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Brief communication

Cognitive testing (delayed non-match to sample) during oral treatment of female adolescent monkeys with the estrogenic pesticide methoxychlor

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Abstract

This report presents preliminary data from a study of endocrine disruption in adolescence. A delayed non-match to sample (DNMS) task was used to assess the effects of the endocrine-active agent methoxychlor (MXC; 0, 25, or 50 mg/kg/day oral administration) in adolescent female monkeys 24–36 months of age ($n = 7–8$/group). The testing utilized an automated touchscreen operant system (Cambridge Neuropsychological Test Automated Batteries [CANTAB]) with abstract, trial-unique visual stimuli. Basic performance of the task was established prior to dosing, training with simultaneous presentation of sample and choice stimuli continued during dosing, and delays (1/2, 1 or 2 s) were introduced near the end of the dosing period. The MXC50 group performed more poorly than controls during delay testing at the end of the dosing period, as well early in training with simultaneous presentation. No interaction between treatment group and delay interval was found. No significant differences from controls were observed at the lower dose (MXC25). Thus, MXC at a daily dose of 50 mg/kg appeared to interfere in a general way with performance of this visual discrimination and memory task. Further investigation will be needed to identify the impairment leading to this performance deficit. © 2002 Published by Elsevier Science Inc.

Keywords: Monkey; Methoxychlor; Endocrine disrupter; Delayed non-match to sample; Adolescent

1. Introduction

One of the issues addressed in endocrine disruption research is possible effects on brain function from low-dose exposure to exogenous estrogenic agents. Estrogen is known to be important for brain function. Considerable information has been developed on the interaction of estradiol with cellular processes in the nervous system that helps explain how estrogen can influence brain function, including cognitive function [8].

There is extensive information on effects of estrogen administration on cognitive function in women. However, almost all human studies of estrogen effects on cognition use situations where background estrogen production is altered, e.g., changes in the menstrual cycle, changes at menopause and with hormone replacement therapy, or estrogen therapy after ovariectomy or for hypogonadal syndromes. These studies address the value of estrogen therapy to brain function in estrogen-deficient states [9,11]. Studies of memory have been particularly common and were one of the reasons for selecting a memory task for the current study. Based on human studies, it appears that exogenous estrogen can maintain verbal memory or restore verbal-memory deficits that appear with estrogen loss in women. An enhancement of verbal memory in the absence of loss of endogenous estrogen has not been demonstrated. Effects on nonverbal memory (spatial, visual) have not been shown; however, one study reported associations between endogenous estradiol and visual memory in men [7].

The question with respect to environmental estrogens is different; here, we would like to know whether exogenous estrogen influences brain function when the exogenous estrogen is examined against the background of an initially normally functioning endocrine system. Under these conditions, the exogenous agent could act synergistically with endogenous estrogen or could interfere with the action of endogenous estrogen through antagonistic or feedback mechanisms. Experience with selective estrogen receptor modulators also suggests that only some of exogenous estrogen functions might be influenced [9]. Finally, toxicants with estrogenic activity might have other biological

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actions that could influence cognitive function independent of pathways used by endogenous estrogen.

We examined this complex issue during the pubertal adolescent period when endocrine system maturation presumably provides a sensitive target for disruption by exogenous estrogens. During adolescence, the final stages of brain development occur under the influence of steroid hormones.

The agent selected for study was the estrogenic pesticide methoxychlor (MXC) [2]. Behavioral endpoints have been examined in studies of MXC toxicity in rats and mice [1,3,6,10]. The emphasis has been on administration of MXC during prenatal and/or early postnatal development, and on endpoints reflecting sex-differentiated behavior. Standard observational neurotoxicity tests have also been conducted.

Only one instance of cognitive assessment is available from rodent studies [1]. No effects of developmental MXC were found on one-trial passive avoidance with 24-h and 2-week retention tests administered to adolescent rats exposed from gestation day 14 through weaning.

This report presents preliminary data from a delayed non-match to sample (DNMS) test used to assess learning and memory in adolescent female monkeys treated with MXC. (A full report of the DNMS task will be provided after ongoing testing is completed.) The animals were tested during MXC dosing from 24 to 36 months of age, 6 months prior to 6 months after the anticipated age at menarche [12,13]. Adult levels of performance at this task are reached at about 24 months of age in rhesus monkeys [15]. A preliminary series of 70 training sessions with simultaneous presentation of sample and choices was followed by a series of 32 sessions using delay intervals of 1/2, 1, and 2 s prior to completion of dosing. Testing is currently continuing using this task during the recovery period.

2. Methods

Female adolescent rhesus monkeys (*Macaca mulatta*) were dosed daily for 1 year (ages 24–36 months) with MXC (98% pure, ICN Biomedicals, Aurora, OH) at one of two doses (50 or 25 mg/kg/day) or vehicle control ($n = 8$/group). Doses were individually weighed, mixed with fruit-flavored baby food vehicle in a syringe, and administered orally. If monkeys did not accept the baby food, the dose was placed in a marshmallow or piece of fruit. The basal diet (LabDiet 5047, PMI Nutrition International, Brentwood, MO), the commercial baby foods, and all other food items available to the monkeys were screened for phytoestrogen content. Body weight and food intake were monitored regularly and endpoints related to growth and reproductive and skeletal maturation were assessed at intervals. These data will be reported separately.

Monkeys were housed in pairs, but separated for behavioral testing. Behavioral testing was conducted in the home cages in 20-min sessions twice a week throughout the dosing period. The Delayed Non-Match to Sample Test (DNMS) from the Cambridge Neuropsychological Test Automated Batteries (CANTAB, CeNeS, Cambridge, UK) was used to assess visual discrimination and short-term memory [5]. In the DNMS test, the initial sample stimulus was a patterned box on the touch-sensitive video screen. After touching the sample box, the monkey was presented with two choices: a matching and non-matching patterned

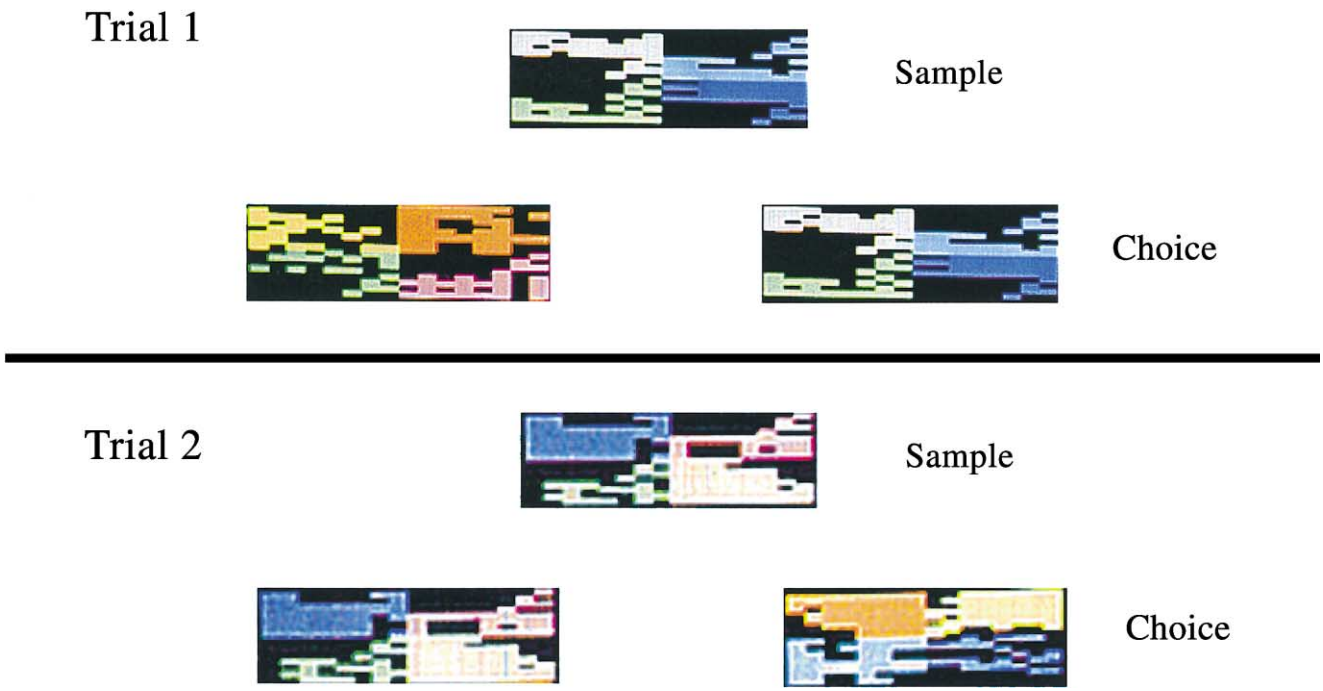

Fig. 1. Example of abstract patterned stimuli used for the DNMS task. The choice stimuli are shown below the sample stimulus.

box (see Fig. 1 for an example). A correct response was recorded when the monkey chose the non-matching box, and they received a sugar pellet reward for the correct choice. A correction procedure was used in which the same sample and choice pairs were repeated if an incorrect choice was made. If the monkey completed 60 trials (correction and noncorrection) prior to the end of the 20-min period, testing was discontinued. For this report, performance was assessed in terms of the percent correct responses of choices made, including both correction and noncorrection trials. Animals were not food deprived for testing; however, the daily ration of food was withheld until the completion of testing.

For all testing sessions, the following parameters were used: maximum length of sample stimulus — 20 s; maximum length of choice stimuli — 10 s; intertrial interval — 1 s; timeout (for incorrect or no response) — 5 s; maximum number of trials — 60. During the first 8 months (training period, 70 sessions), all 60 trials per session were performed in the simultaneous mode. In this mode, after the monkey touched the sample stimulus, the box remained on the screen when the two choice boxes appeared below it. Delays were introduced for the last 4 months of testing (32 sessions). For the first 20 of these testing sessions, 20 trials were performed in the simultaneous setting, 20 trials had a 1/2-s delay after the disappearance of the sample box and before the appearance of the choice boxes, and 20 trials had a 1-s delay between the sample stimulus and appearance of choice boxes. The trials at each delay were randomized throughout the testing session. For the next 8 sessions, a 2-s delay was added to each session with 15 trials simultaneous, 15 trials with a 1/2-s delay, 15 trials with a 1-s delay, and 15 trials with a 2-s delay. Lastly, the 1/2-s delay was removed for the last 4 testing sessions leaving the simultaneous, 1-, and 2-s delays at 20 trials each.

Data were summarized in blocks of four sessions, which provided homogeneous variability indices for repeated measure analysis of variance (RMANOVA). Data from the final three session blocks of the delay testing, after introduction of the 2-s delay, were analyzed separately in a two factor (group, delay) ANOVA. During the delayed choice sessions, the timing of dosing relative to the sessions was changed from 20 to 2 h for a series of sessions. No effect of time of dosing was found, and this variable was not included in the analysis.

3. Results

Monkeys in all groups gained weight during the treatment period, as anticipated. Fig. 2 shows that the mean weight of the three groups did not differ at the beginning or midpoint of the training sessions, at the beginning of delay sessions or at the end of the study. All monkeys reached menarche during the treatment period. Detailed evaluations of growth and maturation are ongoing and will be presented separately. This experiment also contained a DES "positive control" (0.5 mg/kg/day). The DES group was severely growth retarded, had arrested pubertal development, and did not attain menarche [4]. Their data are not included for comparison in this report because of this abnormal developmental profile.

During the 70 training sessions all but one monkey met a "participation" criterion of 90% of trials completed for at least half of the sessions. Data from this monkey (control

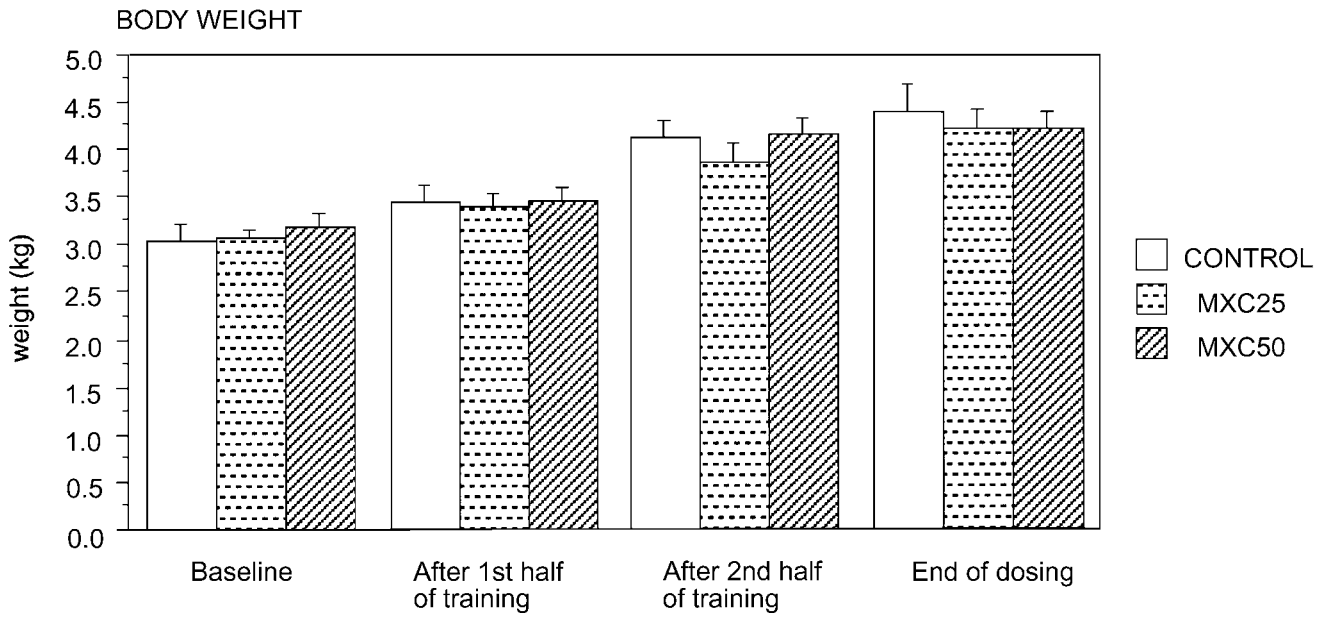

Fig. 2. Comparison of group weights (mean ± S.E.) at different stages of DNMS testing. There were no group differences.

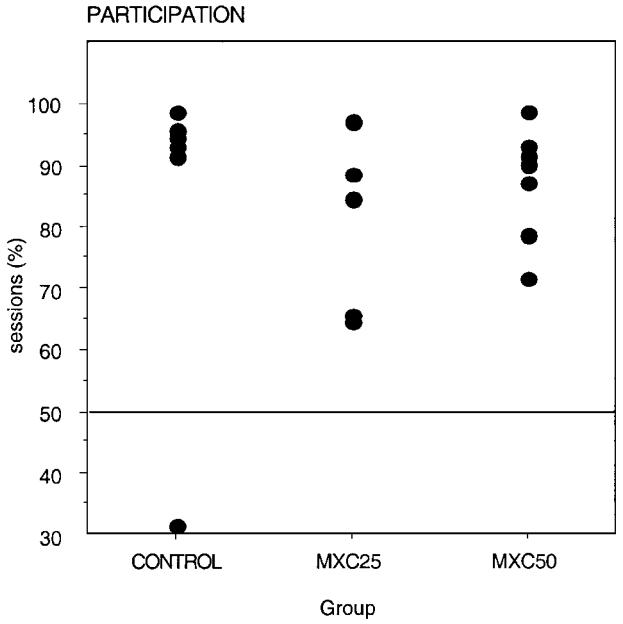

Fig. 3. Participation of individual animals in DNMS training. One control monkey failed to meet a criterion of 90% completion of the session on at least half of the 70-session training series and was eliminated from the data analyses.

group) were excluded from the analyses on this basis. The distribution by individual animal of sessions 90% completed is shown in Fig. 3.

Fig. 4 compares the performance of the three groups during the training period, summarized in four-session blocks. All groups improved their performance during training ($P < .001$, RMANOVA, blocks effect). The groups did not differ significantly in the mean percent correct during the training period ($P = .09$, RMANOVA), although the MXC groups generally had poorer performance than controls, particularly early in training. Further analysis indicated that percent correct during the first half of training was significantly ($P < .05$, Dunnett's test) lower in the MXC50 group than in controls. As indicated in Fig. 4, the MXC50, as well as the MXC25 group, showed an initial period of declining performance, followed by later onset of improved performance. Paired t test of improvement between successive session blocks demonstrated that the control group first showed significantly ($P < .05$) improved performance between Blocks 3 and 4, the MXC25 group between Blocks 4 and 5, and the MXC50 group between Blocks 6 and 7. Thus, the MXC groups were initially slower to show improved performance of this task.

Fig. 5 compares performance (percent correct) during the delayed choice period. The data are summarized in eight 4-session trial blocks. RMANOVA was conducted separately for trials conducted at each delay interval. A signifi-

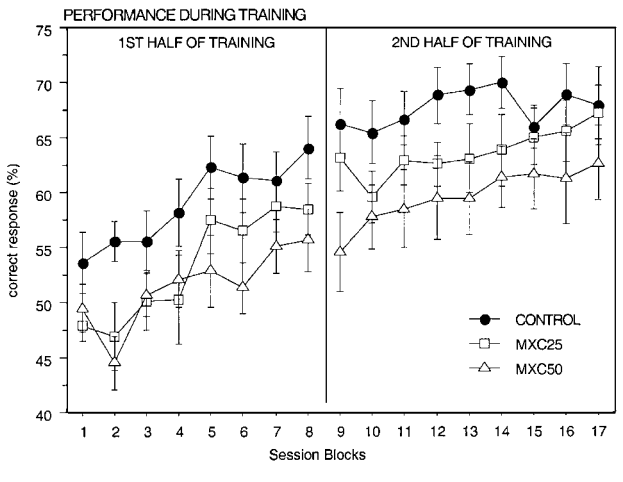

Fig. 4. Comparison of group performance during the 8-month DNMS training period. Percent correct is presented (mean ± S.E.). Each session block contains data averaged over four sessions with the exception of the initial block (five sessions) and the final block (two sessions). See text for statistical analysis.

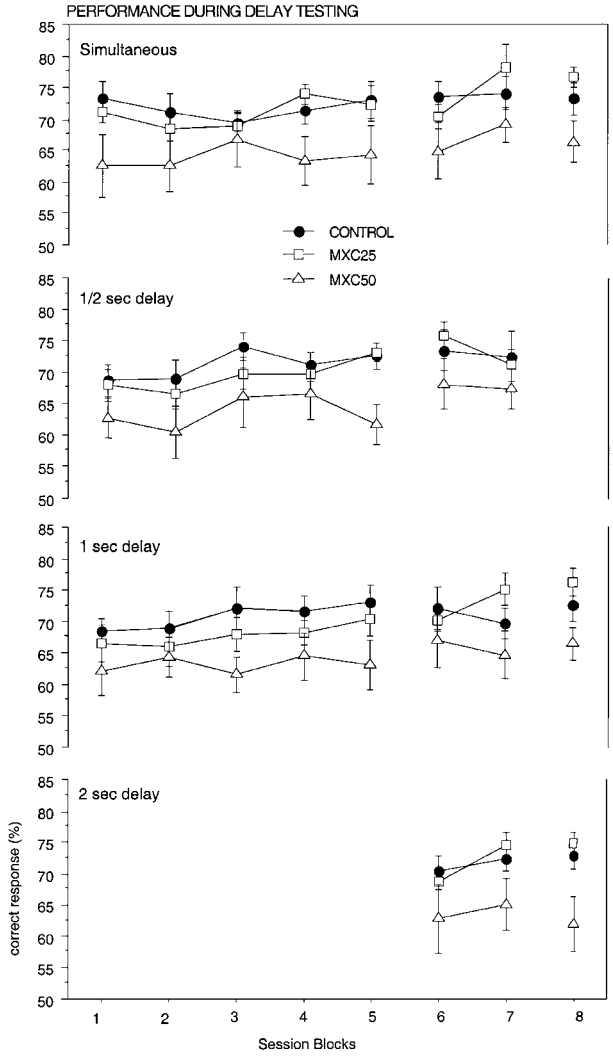

Fig. 5. Comparison of group performance during the 4-month delay testing period on the DNMS task. Each session block contains data averaged over four sessions. The 2-s delay was added after the fifth trial block and the 1/2-s delay was eliminated for the eighth trial block. See text for statistical analysis.

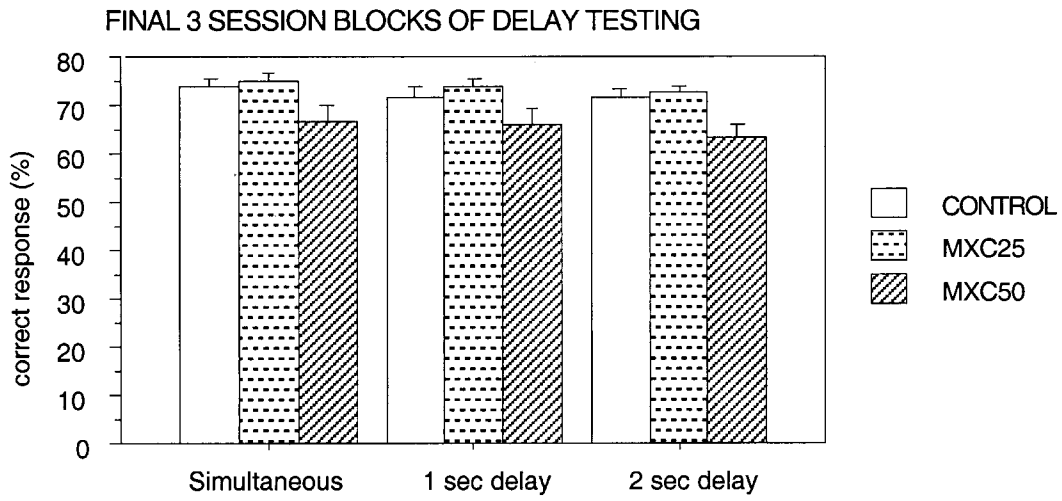

Fig. 6. Performance during the last three trial blocks of DNMS delay testing. Significant effects of group ($P = .03$) and delay interval ($P = .02$) were seen in RMANOVA. Performance of the MXC50 group was significantly below that of controls ($P = .04$).

cant effect of block was seen at the simultaneous and 1/2- and 1-s delays ($P < .001$), but not the 2-s delay. The treatment group effect was not significant in the RM-ANOVA for the 0-, 1/2-, or 1-s delays ($P = .09$, .11, 0.14, respectively). The group effect was significant for the 2-s delay ($P = .01$; Fisher PLSD post hoc tests, control vs. MXC50, $P = .01$; MXC25 vs. MXC50, $P = .01$).

Fig. 6 presents the data averaged over the last three trial blocks for the 0-, 1- and 2-s delays. RMANOVA identified a significant effect of group ($P = .03$) and of delay interval (repeated measure, $P = .02$). Performance was poorer in the MXC50 group than in controls (Fisher PLSD post hoc test, $P = .04$) or the MXC25 group ($P = .01$). Performance was poorer at the 2-s delay than with simultaneous presentation ($P = .004$). There was no significant interaction between group and delay interval.

4. Discussion

The visual discrimination task with trial-unique, abstract stimuli proved difficult for the young monkeys, as has been demonstrated previously [5]; however, all but one monkey reached a criterion of acceptable participation and performance during the 70-session training period. The MXC50 group appeared to perform more poorly than controls, and this comparison was statistically significant for the first half of training. Analysis of improvement between blocks indicated that the MXC groups began to show improved performance somewhat later in training than controls. No deterioration of performance was detected in the MXC monkeys with continued dosing.

During delay testing, the performance of the MXC50 group was consistently lower than that of the other two groups. The overall analysis of performance did not indicate a distinctive short-term memory deficit.

Further analysis of response latencies and performance measures may help indicate whether the poorer performance of the MXC50 group is due to an attention, response bias, or visual discrimination deficit. This information can be integrated with other measures, not reported here, that reflect the estrogenic effect of the MXC in these adolescent females. However, it should be noted that MXC has a different profile of estrogenic action than estradiol, has antiandrogenic effects [14], and may influence the CNS by mechanisms unrelated to its estrogenicity. Thus, it may not be appropriate to generalize from the present findings to the effects of other environmental estrogens.

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