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Animal Use statement:

The Experiments reported here were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No (NIH) 86-23 (1996). All procedures involving live animals were approved by the WPAFB Institutional Animal Care and Use Committee (IACUC) as protocol number F-WA-2005-0082.

Abstract:

In the mid 1990's, the use of tungsten as a replacement for lead and depleted uranium began for the manufacture of small arms munitions and armor penetrator munitions, respectively. Recent reports have demonstrated that tungsten can solubilize in soil and is present in some US drinking water supplies, however, little research has been conducted to determine the human health consequences of exposure. The purpose of this study was to use a battery of tests as an initial screen for potential neurobehavioral effects that may be associated with 70 days of daily tungsten exposure via drinking water. Sprague-Dawley rats were orally dosed with diH_20 vehicle, 5 or 125 mg/kg/day of sodium tungstate for 70 consecutive days. The rats were mated after 14 days and dosing continued through pregnancy up to post-natal day 21. Following sodium tungstate treatment, neurobehavioral tests were conducted on the adult females and their pups. Early neurobehavioral evaluations on the pups were done through tests of the righting reflex and maternal separation distress as measured by ultrasonic vocalizations. The adult females were tested for maternal retrieval, acoustic startle/pre-pulse inhibition (AS/PPI), spontaneous locomotor activity, and navigation in a watermaze. In the pups, a 78% increase in distress vocalizations was observed in the highest dose group as compared to controls and an interaction of sex and dose was found for righting reflex latencies. While there were no treatment related effects for maternal retrieval, AS/PPI or watermaze navigation, dose related effects were observed for measures of locomotor activity. Adult females treated with the low dose showed increased distance traveled, more time in ambulatory movements, and less time in stereotypic behavior than controls or high dose animals. Those receiving the highest dose had more time in stereotypical movements than controls, and less time resting than controls and the lowest exposure group. Results from this initial investigation suggest subtle neurobehavioral effects related to motor activity and emotionality warrant further investigation for oral exposure to sodium tungstate in adult females and their developing pups.

Keywords: Sodium tungstate, reproductive toxicology, rodents, developmental toxicology, neurobehavior, learning and memory, emotionality

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Introduction

In the 1990's, the U.S. Department of Defense started using "greener" alternatives, tungsten nylon and tungsten alloys, as replacement metals for lead and depleted uranium in the manufacture of small arms munitions and armor penetrator munitions, respectively (Koutsospyros et al., 2006). As a result, military personnel as well as civilians are at risk for increased exposure to tungsten on the battlefield either as shrapnel, aerosol plumes or from contact with soil, water or food sources contaminated with soluble tungsten (JOCG Environmental Subgroup, 2006). To date, there has been limited research into the negative health impact of tungsten exposure. With regards to exposure from shrapnel, there is evidence to suggest implanted tungsten alloys are toxic and tumorgenic (Kalinich et al., 2005). Additionally, the results suggested that coincident exposure of tungsten with other heavy metals, such as nickel and cobalt, results in synergistic carcinogenic effects (Kalinich et al., 2005). There is only minimal research into the health effects of oral exposure to tungsten; however tungsten environmental contamination has recently been implicated as a potential contributing factor to a cluster of childhood leukemia in an area around a military base (Seiler, 2004; Sheppard et al., 2007).

In addition to the carcinogenic potential, available reports from the Agency for Toxic Substances and Disease Registry (ATSDR) (2005) suggest oral exposure in pregnant dams can lead to negative end points in developing fetuses. Specifically, administration of a tungsten solution at dose levels as low as 0.005 mg/kg to pregnant rat dams for up to 8 months before and during pregnancy resulted in embryotoxicity and delayed fetal skeletal ossification (Nadeenko and Lenchenko 1977; Nadeenko et al., 1977, 1978). Published reports of neurobehavioral perturbations in animal models suggest tungsten may also have toxic effects in the brain and consequently, neurobehavioral responding. In the first study to report the neurotoxic effects from long term exposure to sodium tungstate (Nadeenko, 1966), the author reports cortical lesions and perturbations in conditioned reflexes. While limited in scope, these data suggest further investigation of the neurotoxic potential of tungsten is needed to more thoroughly delineate the health impact of exposure.

The purpose of the present study was to conduct a screen for neurobehavioral effects resulting from sodium tungstate exposure in adult females and their offspring. A small battery of neurobehavioral tests were selected from the comprehensive Navy Neurobehavioral Toxicity Assessment Battery (NTAB), based on results from earlier work with depleted uranium, one of the compounds being replaced by tungsten (Arfsten et al., 2007). The tests were chosen specifically to assess a range of neurological capacities (reflexive and gross motor responding, emotionality, and complex cognitive capacities) in both the exposed dams and their offspring. In the dams, reflexive motor functions were tested in acoustic startle and pre-pulse inhibition (AS/PPI) responses; emotionality was tested in maternal retrieval responding and spontaneous locomotor activity (SLA); gross motor coordination was assessed in the watermaze test and spontaneous locomotor activity (SLA); and more complex learning and memory functions were assessed in the watermaze. For the pups, emotionality was assessed in maternal separation distress as measured by their emitting ultrasonic distress vocalizations, and motor coordination development was measured in the righting reflex.

By testing the repeated oral exposure in both adult females and their offspring, effects in the developed and developing central nervous systems can be assessed. The results will provide an assessment that begins to fill the gap in neurotoxicological data related to tungsten, and can be used as guidance for selecting more targeted evaluation methods in the future.

Materials and Methods

This study followed methodologies in the USEPA Guideline OPPTS 870.3650 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Study. The protocol was extended beyond the minimum 54 days of treatment to accommodate evaluation of the development of the offspring through postnatal day (PND) 20 as well as their dams following the last dose on day 70.

Chemicals

Sodium tungstate dehydrate, CAS# 10213-10-2 with purity greater than 98%, was purchased from EMD Chemicals, Inc. (Gibbstown, NJ). The powder readily dissolved in a deionized water (diH_2O) vehicle for the concentrations used in this study at 5mg/ml and 125 mg/ml. Fresh solution was made daily and administered via oral gavage. Animals were weighed at the beginning of each study week, and a single bolus of solution was administered daily based on this weight.

Animals and Exposures

Sprague-Dawley rats (8 weeks old), 160 male, 160 female, were obtained from Charles River Laboratories (Wilmington, MA). Upon arrival to the vivarium the adult (e.g., P1) animals were single-housed and acclimated in quarantine for 14 days. At the end of the two week acclimation period, each was randomly assigned to one of three treatment groups (vehicle control, 5 and 125 mg/kg/day) for a total of 40 males and 40 females in each treatment group. Following 14 days of treatment, rats from like-treatment groups were mated. Dosing was continued through the 14 day mating period, the 22 day gestational period, and through to postnatal day (PND) 20 for a total of approximately 70 days of exposure. Prenatal pup exposure was from sodium tungstate crossing through the placenta (Nadeenko et al., 1978), and postnatal exposure was indirect via dams' milk (unpublished data from reporting laboratory).

Litters were culled on PND4 to eight pups. Selection of pups was pseudo-random to maintain a 4 male/4 female ratio whenever possible. Remaining pups were euthanized via CO_2 overdose. All pups were inspected for physical birth defects, including missing digits, however, none were found.

Neurobehavioral Procedures

Neurobehavioral assessments were conducted for the adult females and their pups according to the schedule in Table 1. The pups were tested for righting reflex (Pellis et al., 1991) and separation distress (Bekkedal et al., 1999; Hahn & Lavooy, 2005). The adult females were tested for maternal retrieval responding (Hahn & Lavooy, 2005), Spontaneous Locomotor Activity

(SLA), Acoustic Startle/Pre Pulse Inhibition (AS/PPI) (Buccafusco, 2001), and watermaze navigation using methods based on original Morris water-maze methods (Morris, 1984; Buccafusco, 2001).

Assessments in Pups

Righting Reflex – Development of early motor coordination was assessed in the pups with the test of righting reflex on PND4. Individual pups were placed in a supine position on a Plexiglas platform. The pups were gently held down by positioning an index finger along the abdomen. The finger was removed and the latency for the pup to roll over and obtain the prone posture with all four paws on the platform was timed. The procedure was immediately repeated two more times, for a total of three consecutive tests, and the scores were averaged for statistical analyses. If a pup failed to right within 60 seconds, it was classified as "timed out."

Separation Distress – Emotionality was measured in the pups by recording the ultrasonic distress vocalizations emitted upon separation from the dam and littermates on PND7. Pups were individually taken to a room separate from the home cages and placed in a small cylindrical jar containing enough bedding from the pups' home cage to cover the bottom of the jar. A Petterson Ultrasound Detector (D240) set for 38-40 kHz using heterodyning was attached with stereo audio cables to a Tascam (Da-20 MKII) digital audio tape recorder. The USV detector was hung 15 cm above the center of the jar which contained a small amount of home cage bedding. The total number of ultrasonic vocalizations was recorded for a 60 second period following the first vocalization. Following all data collection, a trained technician replayed all recordings using the same Tascam digital audio tape recorder and counted the number of vocalizations.

Assessments in Dams

Maternal Retrieval – Instinctual maternal responding was evaluated using the test of maternal retrieval on PND2. Home cages were removed from the rack and placed on a hard surface. The dam was momentarily removed from the cage while 3 pups were taken from the nest and moved to the opposite end of the cage. The dam was immediately placed back in the cage. The latency for the dam to retrieve all three pups and return them to the nest was recorded in minutes using a standard stopwatch. Pups and mom were reunited if the pups were not retrieved in 5 minutes and a timed-out was recorded.

Watermaze navigation: The watermaze navigation was used to evaluate motor coordination, learning and spatial memory (Morris, 1984) on days 85-88 (15-18 days post-treatment). The maze was a 4' diameter cylindrical metal tank (2' high) filled nearly to the top with water maintained at 22-25°C and treated with non-toxic, white tempera paint so it was opaque. A round escape platform large enough for the animal to stand on was attached to the floor of the tank, but submerged 1" below the surface of the water. Visual cues were located outside of the tank with a different shape (i.e. triangle, square, circle, diamond) designated for each quadrant of the tank. During training, the animal was placed in the water at a location distal from the escape platform, randomly placed into the different quadrants so that all quadrants equally served as start zones. The rat was allowed to swim until reaching the escape platform or until the 90 second time-out. The rat was removed from the tank, dried with a towel, given a 30 second rest period before the

next trial. Rats were trained for 3-5 trials per day, for three days until they could consistently swim to the platform in less than 15 seconds. On the final day (testing phase, day 88), rats were randomly started in all three of the quadrants where the platform was not located. The distance the animal swam and latency to find the platform were electronically recorded for up to 90 seconds using a Video-Max video tracking system and water maze software (San Diego Instruments (SDI) San Diego, CA).

Acoustic Startle/Pre-pulse Inhibition (AS/PPI): The AS part of the test was used to assess the integrity of the auditory reflex centers of the brainstem, while the PPI component measured the inhibitory controls over the reflex arc which has been shown to be influenced by higher brain centers (Koch, 1997). The AS/PPI test was conducted approximately 8 days after the final dose day (approximately on study day 78). Individual animals were placed inside a plastic testing tube-like enclosure with the head oriented in the direction of an audio speaker. Each animal enclosure rested on a motion sensing base which was hooked up to a San Diego Instruments SR-Lab Acoustic Startle control unit driven by a standard personal computer running DOS based SR-Lab software. The sensing base and testing tube were located inside of a standard Med-Associates operant chamber with an 8"h x 6"w clear plastic window on the front which allowed minimal ambient light and sound. Testing was performed with dimmed room lighting (standard fluorescent rooms lighting dimmed to < 22000 lumens) and software controlled background white noise set at 65dB to further minimize interference from outside stimuli.

The test session consisted of a 300 second adaptation period, followed by 3 baseline startle (115dB) only trials and then a series of 84 trials with inter-trial intervals ranging from 10-25 seconds and averaging 15 seconds. The stimulus presentation for these 84 trials was a random pattern of 1 of 5 conditions: background noise only (65dB), pre-pulse tone (80dB) only, startle tone (115dB) only, paired pre-pulse (70dB) + startle (115dB) or paired pre-pulse (80dB) + startle (115dB). In the paired pre-pulse + startle conditions, the pre-pulse tone preceded the startle tone by 100 msec. All response amplitudes and latencies were detected and recorded by sensors in the movement-sensitive platform. A complete session lasted approximately 25 minutes.

Spontaneous Locomotor Activity (SLA): Gross locomotor movements and exploratory behavior were evaluated in the adult females using the SLA task on day 77. Animals were individually placed in 17" x 17" clear plastic open fields with automated Opto-Varimex Animal Activity Monitors (Columbus Instruments, Columbus, OH). The activity meters had infrared photocells aligned 1 inch apart to detect horizontal movement, as well as differentiate small (stereotypic) movements from large movements. The apparatus was located in a sound attenuated room lit with a low illuminating red light (25-40 W). To begin the test, rats were placed in the center of the open field, and left uninterrupted for the duration of the 30 minute test session. Between each test, all boli were removed and the open fields were washed with a solution of 10% ethanol to remove any olfactory cues that may have been left behind. The following dependent measures were automatically recorded: distance traveled (cm), time spent resting (sec), time spent in ambulatory movements (sec), and time spent in stereotypical movements (sec).

Statistical analysis

All measures were analyzed using ANOVA. Repeated measures ANOVA were used where appropriate. The fiducial limit was $p<0.05$ and post-hoc comparisons were performed using Tukey's HSD analysis for pair-wise comparisons.

Results

During this study, adult rats were orally gavaged for 70 days with 0, 5 or 125 mg/kg/day sodium tungstate to evaluate neurobehavioral effects of dams and their offspring using a small battery of neurobehavioral tests selected from the NTAB. Exposure related effects to oral low and high doses of sodium tungstate were observed in both the pups and the dams. As summarized in Table 2, pup motor reflexes were affected in males only at both doses, but emotionality was affected only at the high dose. In the dams, opposing exposure effects were observed in the low and high dose groups related to locomotor activity and exploration.

Results in the Pups

Righting Reflex: For righting reflex, a significant interaction of treatment group by sex was found ($p < 0.05$) where male pups were generally faster than female pups in the low and high dose groups, but no sex difference was observed in the control group. This interaction is a result of responses in the females showing minimal differences between vehicle (13.5 ± 1.7), 5 mg/kg (14.8 ± 1.6) and 125 mg/kg (12.2 ± 1.5) while showing decreased latencies with increased dose in the males (11.6 ±1.8, 9.8 ±1.5 and 7.5 ± 1.4, respectively) (Figure 1).

Separation Distress: Pups showed exposure effects in the number of ultrasonic distress vocalizations recorded (Figure 2). Specifically, those in the high dose treatment group vocalized significantly more than both control and low dose groups of pups during the 60 second time period (40.5 ± 3.5, 23.9 ± 3.2 and 22.8 ± 2.9, respectively; $p<0.001$).

Results in the Dams

Maternal Retrieval and Watermaze Navigation: No effects of sodium tungstate exposure at either dose were found in the dams for latency of maternal retrieval (Figure 3), or watermaze navigation latency (Figure 4) or distance traveled (Figure 5).

Acoustic Startle/Prepulse Inhibition: For acoustic startle/prepulse inhibition, no significant main effects or interactions related to dose were found for any of the dependent measures. The startle response was reliable attenuated by the two different PPI/startle tone combinations (as measured as a percentage of startle only trials) for all treatment groups (Figure 6). As expected, a significant main effect was found for the startle tone-only trials (Figure 7) where the amplitude of the startle response was greater than for any other stimulus type, and this effect was consistent for all dose groups (startle tone only = 492.6 ± 18.9, 80dB only = 13.3 ± 0.6, 70dB + startle = 73.4 ± 4.7, and 80dB + startle = 39.3 ± 3.9; $p < 0.001$). As indicated by these numbers, the response to the paired pre-pulse (70dB) + startle (115dB) stimulus type was significantly greater than that of the (80dB) only and paired pre-pulse (80dB) + startle (115 dB) ($p < .05$). The response amplitude was still significantly less than that to the startle only suggesting the 70dB pre-pulse was less effective than the 80dB pre-pulse at attenuating the startle response.

Spontaneous Locomotor Activity: Exposure effects in the dams were detected for some of the measures of spontaneous locomotor activity. Specifically, dams treated with the low dose showed significantly more ambulatory time and distance traveled, as well as decreased time in stereotypies as compared to both controls and the high dose. In contrast, dams in the high dose demonstrated significantly less time resting, and more time in stereotypic movements than the vehicle controls or low dose group (Table 3).

Discussion

The series of neurobehavioral tests used for this study were selected to provide a screening-level assessment of a range of neurobehavioral capacities in sodium tungstate exposed dams and their offspring. The tests evaluated reflexive responding, emotionality and higher-level cognitive functions. Exposure-related effects were observed both in the dams and developing pups, and for low and high dose exposures.

These results are consistent with reports that the developing nervous system is preferentially vulnerable to number of neurotoxicants with alteration in cellular physiology resulting in neurobehavioral changes (Bellinger, 2004; Chang et al., 2006). Both tests in the pups, the righting reflex and separation distress, revealed changes. Specifically righting reflex latency showed unexpected sex differences such that increased exposure resulted in faster righting for males, but not females. There are no other reports of sex differences related to sodium tungstate exposure; however, these data suggest follow-up investigation should be completed. In the present study, the test was only conducted on one day, but in the future the pups could be retested on subsequent days to determine the developmental pattern of this effect and ascertain whether the sex difference disappears. The result also suggests that sex is a variable that should be included in all analyses in the event sex differences occur in other assessments.

A sex difference was not apparent in the test of emotionality, separation distress. However, there was a dose effect with those in the high dose group demonstrating a greater number of ultrasonic distress vocalizations when separated from the dam and littermates. Follow-up research could focus on other measures of emotionality that can be used to assess responding as the animals progress in their development, such as juvenile play (Ikemoto & Panksepp, 1992) and spontaneous locomotor behavior in a novel environment (Crawley, 1985).

Further investigation of locomotor behavior is also warranted in the dams as the results suggest increased exploration with the low dose and a contrasting increase in stereotypies with the high dose. The increased exploration is evident from increased ambulatory time and distance traveled. This change from baseline may represent hyperactivity or some other diminution of affective inhibition. Elevated gross motor activity in the low dose group is in opposition to the increased time spent in stereotypic movements in the high dose group. This increased time in stereotypies for the high dose exposure corresponds to a reduction in time spent resting in the high dose group. The altered stereotypical behavior suggests a subtle effect in the motor system that is not apparent in the measures of gross motor movements in the open field, or in the reflexive acoustic startle or prepulse inhibition responses. While this effect appears to be paradoxical, an alternative explanation is that the hyperactivity observed at the low dose is the early manifestation of the

progression towards the more pronounced stereotypies at the high dose. To resolve this issue, subsequent research will need to include additional intermediate doses and monitor for evidence of a progressive degradation towards uncontrollable stereotypic behavior.

The present study is the only report of neurobehavioral development following prenatal and postnatal exposure to sodium tungstate. While it provides evidence for neurological effects, the collection of results are insufficient to delineate a clear dose response in either the pups or dams, and the pattern of behavioral perturbations do not provide a clear indication of areas of the brain that may be more susceptible to the neurotoxic effects. In the pups, only two tests were used, and they measured very early, reflexive behavioral responses. A more thorough assessment covering a longer period of development would help delineate the reversibility of these deficits, and the impact they may have on subsequent neurobehavioral integrity.

For adult rats, the results reported here are consistent with the previous report that indicated oral exposure to sodium tungstate has neurobehavioral consequences. While the original citation provides only a minimal amount of details for study methods and results (Nadeenko, 1966), the author's conclusions indicate both gross morphological effects, as well as deficits in reflexive, classically conditioned responses. The changes were found for doses orders of magnitude lower than in the present study; however, exposure was substantially longer, continuing for eight consecutive months. The lack of detail for the location and extent of cortical lesions precludes the ability to associate them with the observed behavioral changes. Similarly, it is difficult to align deficits in reflex conditioning to the types of tests used in the current study.

This investigation addresses the paucity of neurobehavioral research related to oral exposure to sodium tungstate. The findings are consistent with the previous report that the brain is one of the vulnerable target organs (Nadeenko, 1966), and the effects are measurable in behavioral responses (Nadeenko, 1966; Nadeenko & Lenchenko, 1977). Additionally, the results support previous reports that prenatal and early post natal exposures are detrimental (Nadeenko & Lenchenko, 1977; Nadeenko, 1978), as are exposures in adults. Furthermore, it provides the basis for determining appropriate neurobehavioral tests that can be used to more clearly describe the neurotoxicological effect of sodium tungstate.

Conclusions

Results from this investigation provide a preliminary evaluation of neurobehavioral effects of sodium tungstate administered by oral gavage in adult rats and their progeny. Effects were apparent in both pre- and post-natally exposed pups and their dams, and at both doses tested. This supports the postulation that environmental exposure to sodium tungstate at the doses tested has neurobehavioral consequences. Furthermore, these results provide guidance for where to focus future efforts to more clearly delineate the developmental and long term neurobehavioral consequences of oral exposure to sodium tungstate.

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Table 1: Neurobehavioral Testing Schedule.

Test	Maternal Retrieval (Dams)	Righting Reflex (Pups)	Separation Distress (Pups)			SLA (Dams)	AS/PPI (Dams)	Watermaze Training (Dams)	Watermaze Test (Dams)
Test Day	PND2	PND4	PND7	PND20		Post-Dosing Day 7	Post-Dosing Day 8	Post-Dosing Days 15-17	Post Dosing Day 18
Other Procedure		Cull Litters		Weaning of Pups	Last Dosing (Day 70)				

Table 2: Summary of Results from Neurobehavioral Tests in Pups and Dams.

Neurobehavioral Tests	Sodium Tungstate (mg/kg/day)	
	5	**125**
Righting Reflex (pups)	reduced latencies in males	reduced latencies in males
Separation Distress (pups)	no effect	increased counts
Maternal Retrieval (dams)	no effect	no effect
Watermaze (dams)	no effect	no effect
Acoustic Startle/Prepulse Inhibition (dams)	no effect	no effect
Spontaneous Locomotor Behavior	increased exploration	increased stereotypy

Table 3: Spontaneous Locomotor Activity (SLA) (Adults).

Neurobehavioral Tests	Vehicle Control	Sodium tungstate (mg/kg/day)	
		5	**125**
Distance Traveled (cm)	5521.46 ± 688.75	8640.22 ± 420.73*	5532.32 ± 293.34
Resting Time (sec)	933.49 ± 23.91	925.64 ± 26.72	829.12 ± 29.05†
Ambulatory Time (sec)	131.42 ± 30.08	306.21 ± 12.85*	82.09 ± 4.34
Time in Stereotypic Movements (sec)	735.09 ± 26.25	568.15 ± 19.83*	888.79 ± 25.99#

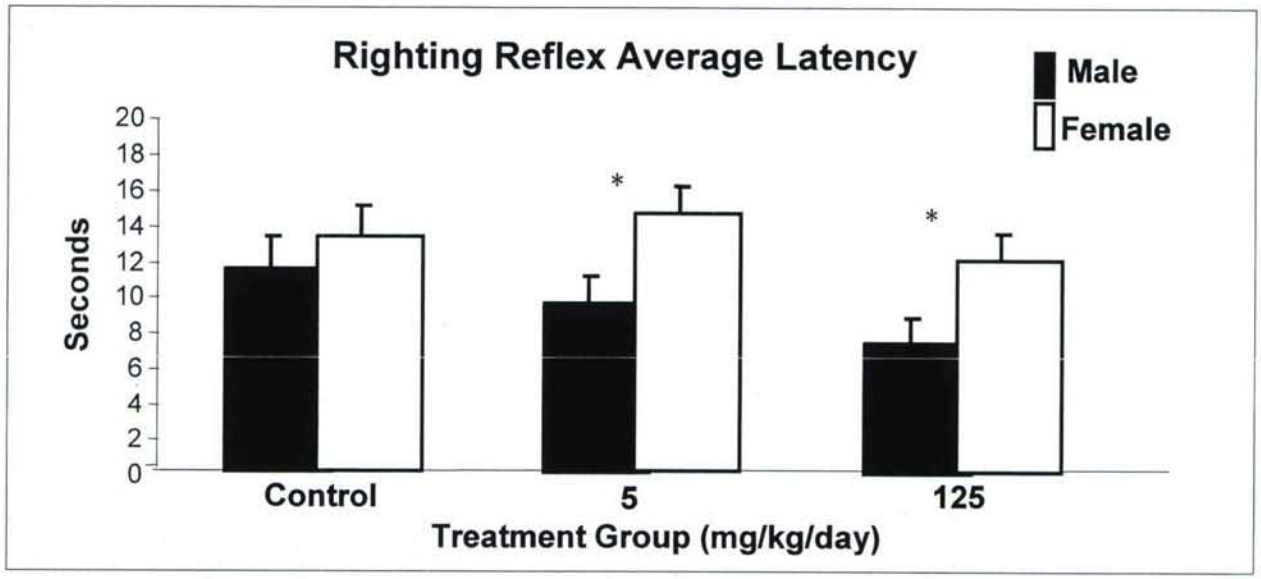
Means and Std Errors for Activity:

* = Significantly different from control and 125 mg/kg/day. **p <0.001**

† = Significantly different from control and 5 mg/kg/day. **p <0.05**

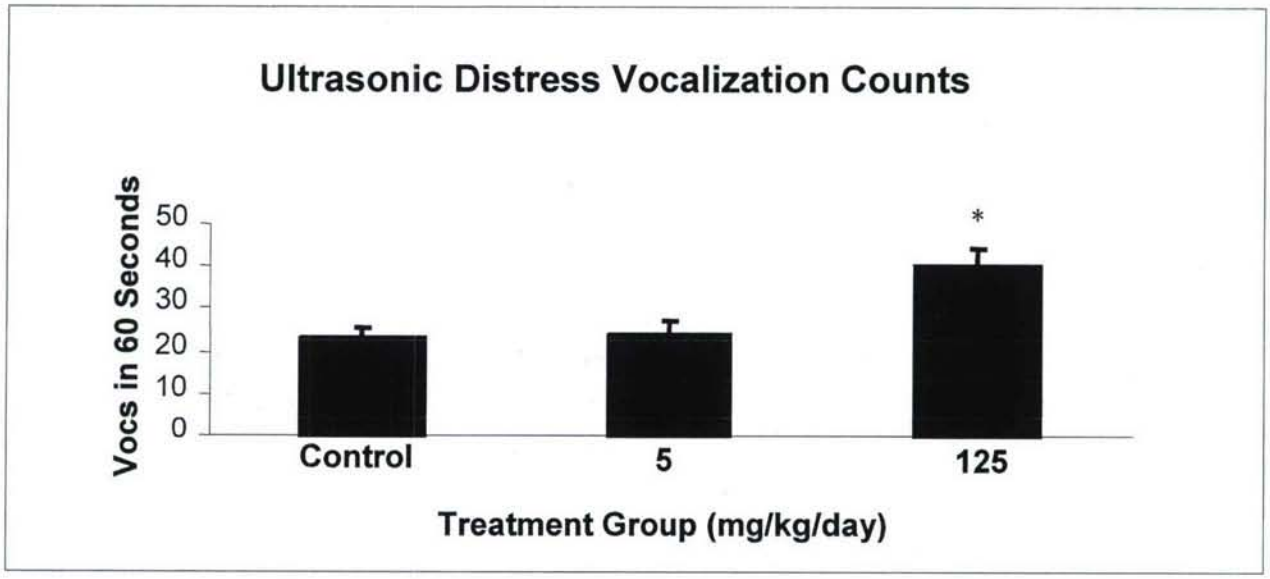
= Significantly different from control. **p <0.05**

Figure 1. Righting Reflex Average Latency for Pups.



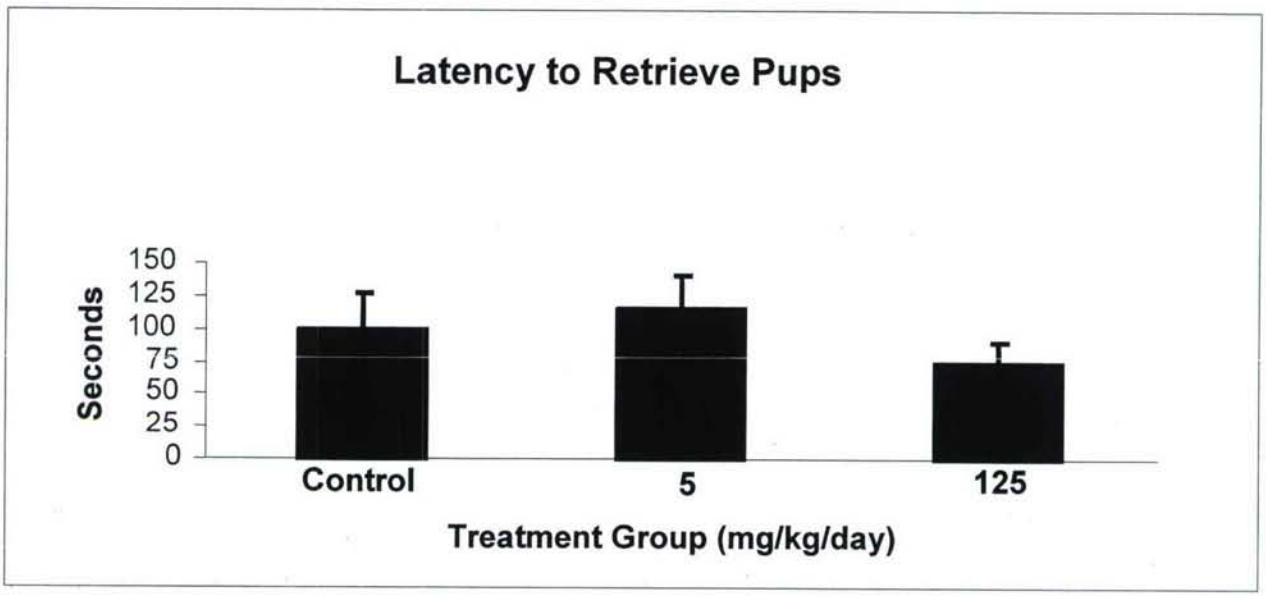
Average latency for the PND4 pups to roll from supine position to have all four paws on the platform. There was a significant interaction of treatment group x sex resulting from a relatively stable latency in the females, and a decreasing latency with increased dose in the males. * = significant sex difference ($p<0.05$).

Figure 2. Ultrasonic Separation Distress Vocalization Counts for Pups.



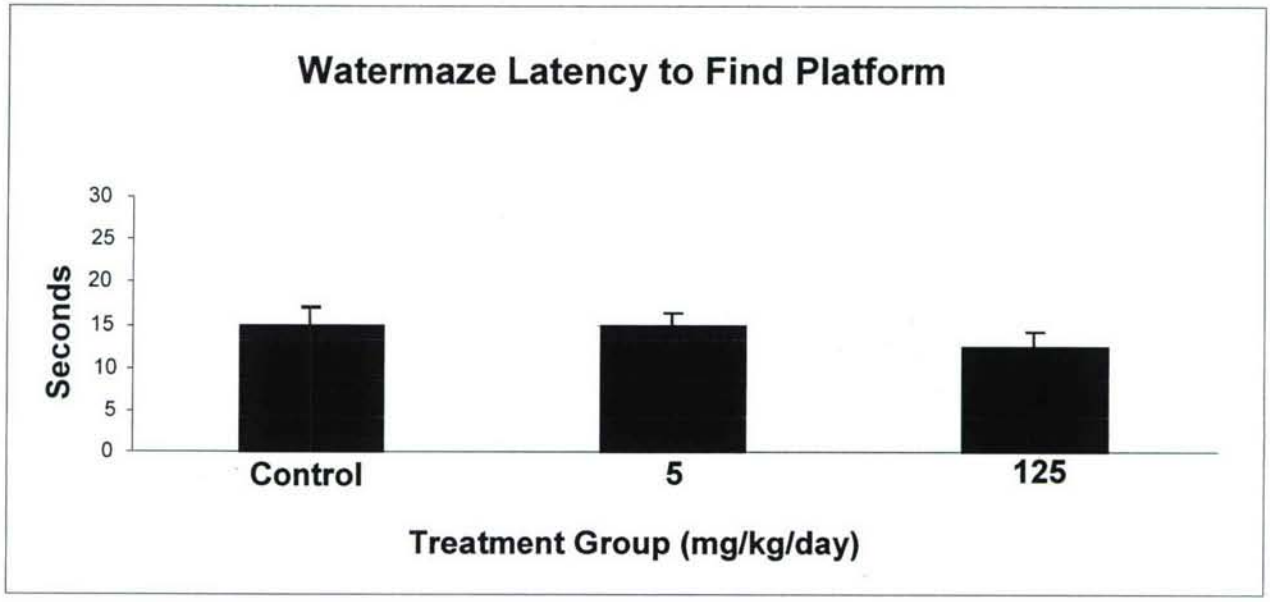
The number of ultrasonic vocalizations during a 60 sec time was recorded for individual pups on PND7. There was a significant main effect for treatment where high dosed pups vocalized significantly more than the control or low dose groups. * = significant difference from control and 5mg/kg/day groups. ($p<0.001$).

Figure 3. Latency to Retrieve Pups.



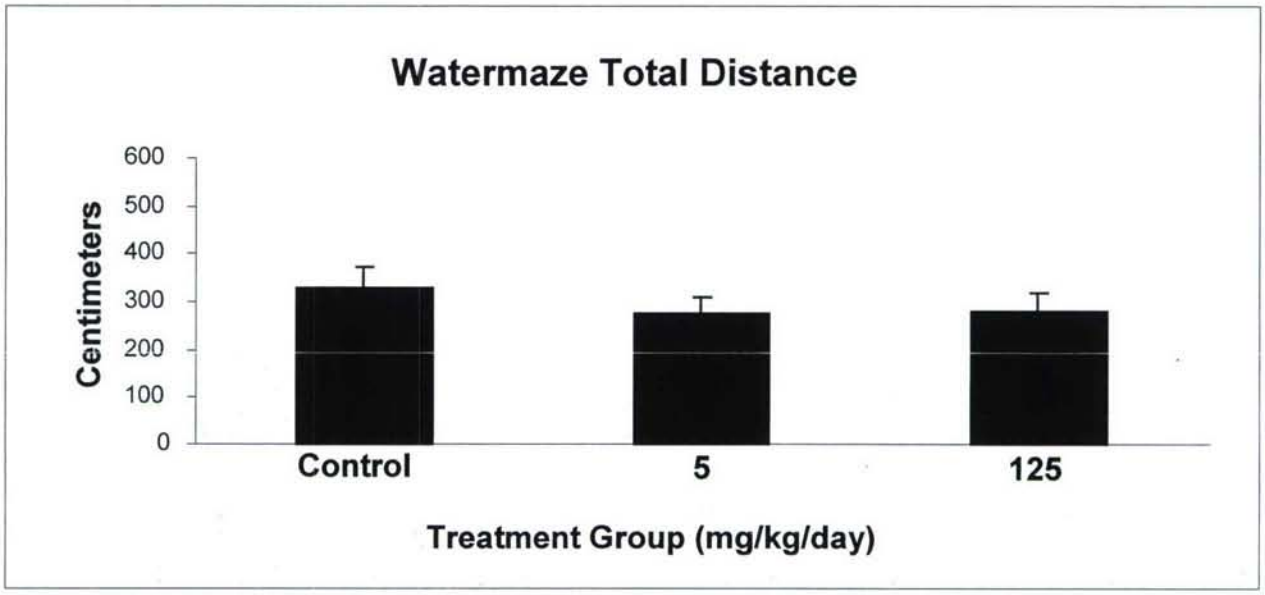
The latency for dams to return pups to the nest following separation from the rest of the litter was recorded. No significant treatment effects or interactions were observed.

Figure 4. Watermaze Latency to Find Platform for Dams.



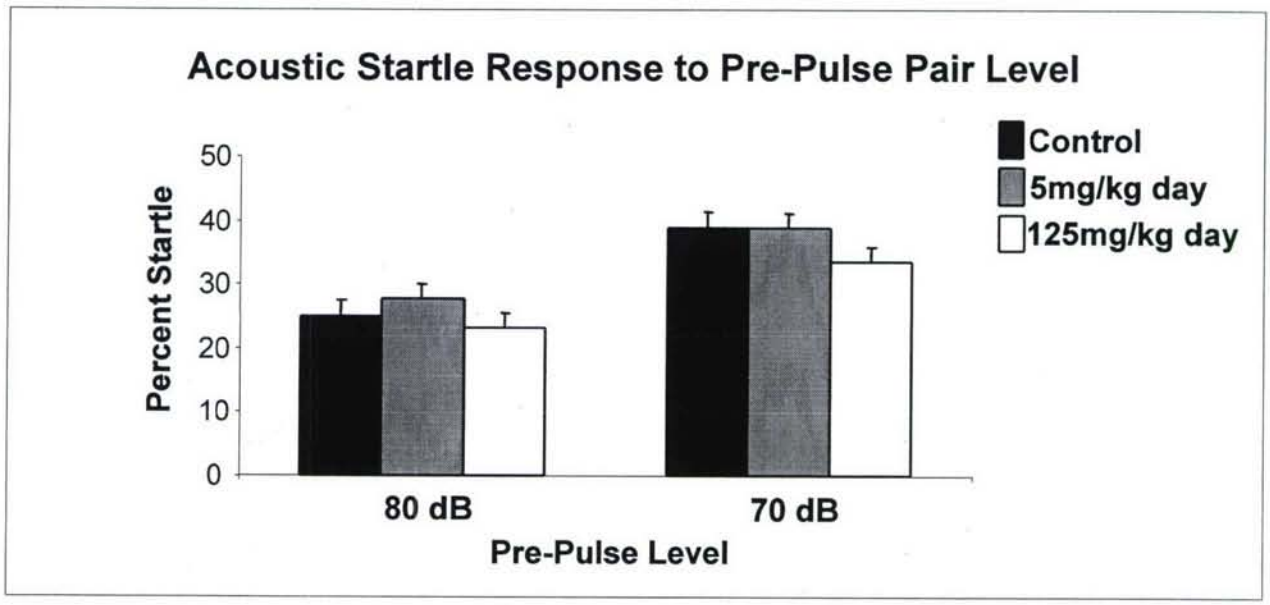
Rats were trained to use visual cues to locate a submerged platform in the water tank. The latency to swim to and stand on the hidden platform on the test day was recorded with a tracking system and software. There were no significant main effects or interactions for either dose group.

Figure 5. Watermaze Total Distance for Dams.



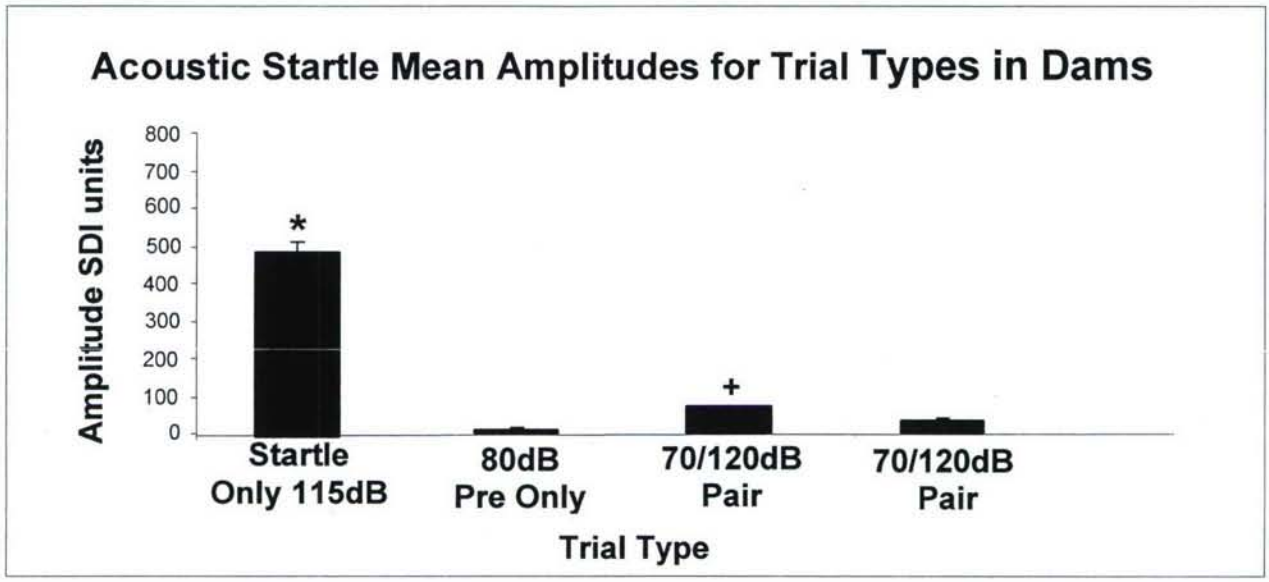
Rats were trained to use visual cues and swim to locate a submerged platform in the water tank. The total distance traveled to find the hidden platform was recorded. There were no significant main effects or interactions in either dose group.

Figure 6. Acoustic Startle Response to Pre-Pulse Pair Levels in Dams.



For the acoustic startle task, two different pre-pulse tones were paired with the startle tone, and the attenuation of the startle response was assessed. Both paired PPI + startle tones responses, measured as a percentage of the response to startle only tone, showed no main effect or interactions related to dose.

Figure 7. Acoustic Startle Mean Amplitudes for Trial Types in Dams



The amplitude of the reflex response shows the expected effect of a significantly greater amplitude for the startle tone only trials than for any other stimulus type. There was also a statistically significant effect for response amplitude in the 70dB + startle paired trials. The difference reflects an attenuation of the startle response, however, less than was observed for the 80db + startle paired trials. There was no main effect or interactions related to dose. * = significantly different from all other stimulus types. ($p < 0.001$). + = significantly different from 80dB and 80/115 dB Pair. ($p < 0.001$).

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