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Amphetamine-Induced Fos is Reduced in Limbic Cortical Regions but not in the Caudate or Accumbens in a Genetic Model of NMDA Receptor Hypofunction

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A mouse strain has been developed that expresses low levels of the NR1 subunit of the NMDA receptor. These mice are a model of chronic developmental NMDA receptor hypofunction and may therefore have relevance to the hypothesized NMDA receptor hypofunction in schizophrenia. Many schizophrenia patients show exaggerated behavioral and neuronal responses to amphetamine compared to healthy subjects. Studies were designed to determine if the NR1-deficient mice would exhibit enhanced sensitivity to amphetamine. Effects of amphetamine on behavioral activation and Fos induction were compared between the NR1-deficient mice and wild-type controls. The NR1 hypomorphic mice and controls exhibited similar locomotor activation after administration of amphetamine at 2 mg/kg. The mutant mice showed slightly reduced peak locomotor activity and slightly increased stereotypy after 4 mg/kg amphetamine. There were no differences in Fos induction in response to amphetamine in the caudate putamen, nucleus accumbens, medial or central amygdala nuclei, or bed nucleus of the stria terminalis. However, amphetamine-induced Fos was substantially attenuated in the medial frontal (infralimbic) and cingulate cortices, basolateral amygdala, and in the lateral septum of the mutant mice. The results suggest a neuroanatomically selective activation deficit to amphetamine challenge in the NR1-deficient mice.
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INTRODUCTION

Acute administration of *N*-methyl-D-aspartate (NMDA) receptor (NMDA-R) antagonists such as phencyclidine (PCP) and ketamine can produce positive, negative, and cognitive schizophrenia-like symptoms in healthy subjects (Luby *et al*, 1959; Davies and Beech, 1960; Krystal *et al*, 1994; Malhotra *et al*, 1996; Breier *et al*, 1997a). In patients with schizophrenia, NMDA antagonists can precipitate psychotic symptoms (Luby *et al*, 1959; Lahti *et al*, 1995; Malhotra *et al*, 1997). Such clinical observations have led to the hypothesis that reduced NMDA-R function could be a component of the pathophysiology of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle, 1996).

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Pharmacologic challenge tests with NMDA antagonists in humans and experimental animals have provided heuristic pharmacological models of schizophrenia (Newcomer *et al*, 1999; Krystal *et al*, 1994; Moghaddam and Adams, 1998; Duncan *et al*, 1998, 2000; Miyamoto *et al*, 2000, 2001, 2002). Since schizophrenia has, at least in part, neurodevelopmental abnormalities, and a chronic and progressive course in the majority of patients (Lieberman, 1999), the effects of chronically reduced NMDA-R function may better represent some facets of the disease (Jentsch and Roth, 1999; Olney and Farber, 1995; Miyamoto *et al*, 2003).

Functional NMDA receptors are composed of a common NR1 subunit and at least one of four subunits NR2 (NR2A–NR2D) combined in an undetermined ratio to form a heteromeric configuration (Nakanishi, 1992; Monyer *et al*, 1992). A mouse strain with markedly reduced levels of the NMDA R1 (NR1) subunit of the NMDA-R (Mohn *et al*, 1999) has been developed that provides a model of NMDA receptor hypofunction potentially relevant to the pathophysiology of schizophrenia. While mice with complete deletion of the NR1 gene die within a day after birth (Li *et al*, 1994;

Forrest *et al*, 1994), the NR1 hypomorphic mice maintain sufficient expression of the gene to allow them to thrive and survive to adulthood. The NR1-deficient mice display reduced locomotor habituation in a novel environment, increased stereotypic activity, deficits in social interactions, and reduced prepulse inhibition of acoustic startle (Mohn *et al*, 1999; Duncan *et al*, 2004). The behavioral alterations in the mutant mice are consistent with the notion that they provide an experimental model potentially relevant to the pathophysiology of schizophrenia (Mohn *et al*, 1999; Duncan *et al*, 2004).

A substantial proportion of patients with schizophrenia have an enhanced sensitivity to the behavioral effects of psychostimulants (Lieberman *et al*, 1997). A single administration of low doses of dopamine agonists, such as amphetamine and methylphenidate, that would not produce psychotic symptoms in healthy subjects, has been shown to exacerbate psychotic symptoms in patients with schizophrenia (Janowsky *et al*, 1973; Lieberman *et al*, 1987). Furthermore, brain imaging studies have shown that after an acute amphetamine challenge, striatal dopamine release is increased more in schizophrenia patients compared to controls (Abi-Dargham *et al*, 1998; Breier *et al*, 1997b; Laruelle *et al*, 1996), suggesting a dysregulated neuronal responsiveness to amphetamine in schizophrenia. It has been proposed that disinhibition of dopamine activity in the striatum is due to disturbed regulatory inputs in cortical and subcortical pathways involving both dopamine and glutamate (Carlsson *et al*, 1999; Laruelle *et al*, 1999; Weinberger *et al*, 2001). The pathophysiologic basis of this dysregulation, however, remains unclear.

The goal of the present study was to determine if chronically reduced NMDA-R function in the NR1 hypomorphic mice would affect amphetamine-induced functional responses in mice. Behavioral responses and alterations in the immediate early gene (IEG) c-*fos* product (Fos protein) were compared after acute amphetamine administration in wild-type and mutant mice with reduced NR1 subunit expression.

METHODS AND MATERIALS

Animals

A mouse line expressing reduced levels of the NR1 subunit of NMDA receptors was generated from a breeding colony originally established at the University of North Carolina as described previously (Mohn *et al*, 1999). Briefly, NR1-deficient mice were generated from E14Tg2a embryonic stem cells in which one of the NR1 alleles was targeted *in vitro* by homologous recombination. A targeting construct, $Nr1^{neo}$, was designed in which a neomycin resistance gene was inserted into Nr1 intron 20. Targeted clones were used to create chimeric mice. Chimeric males were mated with female B6D2 mice to produce heterozygous mutants. The heterozygous mice were intercrossed and used to establish a breeding colony. All wild-type mice and mice homozygous for the mutant Nr1 gene were obtained from the intercross of identified heterozygotes. All mice were genotyped on the basis of Southern or PCR analysis of tail DNA (Mohn *et al*, 1999). The mice have markedly reduced levels of the NR1 subunit, without introducing point mutations or generating novel transcripts or proteins. There was a marked reduction in ^{3}H-MK-801 binding throughout the brain of the homozygous NR1 $-/-$ mice as described previously (Duncan *et al*, 2002a). The NR1 $-/-$ mice and control NR1 $+/+$ mice were created on a mixed genetic background consisting of alleles derived from 129/Ola, C57BL/6, and DBA/2. To increase the probability that alleles of various strains, including those linked to the Nr1, were included in both populations, mice that had inherited the wild-type 129 allele from the ES chimeras were used in establishing the breeding population (Mohn *et al*, 1999). Consistent with previous reports (Mohn *et al*, 1999), no gross neurological deficit was observed in the homozygous mice. The mice were housed in groups of two to six under a 12 h light–dark cycle with lights on at 0700 and had continuous access to food and water, and tested at 10–14 weeks of age. Experiments were performed on both wild-type ($+/+$) mice and homozygous mutant ($-/-$) littermates. Wild-type ($n=48$) and NR1 $-/-$ groups ($n=48$) were matched for age and gender in all experiments. Separate cohorts of mice were used for the locomotor activity and Fos induction studies described below. All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UNC Institutional Animal Care Committee.

Assessment of Amphetamine-Induced Locomotor Activity

The behavioral effects of amphetamine were assessed in photocell-based activity chambers. Activity measurements were taken under standardized environmental conditions, using a TruScan activity monitor (Coulbourn Instruments, Allentown, PA) with a $25.8 \times 25.8\,cm^2$ Plexiglas chamber and a beam spacing of 1.52 cm. Mice were acclimated to the room in which testing was carried out for at least 48 h prior to testing. Activity data were collected for wild-type ($n=24$) and NR1-deficient ($n=24$) mice over a 180-min time course, beginning when the mouse was first placed in the testing chamber. Amphetamine (2 or 4 mg/kg) or saline was injected i.p. 60 min after placing mice in the chambers. Amphetamine doses were chosen to match those used in the Fos induction studies described below. Data were collected in 5-min intervals at a sample interval of 100 ms. The distance traveled in each 5-min interval was measured as the total of all vectored X–Y coordinate changes. In addition, data were collected for fine movements indicative of stereotypic behaviors. For each group of mice, the mean $\pm$ SEM was calculated for each 5-min time interval.

Immunocytochemical Assessment of Fos-Like Immunoreactivity (Fos-LI)

Mice were transported from the animal quarters to the laboratory and habituated to the experimental environment for seven to 10 days before the experiment. Mice were singly housed in a clear plastic cage ($26 \times 16 \times 13\,cm^3$) and handled each day. On the day of the experiment, mice were injected with amphetamine (2 and 4 mg/kg) or saline i.p. and were returned to their home cages. The doses of

amphetamine were chosen based on preliminary studies showing regionally selective induction of Fos and a more robust response for the higher dose. All experiments were carried out between 1100 and 1500 to avoid the effect of circadian rhythm.

Immunocytochemical procedures were performed according to the previously published protocols (Duncan *et al*, 1993, 1996, 1998). At 2 h after amphetamine or saline injection, mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). This time point was chosen because it is near the 'peak' of Fos expression reported after amphetamine administration (Engber *et al*, 1998; Turgeon and Roche, 1999; Mead *et al*, 1999), and found in preliminary studies. Mice were perfused through the left cardiac ventricle with ice-cold 100 mM sodium phosphate-buffered saline (PBS, pH = 7.4) for 1 min, at a rate of 3 ml/min, followed by 4% paraformaldehyde in PBS for 7 min. Brains were removed immediately after perfusion and postfixed in 4% paraformaldehyde overnight. Coronal sections (50 μm) were cut from each brain using a vibratome and placed in PBS contained in 24-well tissue culture dishes. Sections were treated with 5% normal goat serum (Vector Laboratories, Burlingame, CA) and 0.1% Triton X-100 in PBS for 30 min and then were incubated for 48–72 h at 4°C with a rabbit polyclonal Fos antiserum (1:10 000 dilution) raised against a synthetic peptide corresponding to amino acids 2–17 of a conserved region of mouse and human c-*fos* gene product (Biogenesis Inc., Brentwood, NH). Since it is uncertain whether this antibody recognizes Fos-related antigens as well as Fos, results are described as Fos-LI. After incubation with the Fos antiserum, sections were processed through three rinses of PBS and incubated for 1 h with biotinylated antirabbit IgG (Vector Laboratories). After three rinses with PBS, the sections were incubated with avidin–biotin complex (Vectastain Elite ABC Kit; Vector Laboratories) for 1 h. After an additional three rinses with PBS, sections were placed in a solution containing 0.05% 3,3′-diaminobenzidene tetrahydrochloride, 0.005% cobalt chloride, 0.008% nickel ammonium sulfate, and 0.02% hydrogen peroxide.

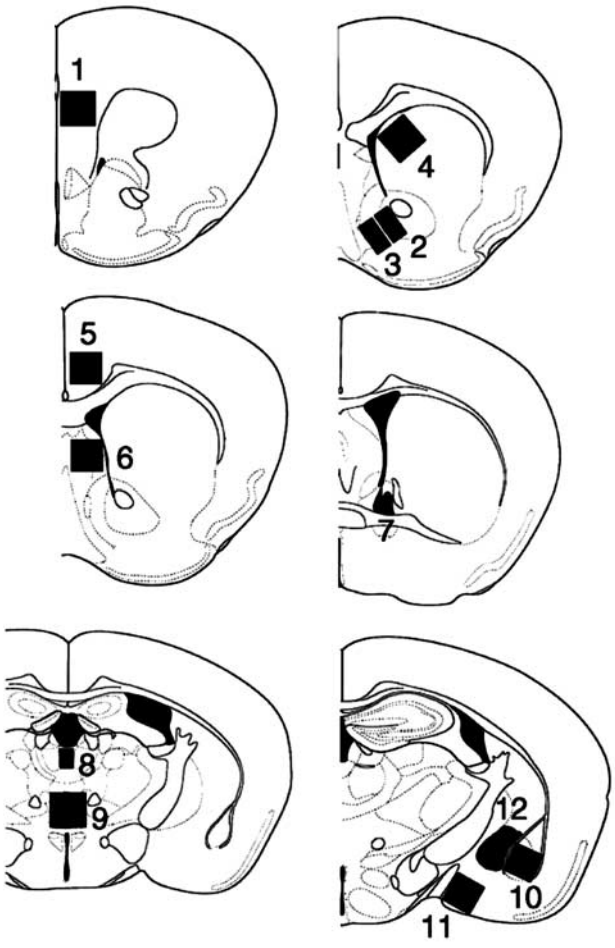

Figure 1 Diagrams of representative coronal sections used for the quantification of Fos-LI-positive neurons in the medial frontal cortex (1), core (2) and shell (3) of the nucleus accumbens, dorsomedial part of the caudate putamen (4), cingulate cortex (5), lateral septal nucleus (6), posterior lateral division of bed nucleus of stria terminalis (7), paraventricular anterior thalamic nucleus (8), midline thalamic nuclei (9), basolateral nucleus of amygdala (10), medial amygdaloid nucleus (11), and central nucleus of amygdala (12). Drawings are from Franklin and Paxinos (1997).

Quantification of Fos-LI

Cells exhibiting nuclear staining for Fos-LI in a 250 × 250 μm^2 area of the selected brain regions were counted at magnification of × 200 by an experimenter blind to the treatment group. From preliminary studies, 12 regions of interest were chosen based on consistent and robust induction of Fos in response to amphetamine. Sections were selected that contained the regions of interest from macroscopic features of the sections. The locations of the areas used within each brain region for assessing Fos-LI-positive cells were taken from Franklin and Paxinos (1997), and are shown in Figure 1. Counts were taken bilaterally in two sections per mouse for each region. For the core and shell of the nucleus accumbens and paraventricular anterior thalamic nucleus, cells were counted in a 125 × 250 μm^2 area due to the small size of the regions. For the posterior lateral division of bed nucleus of stria terminalis, nuclei of the amygdala, and midline thalamic nuclei, cells were counted in the defined nuclear areas clearly apparent from the background nonspecific staining of the sections.

Statistical Analysis

Data for activity and Fos cell counts were analyzed by repeated measures ANOVA using StatView (version 5.0.1, SAS Institute Inc.), with time and brain region as repeated measures for motoric and Fos responses, respectively. For activity, time measures included interval (5-min bins across the 3 h of testing) and hour (preinjection hour, postinjection first hour, and postinjection second hour). In addition to the overall ANOVA, separate repeated measures ANOVAs were performed for each hour, with the factors of genotype and interval. For the Fos-LI results, separate two-way ANOVAs, with the factors of genotype and amphetamine treatment, were conducted on the data for each brain region. Fisher's protected least-significant difference tests were used for comparing group means only when a significant F-value was determined. For all comparisons, significance was set at $p < 0.05$.

RESULTS

Behavioral Effects of Acute Saline or Amphetamine Administration in Wild-Type and NR1-Deficient Mice

Significant overall effects of genotype were evident in the test with an acute saline challenge for the locomotor activity ($F(1,14) = 9.607$, $p = 0.0078$) and number of fine movements ($F(1,14) = 5.345$, $p = 0.0365$). Further analyses of 1 h intervals in the activity chambers revealed that there were no significant differences between the experimental groups in the first hour of the session, before the saline injection. At the time of saline or amphetamine injection (ie, 1 h after placement of mice in the chambers), the levels of activity were similar for the wild-type and mutant mice. However, during the hour following the saline injection, the NR1-deficient mice had higher levels of locomotor activity than the wild-type animals ($F(1,14) = 4.670$, $p = 0.0485$). In the second hour post-saline injection, the NR1 −/− group maintained the increased levels of locomotion (main effect of genotype, $F(1,14) = 10.715$, $p = 0.0055$; and genotype × interval interaction, $F(11,154) = 2.383$, $p = 0.0094$) and also evidenced higher levels of fine movements ($F(1,14) = 9.248$, $p = 0.0088$).

At the 2 mg/kg dose of amphetamine, there were no significant differences between the NR1 +/+ and NR1 −/− mice in the maximal activation or in the time course of the activation for the locomotor measure (Figure 2b). In contrast, the NR1-deficient mice showed significantly higher numbers of fine movements, in comparison to the wild-type group (Figure 3b; $F(1,14) = 10.758$, $p = 0.0055$). Further analyses indicated that the differences in stereotypic activity were not evident for the preinjection hour or the first postinjection hour of the test session, but only emerged in the second hour after drug administration (main effect of genotype, $F(1,14) = 7.862$, $p = 0.0141$; and genotype × interval interaction, $F(11,154) = 2.257$, $p = 0.0142$). This pattern was similar to the increased numbers of fine movements evident in the NR1 −/− mice during the second hour after treatment with saline.

At the higher amphetamine dose (4 mg/kg; Figures 2c and 3c), an overall repeated measures ANOVA revealed significant interactions between genotype and interval for both activity measures (locomotion, $F(11,154) = 1.997$, $p = 0.0321$; and fine movements, $F(11,154) = 4.030$, $p < 0.0001$), and between genotype, hour, and interval (locomotion, $F(22,308) = 1.726$, $p = 0.0240$; and fine movement, $F(22,308) = 2.611$, $p = 0.0001$). Significant differences between the two experimental groups in the locomotor activity were only evident in the hour immediately following the administration of amphetamine, at which time the NR1 mutant animals had a slightly blunted response to the initial stimulant drug effects (genotype × interval interaction, $F(11,154) = 2.796$, $p = 0.0024$). A different pattern was observed for the fine movements measure, wherein the NR1 −/− group had higher levels during the first hour of the test session (genotype × interval interaction, $F(11,154) = 3.531$, $p = 0.0002$) and during the hour following drug administration (genotype × interval interaction, $F(11,154) = 4.135$, $p < 0.0001$). The differences between the two genotypes in both locomotion and fine movements were no longer present during the last hour of the test session.

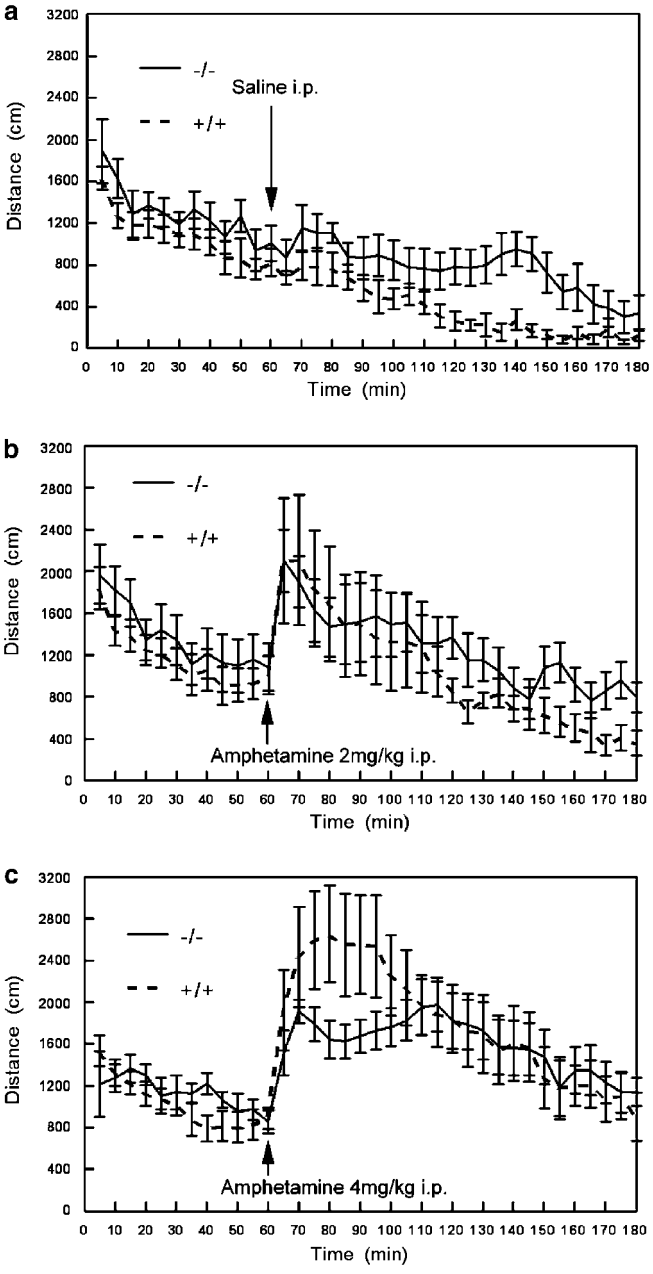

Figure 2 Locomotor activity after amphetamine administration in NR1 +/+ and NR1 −/− mice. Mice were injected with saline (a) or amphetamine (b, 2 mg/kg; c, 4 mg/kg) 60 min after placement in the activity chambers as indicated by arrows. Data are means ± SEMs.

Effects of Amphetamine on Fos-LI in Wild-Type and NR1-Deficient Mice

An overall repeated measures ANOVA revealed highly significant effects of amphetamine treatment ($F(2,42) = 24.330$, $p < 0.0001$) and brain region ($F(11,462) = 24.168$, $p < 0.0001$) on levels of Fos-LI. While genotype did not have an overall effect ($F(1,42) = 3.107$, $p = 0.0852$), there was a significant interaction between genotype and brain region ($F(11,462) = 2.669$, $p < 0.0001$), suggesting that the impact of NR1 deficiency varied across the different areas of the brain. Changes in Fos-LI following the administration of

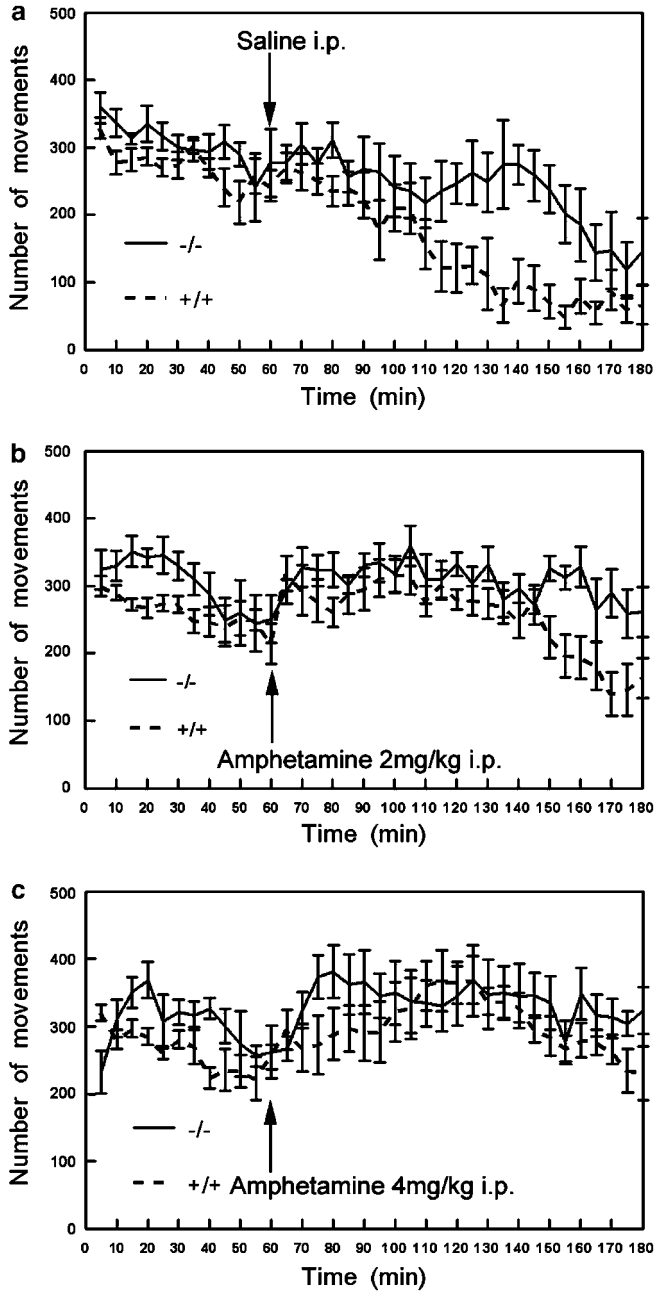

Figure 3 Fine movements after amphetamine administration in NR1 +/+ and NR1 −/− mice. Mice were injected with saline (a) or amphetamine (b, 2 mg/kg; c, 4 mg/kg) 60 min after placement in the activity chambers as indicated by arrows. Data are means ± SEMs.

amphetamine were also dependent on brain region (treatment × brain region interaction, $F(22,462) = 12.587$, $p < 0.0001$).

Repeated measures ANOVAs showed that after the saline injection, there were no significant differences between the wild-type and NR1 hypomorphic mice in Fos-LI induction (Figure 4), although levels varied with brain region ($F(11,165) = 12.517$, $p < 0.0001$). Significant effects of genotype emerged for both doses of amphetamine (2 mg/kg; significant main effect of genotype, $F(1,11) = 29.898$, $p < 0.0009$, and brain region, $F(11,77) = 20.006$, $p < 0.0001$; 4 mg/kg, significant effect of brain region, $F(11,220) = 22.372$, $p < 0.0001$, and significant genotype × brain region interaction, $F(11,220) = 3.511$, $p = 0.0002$).

These results suggested that there was a differential impact of the amphetamine treatment in the NR1-deficient mice, but this effect was only evident in some of the surveyed brain regions. Therefore, separate analyses were run for each area of the brain, looking at both genotype and treatment effects. As depicted in Table 1, amphetamine treatment led to significant increases in Fos-LI in every brain region surveyed (representative immunocytochemically stained sections are shown in Figures 5 and 6). However, the effects of amphetamine were blunted in the mutant mice, in comparison to the wild-type controls, in the medial frontal cortex (infralimic cortex), cingulate cortex, lateral septal nucleus, and basolateral nucleus of the amygdala (Figure 4; see Table 1 for F-values).

DISCUSSION

Many patients with schizophrenia exhibit enhanced sensitivity to psychostimulants (Lieberman *et al*, 1997). The present study was designed to determine if NR1 hypomorphic mice could model this behavioral phenotype-associated enhanced dopaminergic sensitivity. Acute behavioral responses to 2 mg/kg amphetamine were similar between wild-type and NR1 hypomorphic mice. At 4 mg/kg amphetamine, the peak locomotor activation induced by the stimulant was slightly lower in the NR1 hypomorphic mice and stereotypic behaviors slightly higher. The reduced peak locomotor response to amphetamine in the NR1 −/− mice is probably related to the emergence of greater stereotypic behaviors for the higher amphetamine dose. It will be important in future studies to examine the effects of lower doses of amphetamine to better assess behavioral sensitivity in the NR1 −/− mice.

The NR1 −/− mice tested in the present study did not exhibit increased activity in comparison to controls during the first hour after placement in the activity chambers. The main differences between the mutant and wild-type mice were seen during the last hour of the 3-h testing period. Different cohorts of the NR1 −/− mice we have examined show variations in the patterns of locomotor activity, with some exhibiting increased activity relative to controls at early times after placement in the activity chamber, and others showing differences only at later times. The most consistent effect of the NR1 hypomorphic mutation on locomotor activity in different cohorts of mice is reduced habituation. We have seen this pattern of reduced habituation in many groups of mice that did not receive saline injections during the behavioral testing.

Amphetamine-induced Fos in most subcortical structures was similar for the wild-type and mutant animals at both doses examined. However, the NR1-deficient mice exhibited attenuated Fos induction in response to amphetamine in the medial frontal and cingulate cortices, basolateral amygdala, and in the lateral septum. These data clearly show that NR1 hypomorphic mice do not provide a model for enhanced sensitivity to amphetamine with regard to the specific parameters assessed. Accordingly, the mice do not model

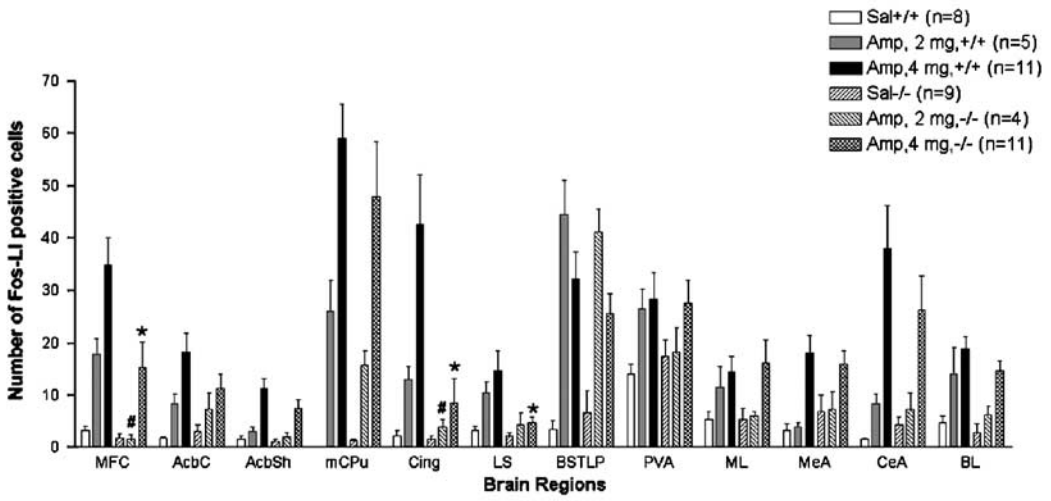

Figure 4 Effects of amphetamine on Fos induction in wild-type (+/+) and NR1-deficient (−/−) mice. Data are means ± SEMs. $^{\#}p<0.05$ compared to 2 mg/kg of amphetamine in wild-type (+/+) mice. $^{*}p<0.05$ compared to 4 mg/kg of amphetamine in wild-type (+/+) mice. Abbreviations: MFC, medial frontal cortex; AcbC, core of the nucleus accumbens; AcbSh, shell of the nucleus accumbens; mCPu, dorsomedial part of the caudate putamen; Cing, cingulate cortex; LS, lateral septal nucleus; BSTLP, posterior lateral division of bed nucleus of stria terminalis; PVA, paraventricular anterior thalamic nucleus; ML, midline thalamic nuclei; MeA, medial amygdaloid nucleus; CeA, central nucleus of amygdala; BL, basolateral nucleus of amygdala.

Table 1 F-Values for the Statistical Analysis of Genotype and Amphetamine Effects on Fos Expression in Different Regions of Brain

Brain region	Genotype, F(1,42)	Amphetamine treatment, F(2,42)	Interaction, F(2,42)
MFC	11.824, $p=0.0013$*	19.002, $p<0.0001$*	3.074, $p=0.0568$
AcbC	0.828, $p=0.3679$	11.851, $p<0.0001$*	1.383, $p=0.2620$
AcbSh	1.465, $p=0.2330$	17.861, $p<0.0001$*	0.765, $p=0.4719$
mCPu	1.149, $p=0.2899$	32.769, $p<0.0001$*	0.478, $p=0.6232$
Cing	7.506, $p=0.0090$*	9.692, $p=0.0003$*	4.833, $p=0.0129$*
LS	6.907, $p=0.0119$*	4.708, $p=0.0143$*	2.009, $p=0.1468$
BSTLP	0.323, $p=0.5729$	28.902, $p<0.0001$*	0.658, $p=0.5230$
PVA	0.264, $p=0.6099$	4.757, $p=0.0137$*	0.633, $p=0.5361$
ML	0.161, $p=0.6904$	5.091, $p=0.0105$*	0.422, $p=0.6459$
MeA	0.411, $p=0.5248$	12.127, $p<0.0001$*	0.687, $p=0.5087$
CeA	0.380, $p=0.5410$	15.316, $p<0.0001$*	0.888, $p=0.4190$
BL	5.368, $p=0.0255$*	18.909, $p<0.0001$*	0.624, $p=0.5408$

$^{*}p<0.05$. For abbreviations, see legend to Figure 4.

the enhanced dopaminergic sensitivity observed in a substantial portion of patients with schizophrenia.

It is unclear as to why a reduction in cortical Fos induction in some brain regions was observed in the NR1 −/− mice, with minimal changes in the behavioral responses to amphetamine, especially for the 2 mg/kg dose of the drug. The data suggest that amphetamine-induced activation of Fos-related mechanisms in the medial frontal and cingulate cortices, and in the lateral septum and basolateral amygdala, can be dissociated from the locomotor activity.

It was surprising that amphetamine-induced Fos was differentially effected in the cortex and striatum of the NR1 hypomorphic mice, since equivalent reduction in ^{3}H-MK-801 binding was found in striatal and cortical regions (Duncan *et al*, 2002). Disruption of NMDA-R function by acute challenge with MK-801 is consistently reported to decrease the effects of amphetamine on striatal *c-fos*, Fos, and zif/268 expression, suggesting possible involvement of NMDA-R in the effects of amphetamine in this region (Snyder-Keller, 1991; Ohno *et al*, 1994; Wang *et al*, 1994; Konradi *et al*, 1996). It is possible that compensatory alterations in dopamine–glutamate interactions occur in the striatum of NR1 hypomorphic mice that make the Fos response to dopaminergic activation less dependent on NMDA receptor activation.

Assessment of the effects of NMDA antagonists on amphetamine-induced Fos induction in the medial frontal and cingulate cortices has been complicated by the fact that NMDA antagonists themselves increase Fos in these regions (Dragunow and Faull, 1990; Duncan *et al*, 1998). The present findings suggest a role of NMDA receptors in

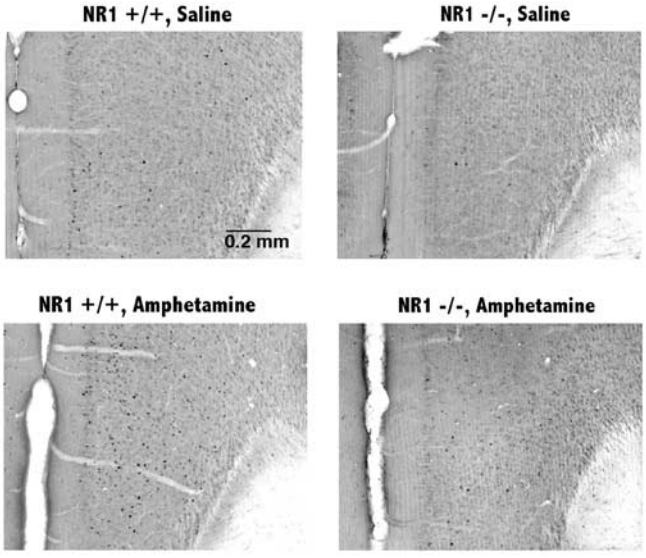

Figure 5 NR1 (−/−) mice display attenuated Fos-LI expression induced by amphetamine in the medial frontal cortex. Representative immunocytochemical staining for Fos-LI in the medial frontal cortex after treatment with saline or 4 mg/kg of amphetamine in both wild-type (+/+) and NR1 (−/−) mice.

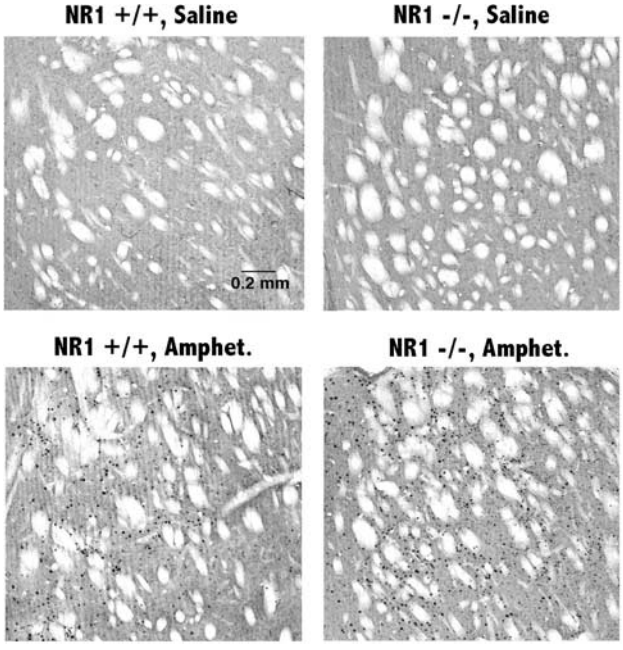

Figure 6 NR1 (−/−) mice display equivalent Fos-LI expression induced by amphetamine in the striatum. Representative immunocytochemical staining for Fos-LI in the dorsomedial part of the caudate putamen after treatment with saline or 4 mg/kg of amphetamine in both wild-type (+/+) and NR1 (−/−) mice. Abbreviation: Amphet.: amphetamine.

amphetamine-induced Fos in the medial frontal and cingulate cortices. The differential effects observed in the cortex and striatum in the NR1 hypomorphic mice after amphetamine suggest that cortical and striatal NMDA–dopamine receptor interactions are affected differently in cortical and striatal regions of the mutant mice.

Amphetamine is well known to induce robustly dopamine release in the striatum (Snyder *et al*, 1972; Zetterstrom *et al*, 1983; Carboni *et al*, 1989; Kuczenski and Segal, 1989). The striatal dopaminergic system in NR1 hypomorphic mice, including basal, unstimulated concentration of extracellular dopamine, the tissue contents of dopamine, and its metabolites, appears to be fairly intact (Mohn *et al*, 1999). In contrast to the apparent lack of effect of the NR1 hypomorphic mutation on striatal dopamine release, chronic treatment with PCP enhanced amphetamine-induced dopamine release in the striatum and medial frontal cortex (Balla *et al*, 2001; Balla *et al*, 2003). It will be of interest to assess dopamine release in the NR1-deficient mice in response to amphetamine in future studies.

It is possible that compensatory changes in non-NMDA glutamate receptors influence the action of the psychostimulant in our mice. However, in the NR1-deficient mice, no compensatory changes in densities of non-NMDA ionotropic glutamate receptors were found (Duncan *et al*, 2002). Future studies measuring the densities and function of dopamine and other glutamate receptors are required to determine potential adaptive neurochemical mechanisms of the mutant mice.

In addition to dopamine, amphetamine also induces the release of norepinepherine and serotonin (Munoz *et al*, 2003; Shoblock *et al*, 2004). Further study will be required to determine the specific neurotransmitter mechanisms responsible for the observed deficits in amphetamine-induced Fos induction in the NR1 −/− mice.

Previous studies reporting the interaction between amphetamine and NMDA-R antagonists in terms of IEG gene induction and behavioral responses yielded somewhat disparate results. For example, amphetamine-induced behavioral responses, including hyperactivity and stereotypies, can be either enhanced (Balla *et al*, 2003; Turgeon and Roche, 1999), inhibited (Greenberg and Segal, 1985), or unchanged (Wang *et al*, 1994) by NMDA-R antagonists such as PCP in rodents. The different effects observed probably relate to different doses, patterns of exposure, and the time interval examined after the administration of the NMDA antagonists. As mentioned earlier, in the striatum, MK-801 has inhibitory effects on amphetamine-induced Fos (Snyder-Keller, 1991; Ohno *et al*, 1994; Wang *et al*, 1994). PCP has a delayed effect of enhancing amphetamine-induced Fos when rats are tested the day after acute or subchronic (4 days) treatment with the NMDA antagonists (Turgeon and Roche, 1999; Turgeon and Case, 2001). The different effects of NMDA-R antagonists on behaviors and IEG induction thus appear to be dependent on the specific paradigms used (Turgeon and Case, 2001).

Although the present results indicate that the NR1 hypomorphic mice do not model enhanced dopaminergic sensitivity, the mice may still be a relevant model for certain phenotypes of schizophrenia. It should be noted that enhanced sensitivity to dopaminergic stimulants is observed in only 40–60% of patients with schizophrenia (Lieberman *et al*, 1987). These findings suggest that a substantial portion of patients do not exhibit a phenotype consistent with dopamine supersensitivity. We recently demonstrated that the NR1 hypomorphic mice exhibit deficits in prepulse inhibition of acoustic startle and showed marked deficits in two different paradigms of species typical

social interactions (Duncan *et al*, 2004). Such data, and the originally observed behaviors of the mice by Mohn *et al* (1999), are consistent with phenotypes observed in schizophrenia patients. The mutant mice may thus provide a useful model to explore neurobiological mechanisms related to certain behavioral phenotypes associated with reduced NMDA receptor function in the context of the NMDA receptor hypofunction of schizophrenia.

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