*Neuroscience* 167 (2010) 985–993

# RELATIONSHIPS AMONG GENDER, AGE, TIME, AND TEMPERATURE IN METHAMPHETAMINE-INDUCED STRIATAL DOPAMINERGIC NEUROTOXICITY

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**Abstract—A neurotoxic regimen of methamphetamine (MA—40 mg/kg ip) administered at 0 (control—MA vehicle), 0.5 and 72 h prior to determinations of striatal dopamine (DA) and DOPAC (3,4-dihydroxyphenylacetic acid)/DA ratios were compared among juvenile and adult female and male mice. Adult females and males showed similar depletions in striatal DA at 0.5 h post-MA, but males showed greater DA depletions and DOPAC/DA ratios at 72 h post-MA. Juvenile mice showed neither sex differences, nor any MA neurotoxicity upon striatal DA or DOPAC/DA ratios. Following MA, body temperatures increased in all mice, but increases in adult males were greater than adult females; juveniles showed no sex differences and body temperature increases were similar to that of adult males. MA-evoked DA output was greater in adult compared to juvenile males and a biologically effective regimen of testosterone to juvenile males neither increased MA-evoked DA output nor decreased MA-induced striatal DA like that observed in adult males. These results demonstrate: (1) Unlike adults, juvenile mice show neither a sex difference for MA-induced neurotoxicity or body temperature increases, nor MA neurotoxicity, (2) Initial effects of MA (0.5 h) in adult females and males are similar, but at 72 h post-MA females show no further striatal DA depletion, (3) Increased striatal DA depletion within adult versus juvenile males may be related to initially higher MA-evoked DA responses, and (4) Testosterone fails to convert juvenile males into adults with regard to MA effects.** 

**Key words: gender differences, nigrostriatal, neurodegeneration, neuroprotection, testosterone.**

Men and women respond differently to methamphetamine (MA) (Dluzen and Liu, 2008), and analogous gender differences are seen in laboratory animals (Liu and Dluzen, 2007). Emergency room deaths resulting from MA are greater in men (Hall and Broderick, 1991) and there exists a correlate in laboratory studies (Dluzen et al., 2002). The degree of nigrostriatal dopaminergic (NSDA) neurotoxicity to MA is greater in males (Dluzen, 2004; Dluzen et al., 2001, 2003; Heller et al., 2001; Miller et al., 1998; Wagner et al., 1993; Yu and Liao, 2000; Yu and Wagner, 1994). Moreover, the amount of striatal dopamine (DA) evoked by MA infusion is greater in males (Dluzen and Salvaterra, 2005; Kunnathur et al., 2006).

In Part 1 of this report, we examined three different parameters (time, age and body temperature) in these gender differences related to MA. Our first major goal was to assess the temporal dependence of sex differences in MA-induced striatal DA neurotoxicity. To accomplish this goal, the effects of MA upon depletion of striatal DA levels (and DOPAC (3,4-dihydroxyphenylacetic acid)/DA ratios) at a relatively short interval (0.5 h) were compared with that at a more prolonged period (72 h) post-MA treatment. Second, it has been shown that various parameters of MA-induced deficits are attenuated in juvenile rodents, as demonstrated in rats (Cappon et al., 1997; Imam and Ali, 2001; Kokoshka et al., 2000; Pu and Vorhees, 1993; Truong et al., 2005), mice (Miller et al., 2000) and gerbils (Teuchert-Noodt and Dawirs, 1991). However, these juvenile versus adult comparisons have not included considerations of gender differences, despite the marked dissimilarities in MA responsiveness between adult females and males as described above. Therefore, the temporally dependent effects of MA upon striatal DA depletion were also evaluated between juvenile (28–30 day old) male and female mice. Third, the acute increase in body temperature that accompanies MA treatment is believed to represent a significant component of the resultant striatal neurotoxicity to this psychostimulant (Cadet et al., 2007; Miller and O'Callaghan, 1995; Riddle et al., 2006). Accordingly, the third parameter evaluated within Part 1 was that of MA-induced increases in body temperature among male and female adult and juvenile mice.

Collectively, the results from the three experiments of Part 1 revealed that juveniles, in particular males, tend to show relatively unique responses to MA. Unlike that observed in adult male mice treated with MA, striatal DA levels of juvenile males were not different from juvenile females, they do not show a temporally dependent change in striatal DA depletion, nor do they show increases in body temperature that differ significantly from that of juvenile females. Therefore, in Part 2 of this report, we focused upon the juvenile male with the goal of identifying some of

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*Abbreviations:* ANOVA, analysis of variance; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high pressure liquid chromatography; KRP, Kreb's Ringer Phosphate; MA, methamphetamine; NSDA, nigrostriatal dopaminergic; °C, degrees celsius/centigrade.

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doi:10.1016/j.neuroscience.2010.02.076

the bases for these distinctive response profiles observed in response to MA. First, MA-evoked DA responses from superfused striatal tissue were compared between adult and juvenile male mice. With this experiment it is possible to determine whether initial responses to this psychostimulant may be present and thus contribute to the differences observed between juvenile and adult male mice. Since significant differences in MA-evoked DA output were obtained between juvenile and adult males, we next compared MA-evoked DA responses between juvenile males treated or not with testosterone as a means to assess whether the immature state of gonadal function might be involved with differences between juvenile and adult males. Lastly, the capacity for MA to produce striatal DA depletion was compared between juvenile males treated or not with testosterone. Since testosterone treatment enhances striatal DA depletion to MA in adult male mice (Lewis and Dluzen, 2008), the purpose of this final experiment was to determine whether testosterone would exert an enhanced depletion of striatal DA to MA in juvenile males, like that seen in adult male mice.

## EXPERIMENTAL PROCEDURES

### Animals

Female and male CD-1 mice (bred within our facilities) were used in these experiments. Mice were used at either 28–30 days (juvenile) or 90–120 days (adult) of age. Mice were housed individually, had free access to food and water and were maintained under a 12-h light cycle with lights on at 0600 h. Since gender differences in nigrostriatal dopaminergic neurotoxicity exist regardless of estrous cycle stage (Brooks et al., 1989; Dluzen and Horstink, 2003; Dluzen et al., 2003; Freyaldenhoven et al., 1996; Miller et al., 1998; Wagner et al., 1993; Yu and Liao, 2000; Yu and Wagner, 1994), estrous cycles of the adult female mice were not monitored. All treatments comply with the NIH guide for Care and Treatment of Laboratory Animals and were approved by the IACUC at NEOUCOM. All efforts were made to minimize animal suffering, to reduce the number of animals used and utilize alternatives to *in vivo* techniques, when possible.

### General Procedure—Part 1

In the experiments of Part 1, mice received a single i.p. injection of methamphetamine (MA) (Sigma Chemical Company, St. Louis, MO, USA) at 40 mg/kg or an equal amount of its saline vehicle. This MA dose was used as it was demonstrated to be effective in producing a statistically significant decrease in striatal DA levels within adult male mice (Lewis and Dluzen, 2008). At 0 (saline vehicle controls), 0.5 or 72 h post-MA mice were euthanized by rapid decapitation, the striatum removed and assayed for DA and DOPAC levels as described below. This identical protocol was used in adult (Experiment 1) and juvenile (Experiment 2), male and female mice. In Experiment 3, rectal body temperatures were taken from adult and juvenile male and female mice. Three baseline recordings were performed at 60, 30 and 0 min prior to MA (40 mg/kg ip) administration. Immediately after the third recording (0 min), the mice were treated with MA and two additional rectal temperature recordings were performed at 30 and 60 min post-MA. The difference between the last pre-MA recording (0 min) and the highest recording achieved post-MA (either at the 30 or 60 min period) was used to calculate the change in body temperature resulting from the MA treatment.

### General Procedure—Part 2

Three additional experiments were performed which comprise Part 2 of this report. In Experiment 4, DA output responses to an infusion of MA (1 mM) into superfused striatal tissue fragments were compared between adult and juvenile male mice. A 1 mM dose of MA was used in these experiments as this relatively high dose can clearly discriminate DA output responses between female and male mice and it was anticipated that juveniles would show attenuated MA-evoked DA responses (Dluzen and Salvaterra, 2005). In this way, it was possible to identify any potential differences in initial DA responses to this psychostimulant, which could then be related with differences observed for MA-induced neurotoxicity. Since a significant difference was obtained for these initial MA-evoked DA responses as determined in Experiment 4, and one of the most salient differences which exists between adult and juvenile males is that of their gonadal state, in Experiments 5 and 6 we attempted to determine whether a deficiency in testosterone might contribute to the dissimilarities in dopaminergic responses to MA. In Experiment 5, juvenile male mice were treated with either testosterone propionate (Sigma; 5 $\mu$g in sesame oil, s.c.) or the sesame oil vehicle at 24 h prior to superfusion. MA-evoked DA output responses were then compared between the superfused striatal tissue of these testosterone- versus vehicle-treated juvenile male mice. Finally, in Experiment 6 we examined the potential for testosterone to enhance MA-induced neurotoxicity in juvenile males. When testosterone is administered at 24-h prior to treatment with MA, the degree of striatal DA depletion resulting from MA is significantly increased within adult male mice (Lewis and Dluzen, 2008). Whether a similar effect would be present in juvenile male mice was tested in Experiment 6. In this experiment, juvenile male mice were treated with testosterone (as described above), the sesame oil vehicle or remained untreated. At 24-h post-testosterone/vehicle (or no treatment) administration, the mice were treated with MA (40 mg/kg ip) and euthanized at 72-h post-MA for determinations of striatal DA and DOPAC/DA ratios. The paired testes weights (in mg) were measured in these juvenile mice treated with testosterone or the oil vehicle as a means to verify a biological effect of this treatment.

For all experiments the striatum was dissected from each mouse. The brain was bisected, ventricles pried open and the cortex cut away to reveal the striatum. The striatum was dissected out from within the periphery of the corpus callosum. For determinations of striatal tissue levels of DA and DOPAC, the tissue was weighed and placed in 500 $\mu$l cold (4 °C) 0.1 N perchloric acid. These tissue samples were sonicated, centrifuged and an aliquot removed for assay of DA and DOPAC. Striatal DA and DOPAC were expressed as pg/mg of tissue weight. When used for superfusion, the striatal tissue was removed as described above and prepared for superfusion.

### *In vitro* superfusion

The striatum was placed within a breaker containing cold (4 °C) Kreb's Ringer Phosphate (KRP) medium. The striatum was sliced into tissue fragments (approximately $0.5\times0.5\times0.5$ mm$^3$) prior to placement into the superfusion chamber. The superfusion medium was a modified KRP medium: 120 mM NaCl, 4.8 mM KCl, 0.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10.2 mM $Na_2HPO_4$, and 0.18% glucose at a pH of 7.35–7.4. The KRP medium was filtered (0.45 $\mu$m, Milipore Filter) prior to use. Each of the superfusion chambers housing the striatal tissue fragments consisted of a tuberculin syringe cut off at the 0.3 ml level and was inserted into a 22 gauge lumbar puncture needle. The top of the chamber was sealed with a rubber stopper containing an entry port for filtered humidified air to oxygenate the tissue and an exit port for the perfusate. The tissue fragments were suspended in the chamber on cellulose filter paper and were superfused with KRP at a flow rate of approximately 25 $\mu$l/min. The chambers were maintained in a