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Research Report

Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding

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ABSTRACT

Epidemiological evidence indicates that prenatal nutritional deprivation may increase the risk of schizophrenia. The goal of these studies was to use an animal model to examine the effects of prenatal protein deprivation on behaviors and receptor binding with relevance to schizophrenia. We report that prenatally protein deprived (PD) female rats showed an increased stereotypic response to apomorphine and an increased locomotor response to amphetamine in adulthood. These differences were not observed during puberty. No changes in haloperidol-induced catalepsy or MK-801-induced locomotion were seen following PD. In addition, PD female rats showed increased ³H-MK-801 binding in the striatum and hippocampus, but not in the cortex. PD female rats also showed increased ³H-haloperidol binding and decreased dopamine transporter binding in striatum. No statistically significant changes in behavior or receptor binding were found in PD males with the exception of increased ³H-MK-801 binding in cortex. This animal model may be useful to explore the mechanisms by which prenatal nutritional deficiency enhances risk for schizophrenia in humans and may also have implications for developmental processes leading to differential sensitivity to drugs of abuse.

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1. Introduction

Considerable evidence suggests that schizophrenia may be a neurodevelopmental disorder in which early abnormalities in

brain development result in brain malfunction and symptomatology that emerges in early adulthood (Ursula et al., 2006; St Clair et al., 2005; Arnold 1999; Pilowsky et al., 1993; Weinberger 1996). Prenatal nutritional deficiency has been

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hypothesized to be a risk factor for schizophrenia since the 1950s (Pasamanick et al., 1956). A series of studies of the Dutch Hunger Winter showed that birth cohorts exposed to famine during early gestation had a two-fold increase in risk of schizophrenia in male and female offspring (Susser et al., 1998; Hoek et al., 1998; Susser et al., 1996; Susser and Lin, 1992). These findings have been replicated in a much larger cohort in China (St. Clair et al., 2005). Further, there was an increase in schizoid personality disorder when male offspring from the Dutch Hunger Winter were studied (Hoek et al., 1996). A recent seroepidemiologic study from a large birth cohort in northern California revealed an association between elevated maternal homocysteine, a marker of folic acid deficiency, and schizophrenia (Brown et al., 2007). Other epidemiologic and laboratory studies of schizophrenia have found evidence of other potential pre- and perinatal etiologies, including prenatal exposure to infections including influenza toxoplasmosis and genital/reproductive infection (Romero et al., 2008; Smith et al., 2007; Brown, 2006) and obstetric complications (Hultman et al., 1999; McNeil et al., 2000a). In addition to epidemiological studies, neuroimaging studies have found that ventriculomegaly and decreased hippocampal volume in schizophrenia are related to obstetric complications (McNeil et al., 2000b) and neuropathology studies have found cytoarchitectonic abnormalities in temporal lobe and prefrontal cortex in post-mortem brains of patients with schizophrenia indicative of prenatal damage and possibly altered neuronal migration (Akbarian et al., 1996, 1993).

Previous studies show parallels between long-term effects of prenatal protein deprivation in animals and clinical studies of schizophrenia. For instance, clinical studies as well as animal studies show altered dopamine neurochemistry

(Almeida et al., 1996a; Butler et al., 1999; Chen et al., 1995, 1997; Toda and Abi-Dargham, 2007), changes in morphology of the hippocampal formation, including dendritic abnormalities (Cintra et al., 1997; Diaz-Cintra et al., 1991, 1994; Rosoklija et al., 2000), deficits in learning and memory (Tonkiss and Galler 1990; Tonkiss et al., 1990, 1991; Goldman-Rakic 1994; Barch and Smith 2008; Ranganath et al., 2008) and changes in anxiety-like behavior and physiological parameters (Watkins et al., 2008). The few studies to have examined the effects of prenatal protein deprivation on dopamine and glutamate mediated behaviors have shown increased sensitivity to dopamine agonists (Brioni et al., 1986; Shultz et al., 1999) and NMDA antagonists (Tonkiss et al., 1998). However, these studies examined behaviors only in adulthood. In our previous study, we identified deficits in pre-pulse inhibition and accompanying changes in NMDA receptor binding following prenatal protein deprivation in female, but not male rats (Palmer et al., 2004); these changes were present at postnatal day 56 (PDN 56; young adult) but not PND 35 (puberty).

Another example of a developmental model examining long-term effects of a perinatal lesion is the work of Lipska and colleagues (Al-Amin et al., 2000; Lipska et al., 1993, 1995; Lipska and Weinberger 1993; Tseng et al., 2007). These investigators and others found that ventral hippocampal lesions in seven day old rat pups increased dopamine- and glutamate-mediated behaviors (Al-Amin et al., 2000; Lipska et al., 1993; Lipska and Weinberger 1993), produced deficits in learning and memory (Le Pen et al., 2000) and produced alterations in prepulse inhibition of startle (Le Pen et al., 2000; Lipska et al., 1995) that emerged in post- rather than pre-pubertal rats. However, some cognitive impairment and deficits in social behavior occurred pre- as well as post-pubertally (Chambers et al., 1996; Sams-Dodd et al.,

Table 1 – Offspring born, number surviving, and distribution for testing

Group	# Dams mated	# Gave birth	Mean # offspring	# Litters cross-fostered	Culled to	# Survived	# Tested for behavior
Protein deprived							
Group 1	10	7 (70%)	13.4	6	26M 22F	19M 68% 13F 59%	11M 7F 1–3 of each sex from 4 litters
Group 2	10	9 (90%)	13.7	6	24M 24F	19M 79% 20F 83%	10M 10F 1–2 of each sex from 6 litters
Pd total	20	16 (80%)	13.6	12	50M 46F	38M 76% 33F 72%	21M 17F 1–3 of each sex from 10 litters
Non-deprived							
Group 1	20	13 (65%)	14.2	6	24M 24F	24M 100% 24F 100%	15M 17F M: 3/litter from 5 litters F: 2–3/litter from 6 litters
Group 2	20	12 (60%)	15.3	6	24M 24F	24M 100% 24F 100%	10M 10F 1–2 of each sex from 6 litters
Nd total	40	25 (63%)	14.7	12	48M 48F	48M 100% 48F 100%	25M 27F M:1–3/litter from 11 litters F:1–3/litter from 12 litters

All litters were culled to 4 males and 4 females with the exception of one litter in PD Group 1 which was culled to 6 males and 2 females as it only included 2 females.

1997). Ventral hippocampal lesions have also been found to alter dopamine and glutamate receptor binding and to alter pre-frontal dopaminergic and glutamatergic systems post- rather than pre-pubertally (Schroeder et al., 1999; Tseng et al., 2007). Along a similar line, Moore et al. (2006) found that treatment at E17 with methylazoxymethanol altered brain development and later life behaviors that may have relevance for schizophrenia.

In order to further evaluate the behavioral and neurochemical alterations caused by prenatal protein deprivation, we designed these studies to examine changes that may be relevant to schizophrenia. Dopamine-mediated stereotypy, locomotion, and catalepsy were examined before and after puberty because of dopamine involvement in schizophrenia and post-pubertal onset of schizophrenia (Abi-Dargham et al., 2000; Carlsson and Lindqvist 1963; Laruelle and Abi-Dargham 1999; Stone et al., 2007). Glutamate-mediated locomotion was also examined pre- and post-puberty because of glutamate involvement in schizophrenia (Javitt and Zukin 1991; Olney et al., 1999; Stone et al., 2007; Javitt et al., 2008). Dopamine and NMDA receptor function were examined because of evidence of alterations in these neurotransmitter systems in schizophrenia (Stone et al., 2007; Abi-Dargham 2004; Javitt and Zukin 1991; Laruelle and Abi-Dargham 1999; Meador-Woodruff and Healy 2000; Olney et al., 1999; Coyle and Tsai 2004).

2. Results

2.1. Litter size, composition and body weight

Table 1 summarizes the number of offspring born, number surviving, and related parameters. Rats were tested before (PND 34–42) and after (PND 56–64) puberty. Rats in Group 1 received apomorphine, amphetamine, and haloperidol; while rats in Group 2 received apomorphine, amphetamine, and MK-801 (Table 2). Each rat in each group received all conditions at each age. PD males and females for Groups 1 and 2 were drawn from a total of ten litters while ND males and females for Groups 1 and 2 were drawn from twelve litters (Table 1).

PD female rats had significantly decreased body weights compared to ND females at most time points (Table 3). For

Table 3 – Body weight of rats as a function of age and comparisons of male and female body weight between ND and PD groups

	Female PD		Female ND		T	df	P
	N=17		N=27				
PND 1	5.4±0.2	6.4±0.1	4.2	42	<0.001		
PND 3	7.4±0.5	8.8±0.2 (n=25)	2.9	40	0.014		
PND 34	132.9±3.8 (n=14)	145.7±2.8	2.7	39	0.01		
PND 37–39	154.0±4.1	163.5±2.8	1.9	42	0.06		
PND 40–42	174.9±4.7	183.8±3.1	1.6	42	0.11		
PND 56	234.8±8.9	256.3±5.0	2.3	42	0.03		
PND 59–61	249.9±9.4	277.5±4.7 (n=26)	2.9	41	0.006		
PND 63–64	261.6±9.8	288.7±5.4 (n=26)	2.6	41	0.01		
	Male PD		Male ND				
	N=21		N=25				
PND 1	5.9±0.2	6.8±0.1	4.3	44	<0.001		
PND 3	8.1±0.3	9.3±0.2 (n=24)	3.1	43	0.003		
PND 34	158.8±5.5	162.7±2.5	0.6	44	0.5		
PND 37–39	184.8±5.5	192.2±2.6	1.2	44	0.2		
PND 40–42	218.1±6.4	225.0±3.6	1.0	44	0.4		
PND 56	367.3±9.7	378.1±5.3	1.0	44	0.3		
PND 59–61	396.2±11.7	409.2±6.1	1.0	44	0.3		
PND 63–64	429.0±13.1	436.1±7.4	0.5	44	0.6		

A range for the postnatal day (PND) is given for some days because rats were weighed and tested over that age range. Where some data are missing the number of subjects at that time point is indicated in parentheses.

male rats, the difference between PD and ND rats was only significant at the earliest time points; nevertheless, the mean body weight of PD male rats showed a non-significant trend towards being lower than ND males at all time points (Table 3).

2.2. Apomorphine-induced stereotypy

At PND 34, apomorphine-induced stereotypy of PD and ND female rats did not differ significantly (Fig. 1A). However, at PND 56, PD female rats showed a significant increase in stereotypy compared to ND females ($F_{[1,39]}=7.20, p=0.011$), with differences at almost every time point after injection (Fig. 1B). PD males did

Table 2 – Measures used

	Pre-pubertal			Post-pubertal			
	PND 34	PND 37–39	PND 40–42	PND 56	PND 59–61	PND 62–64	PND 80
Group 1	apomorphine-stereotypy ¹	novel ² , and amphetamine ¹ -locomotion	haloperidol ¹ -catalepsy	apomorphine ¹ -stereotypy	novel ² - and amphetamine ¹ -locomotion	haloperidol ¹ -catalepsy	³ H-Haloperidol ⁴ , ³ H-MK-801 ⁵ , ³ H-GBR-12935 ⁶ binding
Group 2	apomorphine-stereotypy ¹	novel ² - and amphetamine ¹ -locomotion	novel ² , and MK-801 ³ -locomotion	apomorphine ¹ -stereotypy	novel ² - and amphetamine ¹ -locomotion	novel ² and MK-801 ³ -locomotion	

¹Dopamine-mediated behaviors.

²Behavioral alterations to stress.

³Behaviors mediated by the NMDA.

⁴Dopamine binding.

⁵Binding to the NMDA glutamate receptor.

⁶Binding to the dopamine transporter.

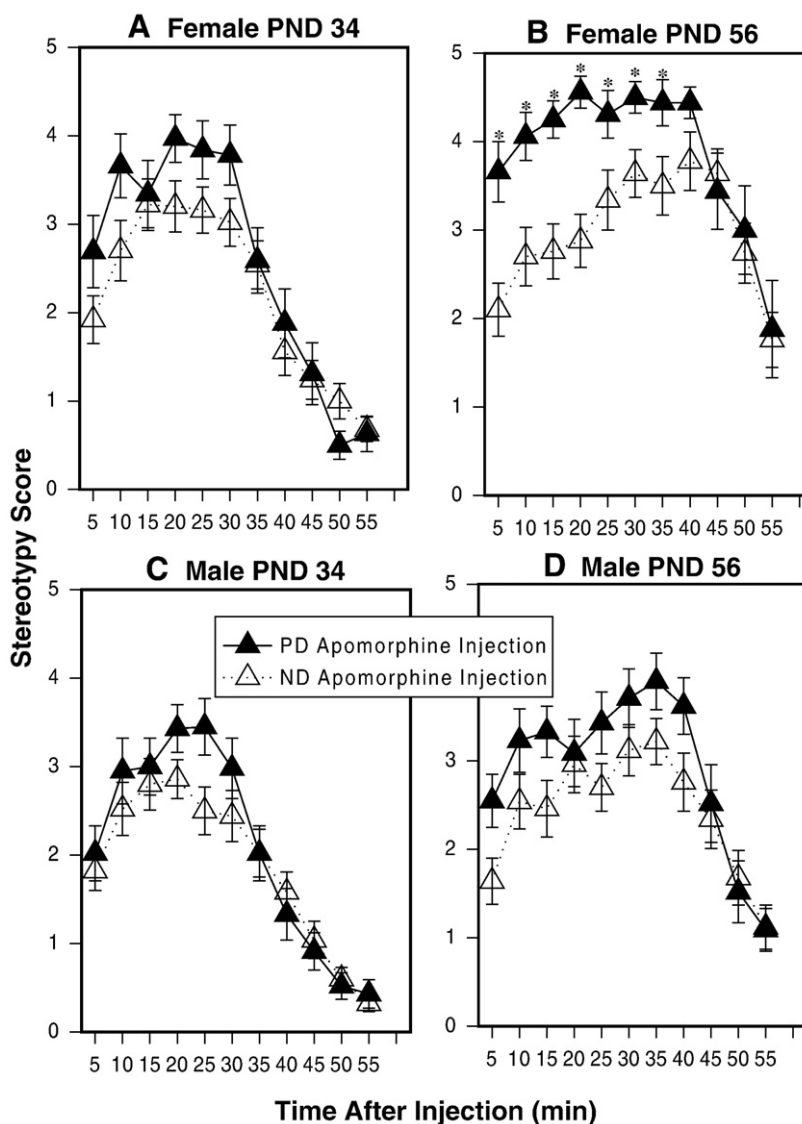


Fig. 1 – Apomorphine-induced (0.75 mg/kg) stereotypy in females at (A) PND 34 and at (B) PND 56 and in males at (C) PND 34 and at (D) PND 56. Values are mean \pm sem. * $p < 0.05$ (post hoc t tests). PD females ($n = 16$); ND females ($n = 25$); PD males ($n = 21$); ND males ($n = 25$).

not show significant differences from ND males on apomorphine-induced stereotypy at either PND 34 or 56 (Figs. 1C and D). Body weight of PD females was not correlated to total stereotypy score for either test age; however, for ND females there was a significant correlation between body weight and total stereotypy score at PND 35 ($r = -0.48$, $n = 27$, $p = 0.012$), but no significant correlation at PND 56. Body weight of PD males was not significantly correlated with total stereotypy score for either test age; however, weight of ND males was significantly correlated with the total stereotypy score at PND 56 ($r = -0.46$, $n = 25$, $p = 0.02$), but not at PND 35.

2.3. Amphetamine-induced locomotion

At PND 37–39, the ambulatory locomotor activity of PD and ND female rats did not differ significantly following exposure to a novel environment or D-amphetamine injection (Fig. 2A).

However, at PND 59–61, while the PD and ND females did not differ significantly after exposure to a novel environment, PD female rats showed a significant increase in D-amphetamine-induced locomotion compared to ND rats (main effect for diet $F_{[1,36]} = 5.47$, $p = 0.025$), with differences at several time points after injection (Fig. 2B). PD males did not differ from ND males on ambulatory activity following exposure to a novel environment or D-amphetamine injection at either age (Figs. 2C and D).

Body weight of PD female rats was not significantly correlated to total novel or amphetamine induced locomotion at either age. However, body weight of ND female rats was significantly related to total amphetamine locomotion at PND 59–61 ($r = -0.46$, $n = 21$, $p = 0.04$) but was not related to amphetamine locomotion at PND 37–39 or to novel locomotion at either age. Decrease in amphetamine locomotion thus does not appear to be related to decreased weight since there was no correlation between weight and amphetamine locomotion in the PD deprived group.

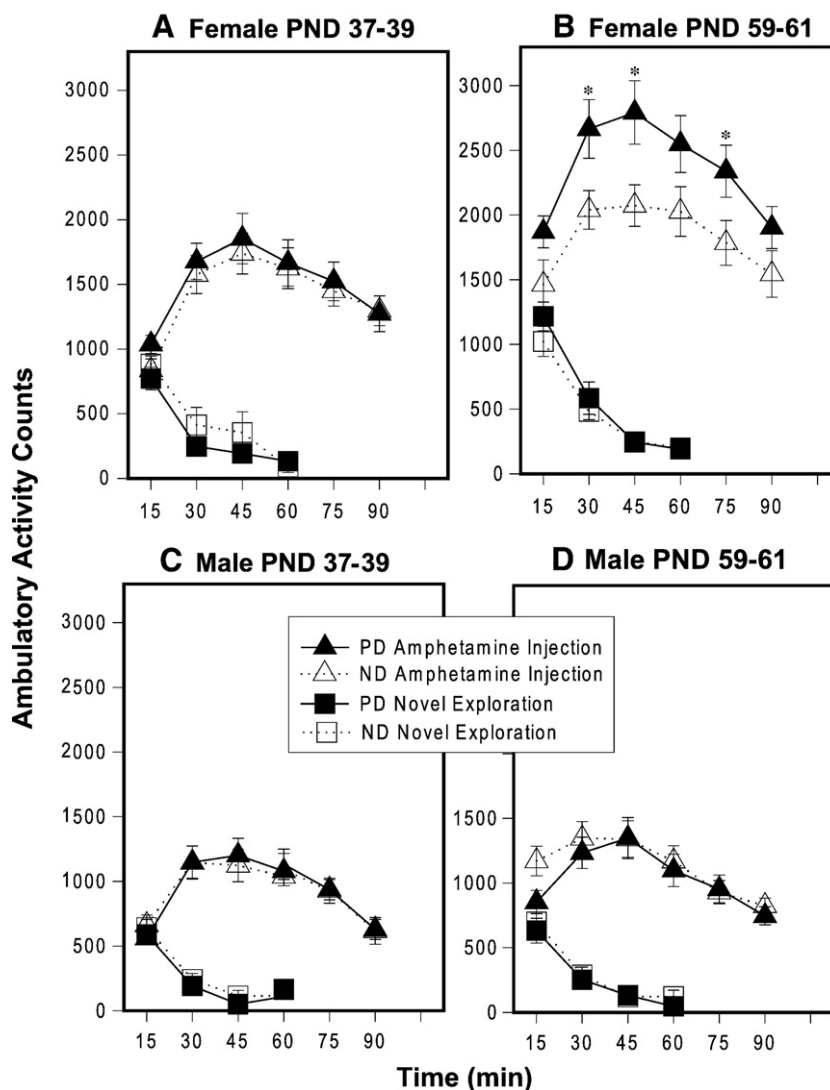


Fig. 2 – Novel and Amphetamine-induced (1.5 mg/kg) ambulatory activity measured in photocell cages in females at (A) PND 37–39 and at (B) PND 59–61 and in males at (C) PND 37–39 and at (D) PND 59–61. Values are mean \pm sem. * $p < 0.05$ (post hoc t-tests). PD females ($n = 16$); ND females ($n = 22$); PD males ($n = 18$); ND males ($n = 22$).

Body weight of PD male rats was significantly correlated with novel ($r = -0.51$, $n = 18$, $p = 0.03$) and amphetamine locomotion ($r = -0.48$, $n = 18$, $p = 0.04$) at PND 59–61 but not at PND 37–39. Body weight of ND male rats was significantly correlated with amphetamine locomotion ($r = 0.45$, $n = 23$, $p = 0.03$) at PND 37–39 but no other significant correlations were seen with this group and novel or amphetamine locomotion at either age.

2.4. Haloperidol-induced catalepsy

PD females and males did not differ from their ND counterparts on time spent in a cataleptic position following injection of haloperidol at either age (Figs. 3A–D).

2.5. MK-801-induced locomotion

PD females and males did not differ from their ND counterparts on ambulatory activity following exposure to a novel environment or MK-801 injection at either age (Figs. 4A–D).

2.6. Receptor binding

Table 4 shows the results of receptor binding studies. PD females showed significant increases in ^3H -MK-801 binding in striatum (35%; $t_{(10)} = 5$, $p = 0.001$), and hippocampus (67%; $t_{(11)} = 12.04$, $p < 0.001$), but not cortex compared to ND rats. In addition, PD females showed a significant increase in striatal ^3H -haloperidol binding (38%; $t_{(11)} = 2.30$, $p = 0.04$) and a significant decrease in striatal ^3H -GBR-12935 binding (33%; $t_{(11)} = 5.88$, $p < 0.001$) compared to ND females. ^3H -GBR-12935 binds to dopamine transporter sites. In contrast, PD males showed a significant increase in ^3H -MK-801 binding in cortex (22%; $t_{(11)} = 4.59$, $p = 0.001$) compared to ND rats, but showed no other changes in receptor binding.

3. Discussion

The major findings of this study were that prenatally PD female rats showed an increased stereotypic response to

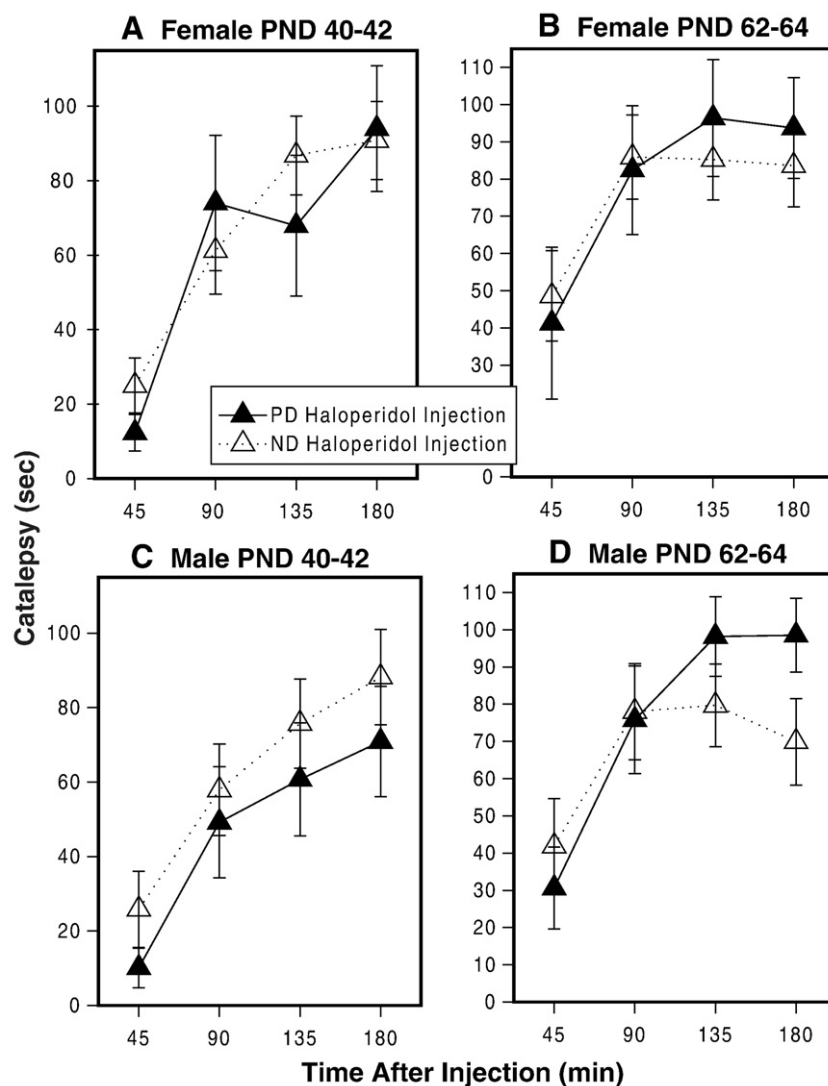


Fig. 3 – Haloperidol-induced (1 mg/kg) catalepsy in females at (A) PND 40–42 and at (B) PND 62–64 and in males at (C) PND 40–42 and at (D) PND 62–64. Values are mean \pm sem. PD females ($n=7$); ND females ($n=17$); PD males ($n=11$); ND males ($n=15$).

apomorphine and an increased locomotor response to amphetamine which reached significance only in adulthood, and not during puberty. In addition, increased ^3H -MK-801 binding was found in the striatum and hippocampus, but not cortex, of PD female rats and increased ^3H -haloperidol binding and decreased dopamine transporter (i.e., ^3H -GBR-12935) binding were found in striatum of PD female rats. PD females did not differ from ND females in MK-801-induced locomotion or haloperidol-induced catalepsy. No statistically significant behavioral or receptor binding changes were found in males with the exception of increased ^3H -MK-801 binding in cortex.

These results extend those from our earlier study which showed that PD female rats developed deficits in prepulse inhibition at PND 56 that were not observed at PND 35. The present results add apomorphine induced stereotypy and amphetamine induced locomotor stimulation to the list of adult-onset changes observed following prenatal protein deprivation. Previous studies have examined the effects of early protein deprivation on dopamine mediated behaviors and have examined these behaviors only in adulthood (Brioni

et al., 1986; Leahy et al., 1978; Shultz et al., 1999). Brioni et al. (1986) found an increase in the locomotor stimulant response to repeated injections of amphetamine in PD male rats tested following pre and postnatal PD with a period of nutritional rehabilitation prior to testing. Similarly, Shultz et al. (1999) found increased sensitization to the stereotypic effects of repeated injections of cocaine in PD male and female rats tested following prenatal PD with nutritional rehabilitation until testing. In contrast, Leahy et al. (1978) found no differences between PD and ND rats on amphetamine-mediated locomotion but a decrease in apomorphine-mediated stereotypy in PD male rats following a single injection of drug when protein deprivation began prenatally and was continued until the time of testing. As stated above, our effects were found only in females and we used only a single injection of each substance at each age. Based on the studies of Brioni et al. (1986) and Shultz et al. (1999), it may be that in males, behavioral sensitivity to dopaminergic agents requires repeated injections and/or a period of nutritional rehabilitation.

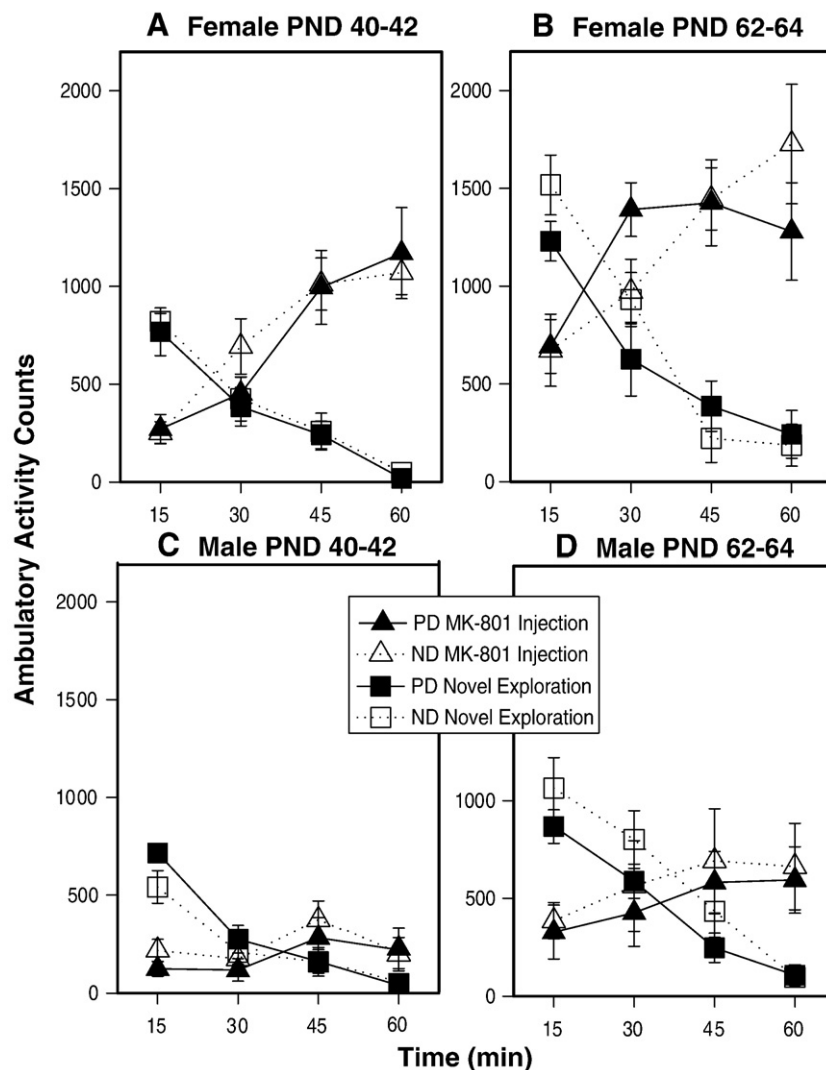


Fig. 4 – Novel and MK-801-induced (0.1 mg/kg) ambulatory activity measured in photocell cages in females at (A) PND 40–42 and at (B) PND 62–64 and males at (C) PND 40–42 and (D) PND 62–64. Values are mean \pm sem. PD females ($n=10$); ND females ($n=9$); PD males ($n=8$); ND males ($n=9$).

Unlike amphetamine and apomorphine, haloperidol did not alter behavior in PD rats. In their developmental model of schizophrenia, Lipska and Weinberger (1993) found decreased haloperidol catalepsy in adult rats receiving neonatal ventral hippocampal lesions, perhaps due to increased dopaminergic tone. Similarly, we hypothesized that we would observe a decrease in haloperidol catalepsy in prenatally PD rats in adulthood. The lack of a decrease in haloperidol catalepsy in our study may be related to our finding of increased ^3H -haloperidol binding in striatum of PD female rats. This difference might counteract the expected effect by increasing haloperidol-induced catalepsy.

The increase in ^3H -haloperidol binding in the striatum of PD females (Table 4) may be functionally related to the observed increases in dopamine mediated behaviors in adult PD females; however in our previous study of prenatal protein deprivation, we observed significant differences in prepulse inhibition in PD females at PND 56 (but not at PND 35), but no

differences in haloperidol binding in the striatum in either male or females rats (Palmer et al., 2004), suggesting that certain PD-induced behavioral differences can be dissociated from differential dopaminergic binding in the striatum. That study used Wistar Kyoto rats, whereas the present study uses Sprague Dawley rats, so the discordant binding results could be due to genetic differences in the response to protein deprivation. Haloperidol is predominantly a D_2 antagonist, but also binds to D_3 and D_4 receptor subtypes as well as to serotonin and noradrenergic receptors. Brioni et al. (1986) reported increased dopamine receptors in PD male rats who had received repeated amphetamine injection in adulthood following pre- and postnatal PD. While brain dopamine and metabolite levels have not been reported to be altered in striatum of PD rats (Chen et al., 1997), our finding of increased ^3H -haloperidol binding in striatum of PD female rats may be related to decreased tyrosine in this brain region following prenatal protein deprivation (Chen et al., 1997).

Table 4 – Receptor and transporter binding following prenatal protein deprivation

	Females		Males	
	ND (n)	PD(n)	ND (n)	PD (n)
³ H-MK-801				
Striatum	132.3±7.1 (7)	178.9±4.6 (5)**	169.5±5.8 (7)	157.0±6.1 (5)
Hippocampus	250.9±7.9 (8)	419.4±12.5 (5)**	49.9±1.2 (8)	49.1±1.9 (5)
Cortex	399.0±10.7 (8)	380.9±8.2 (5)	134.5±4.0 (8)	164.2±5.1 (5)**
³ H-Haloperidol				
Striatum	759.0±72.6 (8)	1048.7±109.8 (5)*	522.9±25.8 (7)	552.9±17.1 (5)
³ H-GBR12935				
Striatum	2675.9±110.5 (8)	1793.6±62.8 (5)**	460.9±111.4 (6)	761.3±53.2 (5)

Results are expressed as pmol of ³H-radioligand bound/mg protein. **p* < 0.05; ***p* ≤ 0.001.

Our findings of increased dopamine-mediated behaviors as well as our finding of increased striatal ³H-haloperidol binding in PD female rats are relevant to long-standing theories of dopamine dysfunction in schizophrenia (Stone et al., 2007; Carlsson and Lindqvist 1963; Laruelle and Abi-Dargham 1999; Abi-Dargham 2004; Seeman and Lee 1975). Increases in striatal D₂ receptor binding have been reported in post-mortem brains from schizophrenia patients (Joyce et al., 1988; Seeman et al., 1987) and imaging studies have provided further evidence that differences in dopamine dynamics play an important role in the pathophysiology of schizophrenia; nevertheless multiple neurotransmitter systems are clearly important for schizophrenia (Abi-Dargham 2004; Toda and Abi-Dargham, 2007; Stone et al., 2007).

The increase in binding of the NMDA antagonist ³H-MK-801 in striatum and hippocampus of PD female rats in our study suggests that there may be decreased glutamatergic activity, leading to a compensatory increase in binding. In our previous study of PD rats we observed similar differences in ³H-MK-801 in the striatum but not in the hippocampus (Palmer et al., 2004). This observation may be related to theories of NMDA receptor hypofunction in schizophrenia (Stone et al., 2007; Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle and Tsai, 2004).

In light of our finding of increased ³H-MK-801 binding in PD female rats, it was surprising that we did not find increased MK-801-induced locomotion in these animals. Tonkiss and colleagues (Tonkiss et al., 1998), using the same diet and protein deprivation regimen, found increased sensitivity to MK-801 in a differential reinforcement of low rates task in prenatally PD female, but not male, rats in adulthood. Very low doses of MK-801 were needed to disrupt learning in that study (e.g., 0.032 mg/kg) whereas higher doses are needed to increase locomotion (e.g., 0.1 mg/kg used in our study; (Fleischmann et al., 1991; Honack and Loscher, 1993; Lipska et al., 1998). A ceiling effect may have occurred in our study because at the dose necessary to produce locomotion, ataxia is also seen. Ataxia could have prevented a further increase in locomotion in the PD rats.

Lipska and colleagues (Lipska et al., 1993, 1995; Lipska and Weinberger, 1993; Sams-Dodd et al., 1997; Tseng et al., 2007), and others (Chambers et al., 1996; Schroeder et al., 1999) have found increased dopamine- and glutamate-mediated locomotion and stereotypy, decreased dopamine-mediated catalepsy, disrupted prepulse inhibition of startle, cognitive and social deficits, as well as dopamine and

glutamate binding and cell excitability changes in adult rats following neonatal ventral hippocampal lesions. Others have found similar results following amygdala lesions (Hanlon and Sutherland, 2000), social isolation (Paulus et al., 1998), and neonatal viral infection (Rothschild et al., 1999). Our model extends this work, and, like viral infection (Rothschild et al., 1999), utilizes a natural exposure that occurs in human populations and has been suggested to be a risk factor in schizophrenia (Susser et al., 1996; Susser and Lin, 1992; St. Clair et al., 2005). Our finding of increased dopamine-mediated locomotion and stereotypy in adult PD female rats is consistent with the idea that prenatal nutritional insults can underlie much later behavioral changes. These behavioral changes were associated with biochemical changes, all of which involve neurotransmitter systems thought to be important in schizophrenia.

The decreased weight in male and female PD are broadly similar to that seen in other studies (Almeida et al., 1996b,c,d; Shultz et al., 1999; Tonkiss and Galler, 1990; Tonkiss et al., 1994), although there are some inconsistencies about whether these differences persist into adulthood, with some showing decreases (Almeida et al., 1996b; Shultz et al., 1999; Tonkiss and Galler 1990; Tonkiss et al., 1991) and others not (Almeida et al., 1996c; Tonkiss et al., 1998; Tonkiss and Galler 1990; Tonkiss et al., 1990, 1994). The differences in performance between PD and ND females on amphetamine-induced locomotion and apomorphine-induced stereotypy are probably not primarily due to weight differences. The correlations between body weight and behavior were relatively weak and inconsistent and would not have been significant had we applied a correction for the number of comparisons performed in this study. While the effect of PD on body weight was strongest in female rats (Table 3), we did not observe correlations between body weight and behavior in this group. Although PD females weighed significantly less than ND females, the magnitude of the difference was small.

Behavioral, receptor binding and body weight changes were most apparent in PD female rats replicating many of our earlier observations (Palmer et al., 2004). One possibility is that these PD females have alterations in the estrus cycle or other aspects of reproductive function. Phase of the estrus cycle was not measured in the current study. The accompanying failure to account for an additional source of variability might obscure, rather than enhance the statistically significant

results reported in this study; however, we cannot be sure that adult PD females had normal estrus cycles, and so it is possible that differences between female PD and ND rats are secondary to an abnormal or absent estrus cycle in PD females. This does not seem to be the case among humans exposed to famine; studies of the children born during and shortly after the Dutch Hunger Winter have not revealed any differences in age of first menstrual cycle, nor in a variety of other measures of reproductive function, with the exception of a small increase in perinatal deaths (Lumey and Stein, 1997). Estradiol is known to alter NMDA receptor binding and hippocampal CA1 dendritic spine density (Woolley, 1998), DA transporter number (Attali et al., 1997; Disshon et al., 1998), D₂ receptors (Bazzett and Becker, 1994), as well as to affect amphetamine-induced behaviors (Becker 1990; Becker and Beer, 1986). Schizophrenia occurs in both men and women with women showing a somewhat later onset. In our study, only female rats seemed to show an effect of PD. In contrast, an increased prevalence of schizophrenia was found in both male and female offspring of mothers exposed to famine during early pregnancy (Susser et al., 1996). This difference represents a limitation of this animal model.

There were several other limitations of this study. First, all animals in a group received all treatments (Table 1). It is possible that exposure to a drug prior to puberty could have altered the outcome after puberty. Similarly, the receptor binding studies were also conducted in animals that had previously received various drugs. Therefore, it is impossible to rule out the possibility that drug administration interacted with treatment to produce the observed binding results. Finally, in both this and our previous study (Palmer et al., 2004), we have treated individuals as the unit of analysis whereas regarding each litter as the experimental unit might have been a more conservative approach. In the present study we took steps to address this final concern by sampling fewer individuals from a larger number of families. Future studies may further address some of these methodological issues.

In conclusion, this study shows that prenatal PD in females produces increased behavioral responsivity to the dopamine agonists amphetamine and apomorphine in adulthood, but not before puberty, and increased ³H-MK-801 and ³H-haloperidol binding post-puberty in PD versus ND female rats. This animal model of prenatal protein deprivation is generally consistent with epidemiological findings showing that prenatal nutritional deficiency is a risk factor for schizophrenia (St. Clair et al., 2005; Susser et al., 1996; Susser and Lin, 1992) and may be a valuable tool to understand aberrant developmental processes that lead to brain pathology in adulthood such as occurs in schizophrenia. Enhanced amphetamine-induced locomotion and other anomalies related to dopaminergic and glutamatergic dysfunction have also been demonstrated in rodent models of maternal infection and immune activation (Shi et al., 2003; Zuckerman et al., 2003; Meyer et al., 2007). Our results are largely consistent with our previous study that examined behavioral and neuroanatomical correlates of prenatal protein deprivation (Palmer et al., 2004). The current results may also be relevant in studying processes that lead to increased responsivity to stimulant drugs such as amphetamine.

4. Experimental procedures

4.1. Nutritional treatment, mating, and cross-fostering

Sprague–Dawley rats were obtained viral and antibody free from Charles River Laboratories (Kingston, NY). Two diets were used, which had exactly the same composition as those utilized by Galler and colleagues (Tonkiss and Galler (1990); Teklad Laboratories, Madison, WI.). The pelleted diets were either of adequate protein content (25% casein) or of low protein content (6% casein) made isocaloric by addition of sucrose and cornstarch to the 6% diet.

Using the procedure of Galler and colleagues (Tonkiss and Galler 1990), one group of female rats (150–175 g) was randomly assigned to receive the adequate protein diet for 5 weeks prior to mating and throughout pregnancy. Another group of female rats (150–175 g) was randomly assigned to receive the low protein diet for 5 weeks prior to mating and during pregnancy. Male rats (300–325 g) received the adequate protein or the low protein diet, according to the females they were assigned to mate with, for 1 week prior to mating to acclimatize them to the diet. Each male was then placed with 2 to 3 females of the same diet group for a 1 week breeding period. Vaginal smears were taken each morning and stained to determine the presence of sperm. The day sperm was present, females were removed from breeding cages and housed singly in birthing cages. Birthing cages were inspected daily between 0800 and 1600 h, and the day pups were born was designated post-natal day 0 (PND 0).

All litters were cross-fostered whole within 36 h of birth to mothers who had received the 25% diet. The experimental group was designated protein deprived (PD) and the control group non-deprived (ND). On the day they were fostered, litters were culled to 8 pups, making sex distribution as even as possible, and toe-clipped for identification. Rats were weaned at PND 25 and housed 2–3 to a cage with other rats of the same nutritional history and sex. There were two groups of rats used in this study, which will be referred to as Group 1 and Group 2. All animals in Group 1 were continued on the 25% diet during and after weaning whereas all animals in Group 2 were continued on standard rat chow during and after weaning, as standard rat chow has not been found to produce differential effects on weight from the 25% diet but is much less expensive (Dr. John Tonkiss, personal communication). Food was available *ad libitum* and animals were maintained on a 12-h light/12-h dark cycle. Offspring were weighed approximately every other day for the first 2 weeks and then again at the time of each behavioral test.

4.2. Stereotypy

Rats were removed from their home cages and placed in steel wire bottom, hanging cages for observation of stereotypy at 0900 h on PND 34 and PND 56. After 2 h of acclimatization, rats were injected with freshly prepared apomorphine (Sigma Chemical Co., 0.75 mg/kg, SC). Beginning 5 min following the injection, rats were observed for 15 s every 5 min during a 55 min rating session. Stereotypy ratings were based on the procedures of Lipska and Weinberger (1993). Rats were scored

from 0 to 5. Zero=no stereotypy, 1=fixed sniffing, 2=short occurrence, 3=occasional bursts, 4=intense intermittent, and 5=continuous intense performance of oral stereotypies of licking, biting, or gnawing.

4.3. *D*-amphetamine-induced locomotion

Ambulatory locomotor activity was examined in two conditions: after exposure to a novel environment and after *D*-amphetamine injection utilizing the procedure of Lipska et al. (1993) on PND 37–39 and PND 59–61. Rats were placed in a clear plexiglass cage (43×43×33 cm) in a novel testing room and ambulatory photocell activity was recorded (Omnitech Digiscan Micro Animal Activity Monitor) every 15 min for 60 min. Then, after a *D*-amphetamine injection (Sigma Chemical Co., 1.5 mg/kg, IP) ambulatory activity was recorded every 15 min for 90 min. Because there were not enough photocell cages to test all rats, locomotor activity scores were not available for all animals. However, these animals received the appropriate injection.

4.4. Catalepsy

Rats in Group 1 were tested for haloperidol-induced catalepsy according to the procedure of Lipska and Weinberger (1993) on PND 40–42 and PND 62–64. A haloperidol solution of 20 mg/ml in 1% lactic acid was made and diluted with distilled H₂O to yield a final concentration of 1.0 mg/ml (pH 5.2). After 3 h of acclimatization to the test environment, rats received haloperidol injections (1 mg/kg, IP) at 1200 h. Beginning 45 min after injection, both front paws were placed on a horizontal plastic bar that was elevated to 9.5 cm for PND 40–42 and 11 cm for PND 62–64. The time spent in a cataleptic position (i.e. both front paws on the bar) for up to 2 min was recorded. This was done four times at 45 min intervals.

4.5. MK-801-induced locomotion

Rats in Group 2 were tested for ambulatory activity in two consecutive conditions: after exposure to a novel environment and after an MK-801 injection on PND 40–42 and PND 62–64. Rats were placed in a clear plexiglass cage (43×43×33 cm) and ambulatory photocell activity was recorded (Omnitech Digiscan Micro Animal Activity Monitor) every 15 min for 60 min. Then, after an MK-801 injection (Sigma Chemical Co., 0.15 mg/kg, IP), ambulatory activity was recorded every 15 min for another 60 min.

4.6. Haloperidol, MK-801, and dopamine transporter binding to regional rat brain membranes

At PND 85, after behavioral testing was completed, animals were sacrificed by decapitation, brains were rapidly removed and cerebral cortex, hippocampus, and striatum were carefully dissected. These brain regions were placed on dry ice and stored at –70 °C until use. PD males and females were drawn from 3 litters. ND males and females were drawn from 5 litters. Binding was done in Group 1 animals. Membrane fractions were prepared as described below and assayed for radioligand binding to cortical, hippocampal, and striatal receptor binding

and transporter sites. Three different radiolabeled ligands were used to quantify the binding of dopamine receptors (³H-haloperidol, NEN specific activity: 15.0 Ci/mmol), NMDA-sensitive glutamate receptors (³H-MK-801, NEN, specific activity: 23.9 Ci/mmol), and dopamine transporter sites (³H-GBR-12935, NEN, specific activity: 22.0 Ci/mmol).

On the day of assay, samples were homogenized in a 60x v/w Tris-EDTA buffer containing (mM): Tris HCl (38.5); Tris-Base (11.5), ethylenediaminetetraammonium (EDTA; 1.0) at pH 7.4. using a Brinkman polytron. The tissue was centrifuged at 30,000 g for 20 min at +4 °C and the resultant pellet was resuspended in 60x v/w radioligand-specific buffer. Tissue for ³H-GBR-12935 binding was resuspended in a Tris-NaCl buffer containing (mM): Tris HCl (38.5); Tris-Base (11.5); NaCl (120); and KCl (5.6) at pH 7.4, while tissue for ³H-MK-801 and ³H-haloperidol binding were resuspended in the above Tris-EDTA buffer. For ³H-GBR-12935, ³H-haloperidol, and ³H-MK-801 binding to rat brain membrane homogenates, 300 μl of membrane homogenate was incubated in the presence of either 600 μl Tris buffer (Tris-NaCl for ³H-GBR-12935 and Tris-EDTA for ³H-haloperidol and ³H-MK-801; total binding) or a final concentration of 10 μM unlabeled nomifensine (RBI, Natick, MA), haloperidol (Sigma, St. Louis, MO), or MK-801 (RBI, Natick, MA) for competition to the dopamine uptake transporter, dopamine receptors, and NMDA receptors, respectively (non-specific binding) for 5 min at room temperature. 100 μl of ³H-MK-801, ³H-haloperidol, or ³H-GBR-12935 (final concentration for each ligand 3 nM) was added and allowed to equilibrate in the dark for 120 min at 22 °C.

The tissue was harvested onto Titertek filtermats, coated with 0.1% polyethylimine (PEI) to reduce non-specific binding, using a Titertek cell harvester and the filters were placed in scintillation vials containing 8.0 ml Liquiscint (National Diagnostics). Samples were counted for 10 min in a Beckman liquid scintillation counter (efficiency: 50%). CPM data were converted to pmol of ³H-radioligand bound/mg protein.

The ³H-MK-801 binding assay was done without the addition of glycine. While some studies have utilized glycine in ³H-MK-801 binding assays to enhance binding or to saturate the glycine site to control for varying amounts of endogenous glycine which can affect ³H-MK-801 binding (Sakurai et al., 1993), previous studies have successfully been carried out without this agent (Woolley et al., 1997) and have found similar results with enhanced or unenhanced binding (McCoy and Richfield, 1996).

4.7. Statistical analysis

The data for apomorphine-induced stereotypy, baseline, saline, and amphetamine-induced locomotion, haloperidol-induced catalepsy, and baseline and MK-801-induced locomotion were analyzed by separate ANOVAs for each sex at each age with repeated measures for test time after injection. We analyzed the data in this manner, rather than starting with more complex multi-way ANOVAs due to our *a priori* interest in these comparisons based on our previous study of protein deprivation in rats (Palmer et al., 2004). Significant main effects for diet or a significant diet×time interactions were followed up using t-tests to compare PD and ND groups. In some instances, behavioral data were missing for one particular test time; in these cases all behavioral data for that animal were excluded from all analyses for that test.

To examine the relationship between body weight and behavior, Pearson's Product Moment Correlation Coefficient was used. Total locomotor or stereotypy scores for each animal were used.

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