cg1	1.3	.26	14	.0008	3.1	.09
Cg3	0.9	.35	12.5	.02	1.1	.31
IL	0.4	.52	10.2	.03	0.7	.40
A1	0.4	.52	3.2	.08	1.6	.22
Striatum						
DLS	1.5	.23	9.8	.004	0.4	.51
DMS	1.2	.29	22.5	.0001	0.1	.70
VLS	0.1	.81	7.6	.009	0.3	.59
VMS	0.1	.96	18.5	.0001	0.4	.53
NACc	2.2	.15	19.9	.0001	5.8	.02
NACs	1.5	.23	4.0	.06	1.0	.32
Hippocampus						
CA1	7.9	.008	4.7	.04	5.5	.02
CA2	6.9	.01	1.9	.18	4.3	.05
CA3	7.6	.009	8.1	.007	3.9	.06
DG	6.7	.01	11.5	.002	5.9	.02

See Table 1 caption for abbreviations.

3. Results

3.1. Overall results

Substantial differences were observed in NMDAR1A mRNA expression between groups that varied by region, and the data is shown in Table 1. Examples of the autoradiographs are shown in Fig. 1 for the prefrontal cortex (a), striatum (b) and hippocampus (c). ANOVA results are shown in Table 2. Generally, when FH rats were compared to Wistar rats, reduced NMDAR1A expression was found across most brain regions, but this interacted in a regionally specific manner with isolation rearing. There were significant main effects of Rearing in hippocampal regions as well, increases in NMDAR1A expression in isolates, but examination of the results, and post hoc statistical analysis, showed that this difference was only observed in FH rats.

3.2. Prefrontal cortex

In medial prefrontal areas (Cg1, Cg3, and IL), but not in the lateral prefrontal cortex (AI), FH rats had lower levels of NMDAR1A mRNA expression than Wistar rats (see Fig. 1a and Table 1 for data and Table 2 for statistics). In medial areas, there was a significant effect of Strain in the ANOVA, but not in the lateral prefrontal cortex. These reductions were much greater in isolation-reared FH rats than socially reared FH rats, relative to Wistar controls. Indeed, post hoc analyses ($P<.05$ comparison between means using Scheffe's post hoc comparison) revealed significant reductions only in FHI compared to WI rats, although the same trends were apparent in FHS compared to WS rats.

3.3. Striatum

NMDAR1A mRNA levels were generally reduced throughout the striatum (DMS, DLS, VMS and VLS) in FH rats compared to Wistar rats (See Fig. 1b and Table 1 for data and Table 2 for statistics). In all of these areas, there was a significant effect of Strain, but in lateral striatal areas, the reduction in NMDAR1A levels was only significant in socially reared FH rats ($P<.05$ Scheffe's comparison). In the NAC, FH rats again had less NMDAR1A mRNA, although this was only significant in the NACc, and, here, there was a different interaction with Rearing than other regions. In the NACc, the levels of NMDAR1A mRNA were far lower in FHI rats than in FHS rats ($P<.05$ Scheffe's comparison), while in the NACs, there was no significant effect of Strain at all.

3.4. Hippocampus

A different pattern of effects emerged in the hippocampus, where FHS rats had lower levels of NMDAR1A mRNA expression that the other three groups (significant Rearing × Strain interaction and significant post hoc differences, $P<.05$ Scheffe's comparison) in all of the areas analyzed (see Fig. 1c and Table 1 for data and Table 2 for statistics). These differences were found across all hippocampal regions examined.

4. Discussion

Isolation rearing did not result in a global down-regulation of NMDAR1A expression, although, in some areas (e.g. medial prefrontal cortical regions), the reduced levels observed in FH rats compared to Wistar rats interacted with rearing. That is, in some brain regions, isolation rearing accentuated the tendency of FH rats to have lower levels of NMDA R1A expression. This was not observed in most striatal areas where social and isolation-reared FH rats both had generally lower levels of NMDAR1A expression, although in the NACc, FHI rats had much lower levels than any other group. Different patterns were observed in the hippocampus where isolation rearing appeared to normalize NMDAR1A levels in FH rats.

The regulation of changes in NMDA receptor subunit mRNA expression is likely to be quite complex and highly regionally and cell type dependent. Even just in the limited circuitry considered in this paper relevant to the glutamate interactions with the NAC, there are relevant glutamate inputs to the NAC from the amygdala (Kelley et al., 1982), hippocampus (Kelley and Domesick, 1982) and prefrontal cortex (Christie et al., 1985b, 1987) in addition to glutamatergic projections to the ventral tegmentum (Christie et al., 1985a). Nonetheless, electrical or chemical stimulation of the ventral subicular outputs of the hippocampus increase DA release in the NAC (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000) and DA cell firing (Floresco et al., 2001; Todd and Grace, 1999), emphasizing the importance of these glutamate outputs for DA activation. However, at least some NMDA receptors in the NAC are autoreceptors (Tarazi et al., 1998), which complicate interpretations of changes in glutamate receptor mRNA expression in this region. There is no evidence regarding subunit expression after hippocampal lesions, although hippocampal lesions do reduce NMDA receptor binding (Tarazi et al., 1998).

The intention of this paper, and several previous publications (Hall et al., 1997a, 1998a,b,c, 2000, 2001a,b), was to examine the effects of isolation rearing simultaneously in two strains of rats that might differ in their sensitivity to the effects of this developmental manipulation. The FH strain was chosen because it exhibits a number of characteristics that are similar to those observed in isolation-reared rats. This comparison to isolation rearing has been strengthened by data demonstrating hyperactivity, increased alcohol consumption, impaired prepulse inhibition and greater responses in models of anxiety in FH rats relative to Wistar rats, the commonly used control strain in studies of FH rats. The initial hypothesis was that FH rats might have greater sensitivity to the effects of isolation rearing since they exhibit many of the same tendencies to start with, but this has not generally been found to be the case. Strain and isolation have been shown to make largely separate contributions to each of the previously observed phenotypes, although there are a few notable exceptions (e.g. Hall et al., 1997a). Although strain had far more pronounced effects on NMDAR1A mRNA levels in the present study overall, the effects often interacted with isolation rearing.

The prediction that reduced NMDAR1A mRNA levels would be seen in both of these circumstances was based on a number of findings, including the potential role of NMDA receptors in ethanol sensitivity and maladaptive responses to ethanol (Faingold et al., 1998; Kumari and Ticku, 2000; Spanagel and Zieglgansberger, 1997) and isolation rearing enhancement of voluntary ethanol consumption (Ellison and Potthoff, 1984; Hall et al., 1998c; Rockman et al., 1989; Schenk et al., 1990; Wolffgramm and Heyne, 1991, 1995). Isolation rearing induced attentional impairments (Bakshi and Geyer, 1999; Geyer et al., 1993, 1999; Varty and Geyer, 1998; Varty and Higgins, 1995; Varty et al., 1999a, 2000; Weiss et al., 1999, 2000; Wilkinson et al., 1994) might very well involve decreases in glutamate function as well.

4.1. Strain

This study found that strain had more consistent effects (or perhaps more general effects) on NMDAR1A mRNA levels than isolation rearing. Across most brain regions, FH rats had reduced NMDAR1A mRNA more than Wistar rats, including all medial prefrontal regions, all striatal regions and all hippocampal regions. However, in each region, strain often interacted with rearing experience in a different way.

4.2. Isolation rearing

Generally speaking, rearing condition did not have widespread effects on NMDAR1A mRNA levels, but interacted with strain. This was most obvious in the hippocampus where the Rearing × Strain interaction was significant for almost all regions examined. FH rats had reduced NMDAR1A mRNA levels, but only if they had been reared socially. In this case, isolation rearing appeared to normalize the reduction in the expression of this glutamate receptor subunit that resulted from strain differences. This is not the way that researchers often look at isolation rearing, but this is not the first time that such an effect has been observed. Both the enhanced acoustic startle responses and diminished prepulse inhibition observed in socially reared FH rats (relative to socially reared Wistar rats) are largely normalized by isolation rearing (Hall et al., 1997a).

This pattern of effects was limited to the hippocampus, however. In the frontal cortex, the opposite occurred. Although FH rats again had low levels of NMDAR1A expression in all medial prefrontal regions (and a strong trend toward reductions in the lateral prefrontal cortex) in the ANOVA overall, post hoc tests revealed that only isolation-reared FH rats exhibited this reduction.

In the striatum, FH rats again had reduced levels of NMDAR1A mRNA. Again, this interacted strongly with

isolation rearing, but in a highly regionally dependent manner. Although for most regions, the overall interaction term in the ANOVA was not significant, post hoc analysis revealed that the reduction in NMDAR1A mRNA levels in FH rats, relative to Wistar rats, was only significant in socially reared FH rats. This was the case in the lateral areas of the striatum (VLS and DLS) in particular, while the reductions in the medial striatum in FH rats were less influenced by rearing condition. While in the NACs no significant differences were observed, in the NACc the reduction in NMDAR1A levels in FH rats was greatly exacerbated by isolation rearing. It is interesting to note that the effects of these factors (strain and rearing) have a similar effect on spontaneous locomotion and exploration under some conditions, but that FH isolates have a greater tendency toward locomotor hyperactivity (Hall et al., 1998b, 2000, 2001a). Furthermore, exploration of novel objects and places, and spontaneous locomotion have been strongly linked to the NACs and core under varying conditions (Maldonado-Irizarry and Kelley, 1994). These accumbens subregions also have different roles in the modulation of prepulse inhibition by glutamate–DA interactions (Wan and Swerdlow, 1996).

4.3. Conclusions

This initial analysis of glutamate function in FH and isolation-reared rats is far from exhaustive. It remains to be seen whether other aspects of glutamate function, in particular the other subunits of the NMDA glutamate receptor and other brain regions such as the ventral tegmentum, have altered expression as well. The differences in expression observed here may also be the result of reduced neuronal survival or reduced numbers of glutamate synapses, which may have a great deal of bearing on these rats as models of schizophrenia (e.g. Geyer et al., 2001 for isolation rearing). Nonetheless, to a large extent, the initial hypotheses proposed from behavioral and pharmacological observations have been supported: FH rats and isolation-reared rats, albeit only in particular circumstances and brain regions, exhibit reductions in glutamate receptor subunit expression. Such reductions in this glutamate marker might indicate that the enhancements in dopaminergic function found in isolation-reared rats and implicated in FH rats are the result of altered glutamate–DA interactions, again supporting these paradigms as animal models of schizophrenia.

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