

Effects of Depleted Uranium on Mouse Midbrain Catecholamines and Related Behavior

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Citation

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Abstract

Depleted uranium (DU) has been shown to have a variety of neurophysiologic effects. Humans demonstrate differences in cognitive testing and serum prolactin levels. Animals exposed to DU demonstrate changes in neurochemistry, development, and behavior. Because catecholamines mediate a number of behaviors we sought to determine if DU affects catecholamine metabolism and related behaviors. Mice were exposed to DU at 0 or 75mg/L in water for 2 weeks. Afterwards the animals were tested in an open-field apparatus. Two days later the animals from each group were injected with apomorphine (10mg/kg) or normal saline and tested again in the open-field. Afterwards, the midbrain concentrations of dopamine, tyrosine, 3,4-dihydroxyphenylalanine (DOPA), norepinephrine, epinephrine, and homovanillic acid were determined by HPLC. We found that DU elevates midbrain tyrosine levels while decreasing DOPA, norepinephrine and epinephrine levels. Dopamine and homovanillic acid levels were unaffected. Previous studies have shown that DU increases brain lipid peroxidation which is correlated with behavioral changes. To determine if lipid peroxidation was related to changes in catecholamines lipid-peroxidation was measured using the thiobarbituric acid method. We found that open-field activity and lipid peroxidation increased with DU exposure. No relationship was found between lipid peroxidation and any of the catecholamine related substances. Lastly, DU exposure did not significantly influence the behavior of mice when challenged with the dopamine agonist apomorphine.

INTRODUCTION

Depleted uranium (DU) is a by-product of uranium enrichment for nuclear energy and nuclear weapons production. DU has a variety of uses but the majority of DU finds its way into military applications. It is by the very nature of military use that DU enters the environment resulting in potential human exposure. Much human research has focused on the health effects of military personnel exposed to DU on the battlefield, especially DU exposure from shrapnel wounds. Despite the emphasis on studying DU exposure through shrapnel wounds it is believed most significant DU exposure comes in the form of dust inhalation¹.

At least one study has found subtle neuropsychological and endocrine effects of DU exposure on servicemen². Less work has been done on the potential effects of the long-term, low-dose exposure of civilians to DU that would remain in the environment after the cessation of hostilities. Studies that have examined the effect of DU on civilian populations have produced contradictory results and often suffer from methodological issues³.

DU is roughly half as radioactive as, but chemically identical to, unprocessed uranium leading many researchers to focus on the chemical toxicity of DU. Animal models of uranium (U) or DU exposure have shown it to accumulate in the brain and interfere with synaptic function^{4,5,6,7}. DU also appears to be active at the estrogen receptor⁸, the vitamin D receptor⁹, the retinoid receptor⁹, and to alter acetylcholine and serotonin activity¹⁰, although these findings are not always consistent¹¹. Our laboratory has consistently shown the DU exposure increases brain lipid peroxidation, a finding replicated by others^{12,13,14}. Brain lipid peroxidation parallels behavioral changes, such as dose related increases in spontaneous activity, in rats and mice exposed to DU^{15,16,17}. Changes in brain lipids may be partially explained by altered gene expression for cholesterol metabolism¹⁸. Interestingly, a variety of physiologic changes have been reported in the intestine of rats being administered DU in drinking water including altered histamine, prostaglandin and NO activity¹⁹.

Behavior is altered in DU exposed animals with changes in spontaneous behavior, grip strength, and working memory¹¹.

^{15, 16, 17, 20}. Others have not found behavioral effects ²¹. It is well-known that spontaneous motor behavior is modulated in part by dopamine (DA) activity and some studies have shown that U alters DA activity in animal brains ^{10, 11}. Others have not found such an effect ²². Humans also demonstrate behavioral responses to DU exposure with one report of Gulf War veterans demonstrating changes in neuropsychological performance ². Importantly, this same study also demonstrated altered prolactin levels in Gulf War veterans, a hormone partially regulated by DA.

The combined data suggesting that DU may alter DA activity, DA mediated hormone activity, and DA-mediated behavior lead us to further examine the effect of DU on DA. Because behavioral regulation is complex we examined the effect of DU on the catecholamine pathway from the tyrosine substrate through 3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), norepinephrine (NE), epinephrine (E) and the metabolite homovanillic acid (HVA.) Behavioral correlates and the effects of lipid peroxidation were also explored.

MATERIALS AND METHODS

Inhalation of DU dust is the typical route of human exposure; ¹ however it is difficult to replicate in the laboratory setting. For this reason, and its common use in other studies ^{10, 19, 22} we have chosen drinking water as the method of DU exposure.

A total of 46 male and female Swiss-Webster mice 30 days of age and housed under standard laboratory conditions were exposed to depleted uranyl acetate dihydrate at concentrations of 0 or 75mg/L in drinking water for 2 weeks. Ten to twelve animals were used in each gender and test group. Based on typical water consumption of 4.5ml/day this produces an estimated daily intake of 6mg/kg of elemental uranium for the experimental group. This dose of DU and the length of exposure have been shown to produce both biochemical and behavioral changes in mice without producing overt toxicity ¹⁷. Previous work in this laboratory, and others ⁷, has not found a significant effect on renal function using this type of design ¹⁷. This experimental protocol was approved by the University of Nebraska at Kearney's Institutional Animal Care and Use Committee.

After two weeks of DU exposure the animals were tested in an open-field apparatus (50cm x 50cm with grid markings) for 5 minutes. While in the open-field, line-crossing behavior was measured. The open-field is a test of spontaneous motor activity and exploration for laboratory

rodents ²³.

Two days after the first open-field test control and DU exposed animals were randomly given injections of either the dopamine D2 agonist apomorphine (10mg/kg) or vehicle. The open-field test was repeated. Differences in line crossing behavior from the first and second session were calculated by simple subtraction to give a single score. The dose of apomorphine was selected because of its documented ability to induce changes in dopamine-mediated behaviors ²⁴.

Two days after the final open-field test, the mice were euthenized with chloroform, heads removed, and brain quickly dissected onto a cold plate and the midbrain and frontal pole were dissected and frozen for chemical analysis. Midbrains were selected for analysis of catecholamine levels because of the location of dopamine containing nuclei. The frontal pole was a reference point, used in previous studies, to gauge the amount of lipid peroxidation in the brain ¹⁷. The two days allowed to elapse between apomorphine injection and brain extraction was more than sufficient time to eliminate any apomorphine effect on the brain (half-life 7-8.5 minutes in mice).

Frozen midbrains were dissected in half, along the midline, and one half was homogenized in a solution of 0.1N perchloric acid. The homogenate was centrifuged for 5 minutes. Concentration of tyrosine, DOPA, dopamine, norepinephrine, epinephrine, and HVA were determined by injection of 20microL of the supernatant into a high-pressure liquid chromatograph. The column consisted of a Zorbax SB C-18 4.6x250mm with 5-micron particles. The mobile phase consisted of 5% acetonitrile with 50mM KH₂PO₄, 100mg/L EDTA, 200mg/L 1-octane-sulfonic acid, pH 3.0 in water. The substances in question were detected with a UV detector at 240nm. This protocol was developed in this laboratory. Concentrations of unknowns were calculated from known concentration standards of tyrosine, DOPA, DA, NE, E. and HVA. All chemicals used for HPLC and the standards were purchased from Sigma Chemical, St. Louis, MO.

Lipid peroxidation was assayed using the thiobarbituric acid (TBA) assay ²⁵. Briefly, a weighed sample of frontal pole was homogenized and incubated with a solution of 3% TBA, 0.4 % SDS and 7.7 % acetic acid, pH 3.5, at room temperature, overnight. After incubation a mixture of butanol and pyridine (15:1) was introduced. The organic layer was removed and absorbance read at 532nm. Concentrations were calculated against known

concentrations of the standard malonaldehyde (MDA) and corrected for protein concentration of the sample, determined by the Coomassie blue method.

Data was entered into a Macintosh computer and analyzed with one-way or two-way ANOVA or correlation using Statview 5 and a statistical significance level of 0.05.

RESULTS

DU exposed animals appeared healthy to casual examination and no deaths resulted from the DU exposure.

EFFECTS OF DU EXPOSURE ON CATECHOLAMINE SYNTHESIS AND METABOLISM.

Midbrain levels of tyrosine, L-DOPA, DA, NE, E and HVA were analyzed by two-way ANOVA with gender and DU exposure as the independent variables. No gender differences were found for any of the examined substances, nor were there significant interactions between DU exposure and gender for any of the substances studied. Therefore, for ease of presentation, only DU dose effects are shown graphically (Figure 1). Significant effects of DU exposure were seen for tyrosine ($F(1,44)=9.75$, $p=.003$); DOPA ($F(1,44)=18.50$; $p<.0001$) and NE ($F(1,44)=15.15$, $p=.0003$). The effect of DU on epinephrine levels approached significance ($F(1,44)=3.67$, $p=.062$). There were no significant differences for DA or HVA.

Figure 1

Figure 1. Effects of DU on Catecholamines

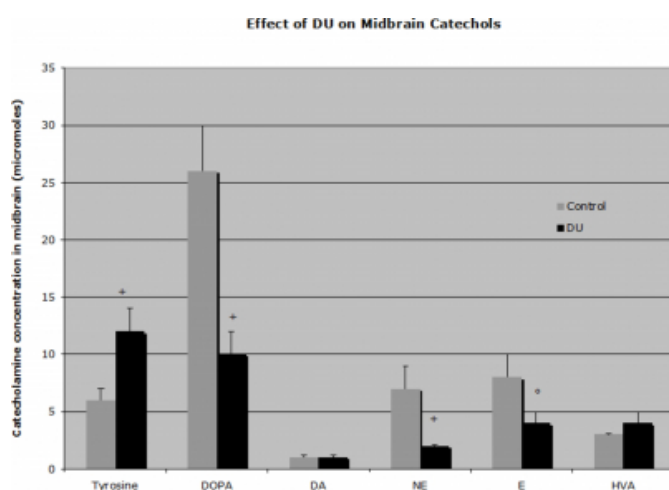


Figure 1. Effect of DU exposure on catecholamine levels in the mouse midbrain. “+” indicates significantly different from control ($p<.05$). “o” indicates $p=.06$. Error bars indicate SEM.

EFFECT OF DU ON LINE CROSSING

Line crossing was effected both by DU dose ($F(1,42)=4.70$, $p=.04$) and by gender ($F(1, 42)=13.04$, $p=.0008$) with a significant interaction between the two ($F(1,42)=6.74$, $p=.01$), females being affected more than males (see Figure 2).

Figure 2

Figure 2. Effect of DU exposure and gender on open-field line crossing.

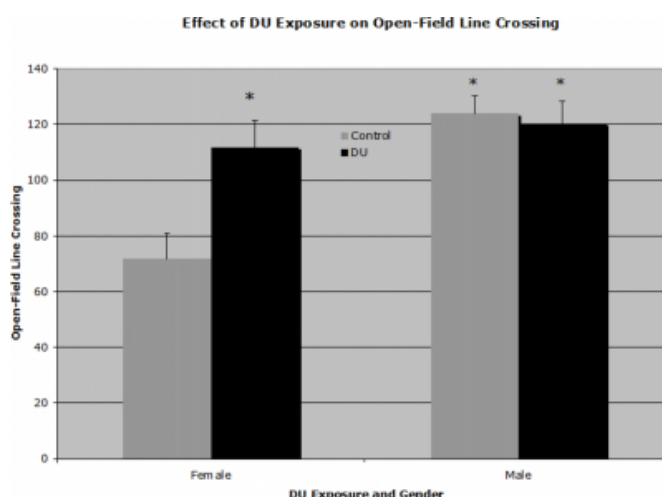


Figure 2. Open-field line crossing behavior was significantly affected by DU exposure and gender with a significant interaction between gender and DU exposure. “*” indicates significantly different from control female animals. Error bars indicate SEM.

EFFECT OF APOMORPHINE ON LINE CROSSING BEHAVIOR.

Open-field line crossing behaviors for the first and second open field test sessions were subtracted to give an indicator of activity change from the first to the second session. Line crossing activity change was analyzed with ANOVA with DU exposure, gender, and apomorphine or vehicle administration as the independent variables. The findings are presented graphically in Figure 3. There was a significant overall increase in behavior with apomorphine exposure when activity between the vehicle and apomorphine groups as a combined whole are compared ($F(1, 38)=3.80$, $p=.05$; data not presented). Moreover, gender effects were borderline ($F(1, 38)=2.87$, $p=.10$) and DU effects were not significant ($F(1, 38)=0.23$, $p=.64$). Most importantly, there was no significant interaction between DU exposure and apomorphine administration ($F(1, 38)=.015$, $p=.9$) which would be expected if DU exposure altered D2 receptor expression or activity in a manner different from control.

This suggests that DU exposure does not have an important effect on the D2 receptor, at least under these experimental conditions.

Figure 3

Figure 3. Effect of apomorphine and DU exposure on activity behavior

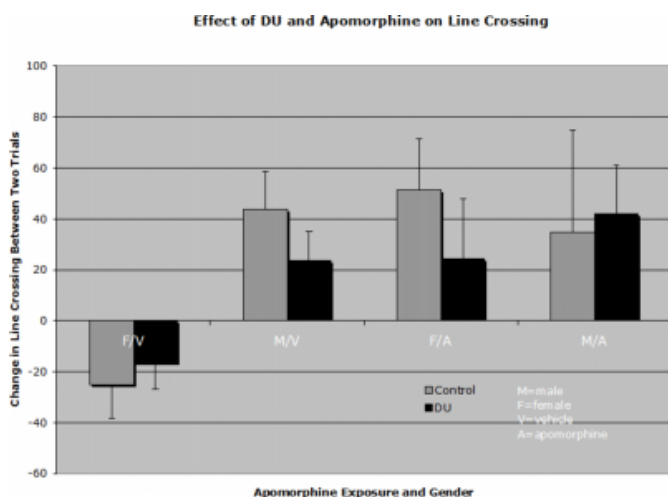


Figure 3. Change in open-field activity, second session compared to first. There was no statistically significant interaction between DU exposure and apomorphine administration. See text for discussion. (F=female; M=male; V=vehicle; A=apomorphine). Error bars indicate SEM.

RELATIONSHIPS BETWEEN DOPA AND BEHAVIOR

Line crossing behavior from the first open-field test forms a significant negative correlation with midbrain DOPA levels $r(44)=-.35$, $p<.05$, $r^2=.12$ (see Figure 4).

Figure 4

Figure 4. Line crossing behavior is correlated with DOPA levels.

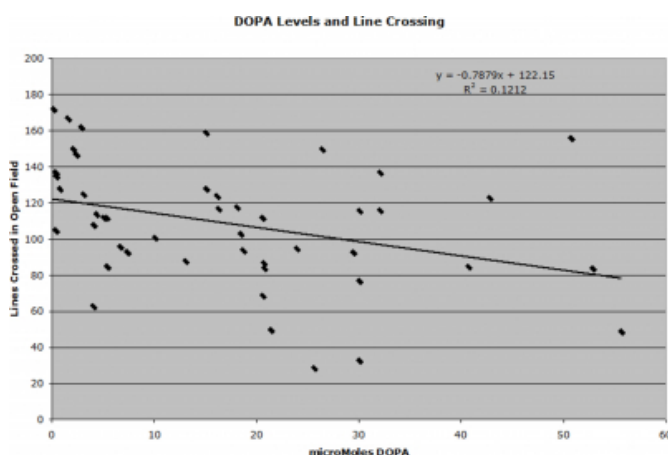


Figure 4. Line crossing and DOPA levels form a significant

negative correlation. $r(44)=-.35$, $p<.05$, $r^2=.12$.

DU EXPOSURE AND LIPID PEROXIDATION

Consistent with previous studies DU increases lipid peroxidation in the brains of DU exposed animals ($F(1,42)=6.31$, $p=.02$; Figure 5). There were no significant effects of gender or an interaction between gender and DU exposure. Lipid peroxidation levels were corrected for the protein content of the sample by dividing the raw lipid peroxidation levels by the sample's protein content. Importantly, lipid peroxidation levels did not correlate with the levels of tyrosine, L-DOPA, DA, NE, E, or HVA (data not shown).

Figure 5

Figure 5. DU exposure and brain lipid peroxidation.

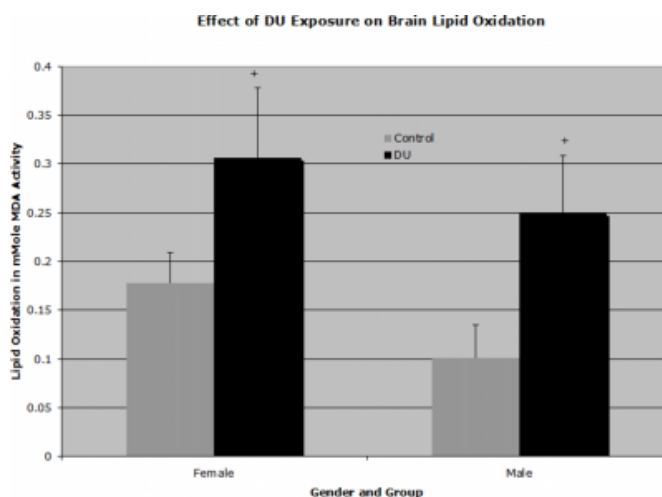


Figure 5. Brain lipid peroxidation levels are significantly increased in DU exposed animals. Lipid peroxidation levels are corrected for the protein content of the sample and expressed in millimoles MDA. "+" indicates significantly different ($p<.05$) from control. Error bars indicate SEM.

DISCUSSION

The animals in this experiment exhibited behaviors and biochemical changes seen in previous work, without exhibiting obvious toxicity from DU exposure¹⁷. In addition, this study provides evidence that DU exposure alters midbrain synthesis and metabolism of catecholamines. The elevated tyrosine levels accompanied by reduced DOPA levels suggests that DU may be inhibiting tyrosine hydroxylase, producing an accumulation of tyrosine with a resulting decrease in DOPA. This reduction in available DOPA supply would then explain the resulting reduction in NE, and the borderline reduction in E. The negative correlation of DOPA with line crossing behavior reinforces

the idea that reduced DOPA has an impact on the catecholamines regulating behavior. The potential effect of DU on the enzymes systems in questions should be examined directly.

There is not a statistically significant reduction in DA levels with DU exposure. This may be due to some of the tyrosine being converted to tyramine and then to DA²⁶. This is hypothetical and would need to be examined directly. The lack of effect on DA from U exposure is similar to the results of previous work¹¹ reporting an initial decrease in striatal DA after U exposure. The same study also reported that after a period of several days the DA levels returned to normal.

Interestingly, there have been reports of significant changes in rat brain DA activity only after 1.5 months of U exposure, with further changes reported after 6 to 9 months of exposure¹⁰. It may be that the length of exposure in our study was not sufficiently long to produce changes in DA activity. This may also explain why some studies found little effect on DA or serotonin from uranium exposure; the length of exposure was only one month²².

HVA levels were not affected in this experiment. Other studies reported no difference in (3,4-dihydroxyphenylacetic acid) DOPAC levels with U exposure¹¹, suggesting that no differences in HVA levels might be expected. HVA is only one breakdown product of the catecholamines, it is possible U exposure did not alter this particular metabolic pathway but does effect other pathways.

D2 receptor numbers were not altered with uranium exposure in a recent study¹¹. In this study the administration of apomorphine did not produce a differential effect of on open-field line crossing behavior that would be expected if DU exposure altered D2 activity or expression. The findings of this study and those of others¹¹ would seem to be in general agreement. It is possible that, if DU has an effect on DA receptors, that the effect is mediated via another receptor subtype.

Despite the seeming lack of DU's effect on DA, behavior was, as shown in Figure 2, altered in these animals. NE levels were altered; with suggestive changes in E levels in DU exposed animals. This is in contrast to the findings of others who did not find changes in NE levels from U exposure¹¹. These contradictory findings may be explained by differences in U exposure protocols. DOPA levels also correlated with line crossing activity suggesting a link

between altered catecholamine synthesis and spontaneous motor activity associated with DU exposure.

Midbrain lipid peroxidation levels were increased in the animals exposed to DU, a finding reported previously and linked to altered behaviors¹⁷. However, the mechanism by which lipid peroxidation may alter behavior is unclear. This study has found no statistically significant relationship between lipid peroxidation and tyrosine, DOPA, DA, NE, E or HVA levels. This suggests that the mechanism by which CNS lipid peroxidation alters behavior is not by this synthetic pathway. Other mechanisms need to be examined.

Overall, this study suggests that DU exposure alters the synthesis of the catecholamines NE and E, with little effect on DA in the short term. Longer lengths of exposure to DU may be needed to produce changes in DA levels. The behavioral changes seen with DU exposure may be explained by way of altered NE and E levels, or other mechanisms. Differences in the literature on the effects of DU and U exposure on brain neurotransmitter levels are probably the result of widely different experimental protocols. DU probably alters CNS activity and behavior by a wide variety of mechanisms.

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