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Gender differences in modulatory effects of tamoxifen upon the nigrostriatal dopaminergic system

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Abstract

It has been demonstrated that the gonadal steroid hormone estrogen can exert neuroprotective effects upon the nigrostriatal dopaminergic (NSDA) system against methamphetamine (MA)-induced neurotoxicity in female, but not male, mice. In contrast, the anti-estrogen, tamoxifen (TMX) can function as a NSDA neuroprotectant within both female and male mice. In an attempt to understand these effects of TMX, the effects of this anti-estrogen upon both behavioral and neurochemical indices of NSDA function were examined within female and male mice following treatment with MA. In general, TMX exerted markedly different (bi-directional) effects upon NSDA function between female and male mice. Notably, treatment with TMX resulted in a relative decrease in striatal dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations within male mice and a relative increase in female mice when treated with MA to produce a significant gender difference. Similar effects were obtained for locomotor behaviors related with NSDA function. That is, TMX produced increases in horizontal activity, number of movements and total distance traveled within MA-treated female mice resulting in statistically significant gender differences for the two former parameters. For non-locomotor behaviors, like time occupying the center and margin of the cage, TMX-treated male mice showed statistically significant increases and decreases compared within TMX-treated female mice, respectively. These results show that in contrast to the similar neuroprotective effects of TMX upon MA-induced NSDA neurotoxicity, a number of other NSDA indices induced by MA show markedly different response profiles between TMX-treated female and male mice.

Keywords: Sex differences; Estrogen; Parkinson's disease; Methamphetamine; Neurodegeneration; Neurotoxicity; SERMs

1. Introduction

There exist marked gender differences in the functioning of the nigrostriatal dopaminergic (NSDA) system (Becker, 1999; Becker and Ramirez, 1981; Crowley et al., 1978; Fernandez-Ruiz et al., 1992; McDermott et al., 1994). In part, these differences can be attributed to dissimilarities in gonadal steroid hormones between the sexes. Testosterone (Dluzen and Ramirez, 1989), progesterone (Ramirez et al., 1985; 1990) and estrogen (Bedard et al., 1981; Di Paolo, 1994; Van Hartesveldt and Joyce, 1986) have all been shown to be capable of altering the NSDA system. The findings that a gonadal steroid hormone, like estrogen, can exert a modulatory effect upon NSDA functioning suggests that other selective estrogen receptor modulators (SERMs) may also possess a similar capacity.

The anti-estrogen, tamoxifen (TMX), represents one such SERM. There exist a number of reports which show that TMX can alter NSDA function (Baksi et al., 1985; Chaurasia et al., 1998; Cyr et al., 2000; Dluzen et al., 2001a,b; Hiemke and Ghraf, 1984; McDermott et al., 1997; 1999, 1998, 1995; Mickley and Dluzen, 2004; Obata and Kubota, 2001; Toney and Katzenellenbogen, 1987). With regard to NSDA function, a particularly intriguing feature of TMX is its ability to function as a neuroprotectant against the NSDA neurotoxin, methamphetamine (MA)

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(Dluzen and McDermott, 2002; Dluzen et al., 2001a,b; Yu et al., 2002). Moreover, unlike estrogen which can function as a NSDA neuroprotectant against MA only within female mice (Dluzen and McDermott, 2000; Gao and Dluzen, 2001a,b), TMX is capable of serving as a neuroprotectant against a neurotoxic regimen of MA within both males and females (Dluzen and McDermott, 2002; Dluzen et al., 2001a,b). The similarity in responsiveness to TMX between male and female mice with regard to NSDA neuroprotection against MA raises the issue of whether other MA-evoked indices of the NSDA system may also be affected in a similar manner. Accordingly, in this report, behavioral and neurochemical responses of the NSDA system to MA were compared between TMX or vehicle-treated male and female mice.

2. Methods

2.1. Animals

Gonadally intact adult (2–3 months of age) CD-1 male and female mice obtained from Charles River Laboratories were used in the experiment. Gonadally intact mice were used in this experiment since we have reported these mice to show a neuroprotective effect against MA-induced NSDA neurotoxicity when treated with TMX (Dluzen et al., 2001a,b). Mice were individually housed and maintained on a 12 h light/dark cycle with free access to food and water within a temperature and humidity controlled colony room. All treatments of the mice complied with the NIH guide for the care and treatment of animals and were approved by the IACUC at NEOUCOM.

2.2. Treatments

Mice received either a single subcutaneous injection of TMX in sesame oil (500 μg) or the sesame oil vehicle. This dose of TMX was selected based upon previous work which indicated it to alter NSDA function within female mice (Mickley and Dluzen, 2004). At 24-h post-TMX/Vehicle administration, all mice were treated with a single intraperitoneal injection of MA (40 mg/kg in saline). This dose of MA was selected since it will produce a mild neurotoxicity (approximately 50% reduction in striatal dopamine concentrations) of the NSDA system (Gajjar et al., 2003). Immediately after the MA injection, the mice were placed in an Omnitech Animal Activity Monitor for a 1-h period to assess locomotor and related behavioral responses. After testing within the Animal Activity Monitor, the mice were placed in their home cage and returned to the colony room. At 1-week post-MA treatment, the mice were sacrificed by rapid decapitation. This time period was chosen since we have reported that striatal dopamine (DA) concentrations were decreased by approximately 50% using this protocol (Gajjar et al., 2003). The corpus striatum (CS) was removed, weighed and prepared for assay of DA and 3,4-dihydroxyphenyl-acetic acid (DOPAC) concentrations.

2.3. Behavioral activity

To evaluate the locomotor and related behaviors of the mice, immediately following MA treatment, mice were placed in an Omnitech Animal Activity Monitor (Columbus, OH).With this apparatus, the following locomotor-related behaviors were measured—Horizontal Activity (total number of beams interrupted in the horizontal sensor), Number of Movements (number of horizontal movements executed as separated by a rest period of at least one second) and Total Distance Traveled (cm). The following (non-locomotor) behaviors were also recorded—Center and Margin Times (the amount of time, in seconds, that the mice occupy the center or periphery of the chamber, respectively), Stereotypy Counts (breaking of the same beam, or set of beams, repeatedly) and Number of Stereotypies (number of stereotyped movements executed as separated by a rest period of at least 1 s). The total scores obtained over a 50-min period of recording were used in the analyses of these behavioral responses. The first 10 min was deleted from the recordings to allow the mice to acclimate after the MA injection.

2.4. Tissue preparation

At 1-week post-MA treatment, mice were euthanized by rapid decapitation. The brain was removed, bisected, the ventricles pried open and the cortex cut away revealing the CS. The perimeter of the CS within the corpus callosum, anterior commissure and internal capsule/cortex was cut and the CS removed with curved scissors. The CS from both hemispheres was weighed and placed in vials containing 500 μl cold (4 °C) 0.1 N perchloric acid. The samples were sonicated, centrifuged and an aliquot was removed from the supernatant for assay of DA and DOPAC. Concentrations of DA and DOPAC were expressed as pg of DA or DOPAC per mg of tissue weight.

2.5. Neurochemical assay

Measurements of DA and DOPAC were determined using HPLC-ECD (ESA-Chelmsford, MA). A 100×4.6-mm, 3-μm C-18 reverse phase column (Biophase ODS, BAS-W, Lafayette, IN) with an isogradient mobile phase (5.75 g citric acid, 4.1 g sodium acetate, 0.5 g sodium hydroxide, 20 mg sodium octyl sulfate, 35 mg EDTA and 7% methanol in 1.0 L of deionized water at a pH of 4.5) was used in this system. The mobile phase was filtered through a Millipore filter (0.45 μm) and degassed prior to use. Standards for DA and DOPAC were stored frozen (−20 °C) in 0.1 N perchloric acid. The DA and DOPAC standards were diluted in the perchloric acid in doses of 12.5, 25, 50,

100, 200, 400 and 800 pg/20 μl to construct a standard curve. DA and DOPAC from tissue samples were determined by comparing with peak heights and retention times of standards. The sensitivity of this assay, as determined by the reliable appearance of chromatographic peaks over baseline noise, was ≤12.5 pg.

2.6. Endocrine bioassay

At sacrifice, pituitary and uterine weights were determined from these mice. These endocrine bioassay measures provided a means to approximate the effects of the TMX treatment administered.

2.7. Analyses

Nonparametric statistics were used to analyze the data. These involved Mann–Whitney *U*-Tests to evaluate whether significant differences were present between responses of male and female mice. A $p<0.05$ was required for results to be considered statistically significant.

3. Results

3.1. Neurochemical results

The data for DA concentrations of the female and male mice treated or not with TMX prior to MA administration is presented in Fig. 1A. Inspection of this figure indicates that TMX exerts divergent effects upon MA-altered DA concentrations as a function of gender. Fig. 1B contains a summary of the data analysis for these DA concentrations expressed as the percent change in DA concentrations of TMX-treated mice as compared with the mean of their respective vehicle-treated controls. This presentation illustrates the overall increase of DA concentrations within MA-treated females and decrease in MA-treated males as a function of TMX administration. Analysis of these percent changes reveals a statistically significant gender difference ($U=2$, $p=0.017$). Fig. 2 contains the corresponding data for DOPAC concentrations. Following TMX treatment, corpus striatal DOPAC concentrations from females increase while that from males decrease (Fig. 2A). Analysis of the percent change in MA-induced DOPAC concentrations of TMX-treated mice as compared with their respective vehicle-treated controls indicates a statistically significant gender difference in response to TMX ($U=3$, $p=0.03$).

3.2. Behavioral results

In Fig. 3 are contained the data from locomotor-related behaviors as measured in the animal activity monitor. The profiles in these locomotor-related behavioral measures are similar to that of the corpus striatal DA and DOPAC concentration profiles. MA-treated female mice show increases in activity following TMX administration while MA-treated male mice show decreases. Analysis of these behavioral responses reveals that the amount of Horizontal Activity ($U=5$, $p=0.037$—Fig. 3A) and Number of Movements ($U=4$, $p=0.025$—Fig. 3B) of MA-treated female mice is significantly greater than that of MA-treated males following TMX administration. No statistically significant gender differences are obtained in vehicle-treated mice. While a very similar profile was observed for the measure of Total Distance (Fig. 3C), these results failed to achieve a statistically significant difference.

In Fig. 4 are contained data from responses in non-locomotor behaviors as a function of gender and TMX treatment. In Fig. 4A and B are presented data regarding

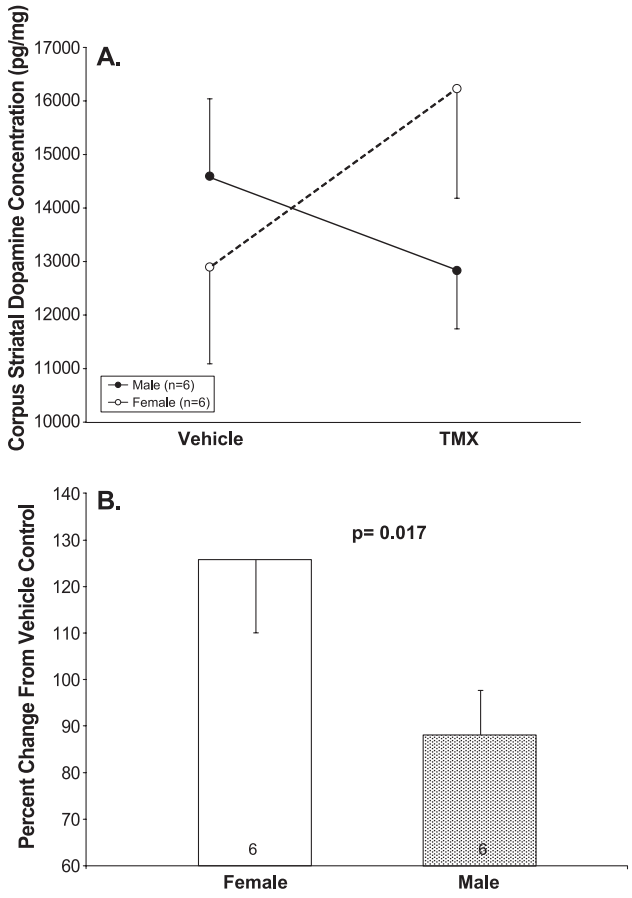

Fig. 1. (A) Corpus striatal dopamine concentrations (Mean±S.E.M. in pg/mg) from female ($N=6$) and male ($N=6$) CD-1 mice that were treated with tamoxifen (TMX-500 μg) or its vehicle at 24 h prior to administration of a neurotoxic regimen of methamphetamine (40 mg/kg). Corpus striatal dopamine concentrations increased by 26% in females and decreased by 12% in males as a result of TMX treatment. (B) Percent change in dopamine concentrations of female and male mice as calculated from values obtained in TMX-treated mice relative to the mean of their respective vehicle controls. This presentation highlights the differences in responses that occur between female and male mice as revealed by the statistically significant ($p=0.017$) greater percentages obtained from female compared with male mice.

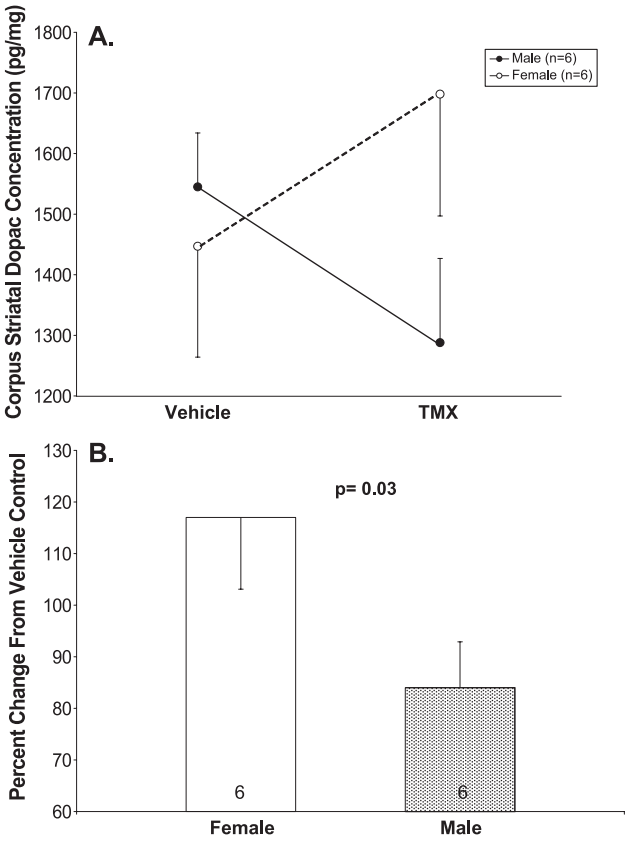

Fig. 2. (A) Corpus striatal DOPAC concentrations for the data presented in Fig. 1. A very similar profile of responsiveness was obtained for DOPAC concentrations as that observed for dopamine in Fig. 1. Corpus striatal DOPAC concentrations increased by 17% in females and decreased by 16% in males as a result of TMX treatment. (B) Percent change in DOPAC concentrations of female and male mice as described in Fig. 1B. The percent change obtained from females was significantly greater (p=0.03) than that of males.

the region within the animal activity monitor chamber where the mouse resides during the test. As can be seen from these figures, TMX has the effect of decreasing the amount of time that MA-treated male mice reside within the center of the cage (Fig. 4A), thereby increasing the amount of time within the marginal/peripheral areas of the cage (Fig. 4B). Analyses of these data reveal that following treatment with TMX, MA-treated male mice spend significantly decreased amounts of time localized within the center of the cage (U=4, p=0.025—Fig. 4A) and significantly greater amounts of time localized within the periphery/margins of the cage (U=4, p=0.025—Fig. 4B) compared with MA-treated females. In contrast to the MA-treated male mice, these localization scores for MA-treated females were virtually identical between females treated or not with TMX. Two additional behavioral responses which indicated some interesting trends, but failed to achieve a statistically significant difference were related to stereotypy scores. For Stereotypy Counts, scores for MA-treated females tended to increase, while those for MA-treated males decrease following TMX treatment (Fig. 4C). With regard to the Number of Stereotypies displayed, overall levels were greater in MA-treated females and showed a slight increase with TMX. The lower levels observed within MA-treated males showed little change as a function of TMX treatment.

3.3. Endocrine bioassay results

Treatment with TMX decreased both pituitary (Mean+S.E.M. in mg: Vehicle=3.05+0.19 versus TMX=2.74+0.19, N=6 for both groups) and uterine (Vehicle=196+26 versus TMX=165+23) weights within female mice, however, neither result achieved a statistically significant difference. Pituitary weights obtained from male mice treated or not with TMX were virtually identical (Vehicle=2.35+0.13

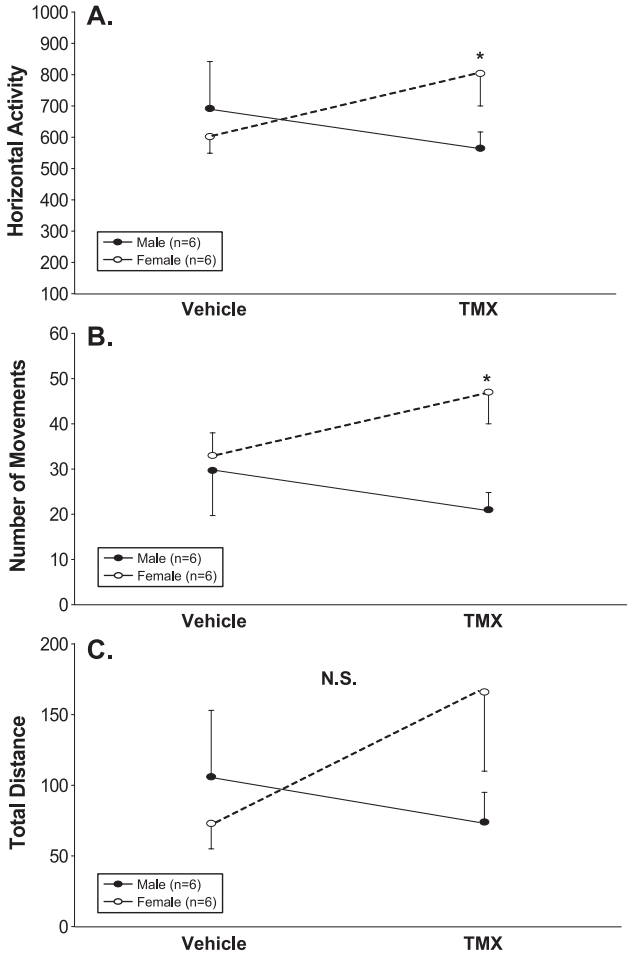

Fig. 3. Methamphetamine-induced locomotor-related activity scores for female and male mice treated with tamoxifen (TMX-500 μg) or its vehicle at 24 h prior to methamphetamine administration. No statistically significant differences between female and male mice were observed with vehicle-treated mice. As a result of TMX treatment, females showed statistically significant (indicated by the *) increases in Horizontal Activity (A—p=0.037) and Number of Movements (B—p=0.025). While the Total Distance (C) scores of TMX-treated females were also increased as compared with males, these differences failed to achieve a statistically significant difference.

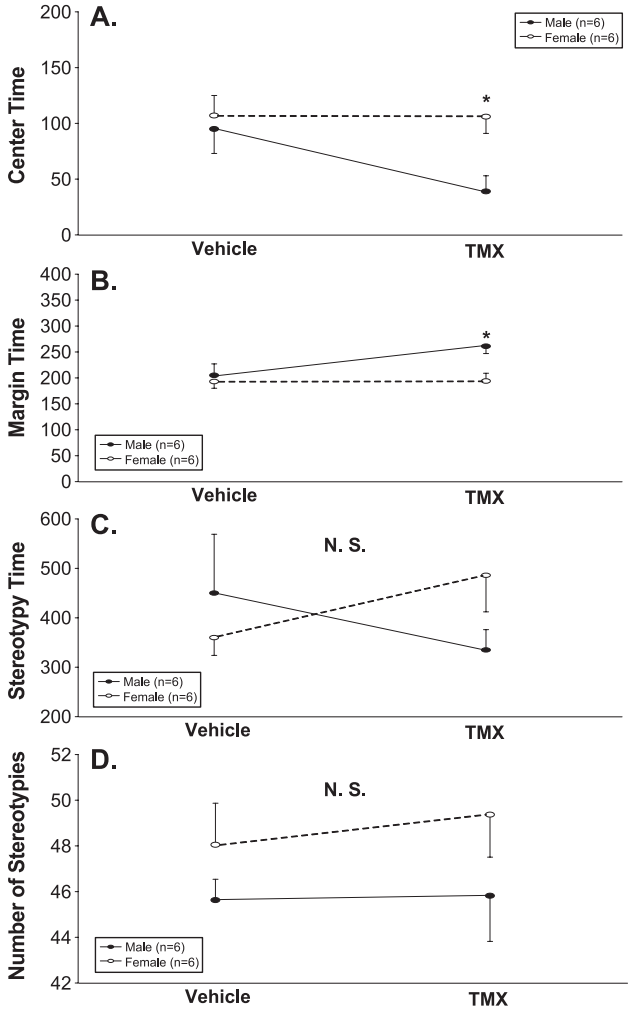

Fig. 4. Methamphetamine-induced non-locomotor-related scores for female and male mice treated with tamoxifen (TMX-500 μg) or its vehicle at 24 h prior to methamphetamine administration. No statistically significant differences between vehicle-treated female and male mice were obtained for any of these behaviors. As a result of TMX treatment, male mice showed statistically significant (indicated by the *) decreases in the amount of time occupying the center of the cage (A—p= 0.025) and significantly greater amounts of time localized to the margin/periphery of the cage (B—p=0.025). Female mice receiving TMX showed a trend for increased number of stereotypy (C) counts and number of stereotypies (D) as compared with males, however, these results failed to achieve a statistically significant difference.

versus TMX=2.38+0.15, N=6 for both groups) and not significantly different.

4. Discussion

The present results reveal some interesting interactions between the effects of the anti-estrogen, TMX and MA-induced actions upon the NSDA system. We, as well as other laboratories, have reported that TMX can function as a NSDA neuroprotectant against MA-induced NSDA neurotoxicity within both female and male mice (Dluzen and McDermott, 2002; Dluzen et al., 2001a,b; Yu et al., 2002). In contrast to the similarity of TMX effects within female and male mice to MA-induced NSDA neurotoxicity, it is clear that marked gender differences are observed to the acute behavioral responses induced by MA as well as in the relative changes in striatal DA and DOPAC concentrations. Similarly, the endocrine bioassay results suggest a gender difference with females showing 11% and 19% reductions in pituitary and uterine weights, respectively, as compared with a 1% increase of pituitary weight within the male. The lack of a statistically significant difference in pituitary and uterine weights between TMX and vehicle-treated female mice may be attributable to the determination of these parameters at eight days post-TMX treatment.

For DA and DOPAC concentrations, differences are present with regard to responses to MA as a function of TMX treatment and gender. In specific, prior treatment with TMX has the effect of increasing striatal DA and DOPAC concentrations within female mice while decreasing these levels within male mice. As a result, when these changes are expressed relative to their respective vehicle control values, a statistically significant gender difference emerges (Figs. 1B and 2B). It should be noted that the absolute values of striatal DA and DOPAC concentrations in TMX-treated mice receiving MA remained elevated (~13,000 pg/mg) indicative of an overall neuroprotective action of TMX since striatal DA concentrations of mice receiving this MA regimen are typically decreased to approximately 50% of control/vehicle levels (~8,000 pg/mg (Gajjar et al., 2003). In this way, MA-induced NSDA neurotoxicity remains attenuated within TMX-treated female and male mice as reported previously (Dluzen and McDermott, 2002; Dluzen et al., 2001a,b; Yu et al., 2002). However, the present results reveal that the relative effects of striatal DA and DOPAC preservation to MA-induced neurotoxicity appear greater in female mice.

Associated with this relative gender difference in TMX preservation of striatal DA and DOPAC concentrations were gender differences in acute behavioral responses to MA as a function of TMX treatment. In response to MA, TMX-treated females show statistically significant increases in horizontal activity and number of movements and a non-statistically significant increase in total distance traveled as compared with males. Such locomotor behavioral responses illustrate acute indices of NSDA responsiveness to MA treatment and show a coordinate relationship with the changes in striatal DA and DOPAC concentrations observed at 1 week post-MA. In this regard, it would appear that females may be more sensitive to the modulatory effects of TMX upon the NSDA system as revealed in both an acute behavioral and chronic neurochemical assay of function.

In contrast to these behavioral responses associated with NSDA function is the absence of changes in the amount of time that these females localize within the center or margins of the cage. Center and margin times for females treated or not with TMX are virtually identical (Fig. 4A and B). For this response, a clear effect is obtained with male mice

where TMX had the effect of decreasing the amount of time within the center of the cage and a concomitant increase in the amount of time spent in the margins (periphery) of the cage. Since rodents prefer to be in the periphery of the cage, positioning or movement into the center of the cage has been interpreted to indicate an anxiety reducing response (Miller et al., 2002; Sullivan et al., 2003; Vendruscolo et al., 2003). Therefore, the increased periphery times produced by TMX within male mice suggest that this anti-E may be producing an anxiety-enhancing effect. In this regard, TMX may serve as an agent with the capacity to create an animal model of anxiety within male mice. The potential for TMX to increase anxiety within the male can be contrasted with the tendency for TMX to increase stereotypy responses within female mice treated with MA. Both stereotypy counts and numbers are increased, albeit not significantly, in MA-treated females receiving TMX while these responses are either decreased or remain the same within male mice. Taken together, these data imply that TMX may also represent an important, gender dependent, modulator of psychological responses not typically associated with the NSDA system thereby suggesting alternative brain systems which may be modulated by TMX in MA-treated mice.

The exact bases for these gender differences in response to TMX are not immediately apparent. An initial cursory explanation would involve the suggestion that TMX is working through a classic anti-E binding mechanism. Gender differences may then reflect a greater potential for anti-E effects within females where greater concentrations of E are present and the data of the endocrine bioassays would support this possibility. While such an explanation has its merits, it would not seem to provide a complete accounting for the results obtained. For example, the striatal area has few, if any, classic E receptors where TMX can exert an anti-E effect (Pfaff and Keiner, 1973). While this TMX-E interaction may occur at sites other than the NSDA system and secondarily modulate NSDA function, it is also possible that TMX may affect the NSDA, as well as other brain, systems, through a means which is independent of its competition with E binding. There are data which demonstrate such alternative effects of TMX action (Allen et al., 2000; Allen et al., 1998; Cabot et al., 1995; Dodds et al., 2001; Hutchinson et al., 2001; Severcan et al., 2000; Smitherman and Sontheimer, 2001; Song et al., 1996) which suggest actions of TMX within the central nervous system that are independent of estrogen receptors (Cyr et al., 2001; Dicko et al., 1999; Gursoy et al., 2002; Phillis et al., 1998). The present data would suggest that these proposed alternative effects retain a distinct gender difference with regard to modulatory MA-induced effects upon the NSDA system.

In summary, the current results reveal that the anti-E, TMX, can exert a significant modulatory effect upon both neurochemical and behavioral NSDA responses evoked by MA within the mouse. Interestingly, these responses show clear differences in direction of this modulation as a function of the gender of the mouse. While our main emphasis was upon the NSDA system, it appears that gender-dependent TMX effects may be exerted upon other brain systems as based upon the behavioral responses observed. It remains to be determined whether these effects of TMX may involve classic anti-E effects and/or alternative mechanisms of action which can alter central nervous system function.

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