Original Article

Uranium modifies or not behavior and antioxidant status in the hippocampus of rats exposed since birth

Philippe Lestaevel, Bernadette Dhieux, Olivia Delissen, Marc Benderitter
and Jocelyne Aigueperse

*Institut de Radioprotection et de Sécurité Nucléaire, Direction de la RadioProtection de l'Homme,
Service de Radiobiologie et d'Epidémiologie, Laboratoire de RadioToxicologie Expérimentale. IRSN, BP 17, 92262
Fontenay-aux-Roses Cedex, France*

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ABSTRACT — In view of the known sensitivity of the developing central nervous system to pollutants, we sought to assess the effects of exposure to uranium (U) — a heavy metal naturally present in the environment — on the behavior of young rats and the impact of oxidative stress on their hippocampus. Pups drank U (in the form of uranyl nitrate) at doses of 10 or 40 mg.L⁻¹ for 10 weeks from birth. Control rats drank mineral water. Locomotor activity in an open field and practice effects on a rotarod device decreased in rats exposed to 10 mg.L⁻¹ (respectively, -19.4% and -51.4%) or 40 mg.L⁻¹ (respectively, -19.3% and -55.9%) in compared with control rats. Anxiety (+37%) and depressive-like behavior (-50.8%) were altered by U exposure only at 40 mg.L⁻¹. Lipid peroxidation (+35%) and protein carbonyl concentration (+137%) increased significantly after exposure to U at 40 mg.L⁻¹. A significant increase in superoxide dismutase (SOD, +122.5%) and glutathione peroxidase (GPx, +13.6%) activities was also observed in the hippocampus of rats exposed to 40 mg.L⁻¹. These results demonstrate that exposure to U since birth alters some behaviors and modifies antioxidant status.

Key words: Locomotor activity, Anxiety, Depression, Oxidative stress, Radionuclide

INTRODUCTION

Uranium (U) is an element belonging to the actinide group, found in rocks, soils, water, air, plants, and animals. The population may be chronically exposed to low levels of U from the environment through food (Kurtio *et al.*, 2002). U may be also introduced into drinking water supplies through the mining and milling of U ore (Cothorn and Lappenbusch, 1983). Moreover, in recent years, the use of U in nuclear power-reactors and munitions has given rise to a new exposure route for this heavy metal (López-Abente *et al.*, 2001; McDiarmid *et al.*, 2004; Abou-Donia *et al.*, 2002). Although nephrotoxicity is considered to be the most serious potential effect of U exposure (Diamond *et al.*, 1989), it may have other possible adverse effects of U on organisms. For example, it has been shown that U is a developmental toxicant with dose-related fetal toxicity (Arfsten *et al.*, 2001). U crosses the blood-brain barrier (Lemerrier *et al.*, 2003) and accumulates in the rat brain after chronic exposure (Pellmar *et al.*, 1999a; Paquet *et al.*, 2006), and several experimental

studies have found that it has a toxic effect on the behavior of adult rats (Briner and Murray, 2005; Bellès *et al.*, 2005; Houpert *et al.*, 2005; Lestaevel *et al.*, 2005). This behavioral toxicity is expressed by increased locomotor activity (Briner and Murray, 2005; Bellès *et al.*, 2005), significantly decreased spatial working memory capacities (Houpert *et al.*, 2005), increased anxiety (Houpert *et al.*, 2005), and shorter paradoxical sleep (Lestaevel *et al.*, 2005). In addition to the cerebral cortex (Lestaevel *et al.*, 2013), the hippocampus also seems to be a major target of U-induced neurotoxicity (Pellmar *et al.*, 1999b).

The hippocampus is a major component of the brain. It belongs to the limbic system and plays a number of important roles. It is involved in mood disorders, such as anxiety and depression (Deacon and Rawlins, 2005; Bannerman *et al.*, 2004). Oxidative stress may be associated with these and other cognitive disturbances (Floyd, 1999; Thiels and Klann, 2002), and the deleterious effects of oxidative insult appear to affect the hippocampus strongly (Bonatto *et al.*, 2005; Serrano and Klann, 2004). Oxidative stress is caused by oxidants, particular-

Correspondence: Philippe Lestaevel (E-mail: philippe.lestaevel@irsn.fr)

ly reactive oxygen species (ROS) and reactive nitrogen species (RNS), which overload the antioxidant defense system developed by cells to counteract oxidation. Oxidative insult may disrupt cell structures and functions that depend on the critical redox balance. The reactive species can inflict significant oxidative damage, especially on membrane lipids, and cause lipid peroxidation (Videla and Fernández, 1988). Previous studies have demonstrated that chronic U ingestion induces lipid peroxidation and antioxidative defense mechanisms in the brain of adult rats (Linares *et al.*, 2007; Lestaevel *et al.*, 2009).

Although immature brains are known to be more susceptible to some toxic effects, only a few published studies describe animals exposed to U during this developmental period. Briner and Abboud have demonstrated that mice exposed to U during development, through exposure of their dams for two weeks before and after mating, show altered learning and habituation at 21 days of age (Briner and Abboud, 2002). Slight effects of U were also observed on the learning profiles of the offspring of adult rats exposed before mating, for passive avoidance and water maze tests (Albina *et al.*, 2005; Sánchez *et al.*, 2006). Certain environmental contaminants can induce and exacerbate developmental neurotoxic effects when these agents are present during a critical period of neonatal brain development (Eriksson, 1997; Eriksson *et al.*, 2010). Rapid developmental and biochemical changes occur during the brain growth spurt (Eriksson, 2008), which for rats is the neonatal, during the first 3–4 weeks after birth. Thus, we decided to investigate the effects of exposure to U, including during this period. Exposure to U started just after the animals' birth and continued for 10 weeks. Their anxiety, depression, motor coordination, and locomotor activities were studied, as well as the redox environment of their hippocampi. This study used dosages of 10 and 40 mg.L⁻¹ of U in drinking water. The highest concentration of U found naturally in mineral water is 12 mg.L⁻¹ (Juntunen, 1991). These dosages were not considered nephrotoxic (Souidi *et al.*, 2005).

MATERIALS AND METHODS

Animals

Experiments were conducted in accordance with the recommendations of the European Animal Care Commission (Act no. 87-848). All procedures were performed by scientists accredited by the French Ministry of Agriculture (license of first author, no. 92-254).

This study used pregnant female Sprague-Dawley rats (Charles River, L'Arbresle, France), individually housed during gestation under standard conditions (21 ± 1°C)

with a 12-hr light/12-hr dark cycle (light-on from 08:00 to 20:00 hr), and their offspring. Animals had free access to food and water *ad libitum*.

Around 24 hr before the birth of the pups, the female rats were subdivided into three groups (n = 14 females for each group). One group was contaminated by mineral drinking water supplemented with U in the form of uranyl nitrate (AREVA), which produced a dose of 10 mg.L⁻¹ (about 0.25 mg.day⁻¹ per rat). The second group was contaminated with U by drinking water at a dose of 40 mg.L⁻¹ (about 1 mg.day⁻¹ per rat). The specific activity of U is 1.4 × 10⁻⁴ Bq.g⁻¹, with an isotopic composition of ²³⁴U = 0.0055%, ²³⁵U = 0.255%, and ²³⁸U = 99.74%. Females were exposed to U throughout the lactation period. Control female rats drank uncontaminated water. After weaning on postnatal day 21, male pups' exposure to U *via* drinking water (10 or 40 mg.L⁻¹) continued until they reached 10 weeks of age. One male offspring per litter was assigned to behavioral tests (n = 12 for each group, the same animal for all four tests). Health variables, *i.e.* body weight, water consumption, and food intake, were measured at the end of the pups' U exposure (at age 10 weeks).

Behavioral tasks

Male pups (n = 12 for each experimental group) underwent behavioral evaluation tests at age 10 weeks. Their behavioral functions were evaluated with four commonly used tests: the open-field test, the elevated plus-maze, the rotarod test, and the Porsolt test. Behavioral tasks were performed in a room with constant temperature (21 ± 1°C) and light conditions (60-W light). No test required food deprivation, reward, or punishment. The tests were recorded by a video camera and were read by a trained observer unaware of the exposure conditions.

Open-field test

During the first day of behavioral testing, locomotor and exploratory activities were assessed with an open-field test, the methodological details of which are reported elsewhere (Prut and Belzung, 2003). Briefly, each rat was moved from its home cage to the center of an open-field (45 cm × 45 cm), equipped with invisible infrared beams, with lines every 2.5 cm and one line 16 cm high, monitored by an automated activity-monitoring system (Bioseb, Vitrolles, France). Counting occurred when the rat moved horizontally, showing predominantly locomotor behavior, or vertically (rearing), showing exploratory activity (Rocha and de Mello, 1994). The numbers of horizontal and rearing movements were recorded over a 20-min period.

Elevated plus-maze

The second day, anxiety-like behavior was evaluated by the elevated plus-maze, an apparatus that consists of two facing open arms (50×10 cm) and two opposed enclosed arms without a roof ($50 \times 41 \times 10$ cm), with an open square (10×10 cm) in the center. The maze is elevated 50 cm above the floor. For the test, each animal was placed in the center of the maze, facing one of the open arms. The number of entries and the time spent in open and closed arms was recorded for 5 min (Dunham and Miya, 1957).

Rotarod test

Motor coordination and a practice effect were evaluated on a rotarod the third day of the behavioral tests (Ugo Basile, Monvalle, Italy). For motor coordination, rats were placed on the rotarod at 5 rpm without previous training. For the practice effect (learning ability), rats were then placed on the bar two more times the same day, again at 5 rpm. The time each animal remained on the rotating rod was recorded. The time limit was set at 3 min (Darnaudéry *et al.*, 2004).

Porsolt test

The forced swimming test (or Porsolt test), which was the most stressful test applied in this study, was performed the fourth day. The rats were individually placed in a glass cylinder (height 60 cm, diameter 40 cm), containing enough water so the hind legs could not reach the bottom of the cylinder but the tail could. The water was maintained at 23–25°C and the rats were left for 10 min. Immobility was measured during the last 5 min of the test. The Porsolt test was administered once. The animal was judged to be immobile when it floated in an upright position and made only minimal movements to keep its head above water (Bourin *et al.*, 2004).

Oxidative stress analyses

After behavioral tasks, animals were anesthetized by inhalation of 95% air/5% isoflurane (Forène, Abbott, Rungis, France). After decapitation, the hippocampus was dissected out in ice, frozen immediately in liquid nitrogen, and stored at -80°C for later biochemical analyses ($n = 8/\text{group}$). The tissues were homogenized in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 1 mM EDTA. The homogenates were centrifuged at 10,000 g for 15 min at 4°C, and the supernatants were collected for analysis. Protein concentrations were determined by the Bradford method, with bovine serum albumin as the standard.

Indicators of oxidative stress

As an index of lipid peroxidation, we measured the concentrations of hydroperoxides. Concentrations were determined with a commercial kit supplied by Cayman Chemical (Interchim, Montluçon, France). This lipid hydroperoxide assay kit measures hydroperoxides that directly utilize redox reactions with ferrous ions to produce ferric ions. The resulting ferric ions were detected with thiocyanate ion as the chromogen. Each assay was performed in duplicate, and concentrations are expressed as μM (Lestaevel *et al.*, 2009).

Highly reactive free radical species can oxidize lipids but also proteins. The most general indicator and by far the most commonly used marker of protein oxidation is protein carbonyl concentration, which we measured with a commercial kit from Cayman Chemical (Interchim). This colorimetric assay kit used the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyls. DNPH reacts with protein carbonyls, to produce the corresponding hydrazine, which is quantified spectrophotometrically at an absorbance of 385 nm. Each assay was performed in duplicate, and concentrations are expressed in nmol/mg of proteins.

Antioxidant enzyme activities

We assessed the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), using commercial kits from Cayman Chemical (Interchim), as previously described (Lestaevel *et al.*, 2009).

SOD activity was estimated with a tetrazolium salt to detect the superoxide radicals generated by xanthine oxidase and hypoxanthine. Enzyme units were recorded as $\text{U} \cdot \text{mg}^{-1}$ protein. The method for CAT activity is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 . The formaldehyde produced was measured by a spectrophotometer, and the CAT activity is expressed in nmol of formaldehyde $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. GPx activity was determined by measuring the rate of NADPH oxidation in a spectrophotometer at 340 nm, and the results are expressed as nmol NADPH oxidized $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein.

Uranium analyses

After addition of concentrated nitric acid (Ultrapur, JT Becker) and hydrogen peroxide 30% (Normapur, VWR), brain samples were digested in a microwave oven (Ethos TC; Milestone, Bergamo, Italy) by the following procedure: microwave power at 1,000 W, ramp 10 min to reach 200°C, and then hold at 200°C for 20 min. U content in mineralized sam-

ples was determined with an inductively coupled plasma mass spectrometer (ICPMS-VGPQ, EXCELL, ThermoElectron, Courtaboeuf, France) with bismuth ($1 \mu\text{g.L}^{-1}$) as an internal standard, according to previously reported methods (Becker *et al.*, 2005). For U, the ICPMS detection limit is $10^{-4} \mu\text{g.L}^{-1}$. Values are expressed as nanograms per gram of fresh sample material. This ICP-MS protocol has been previously described in the literature (Karpas *et al.*, 2005).

Statistical analyses

In all experiments, data are expressed as mean $\pm$ S.E.M., and the effect of U is assessed by one-way ANOVA, followed by a Student-Newman-Keuls *post hoc* test, whenever appropriate necessary. Differences were considered significant if $p < 0.05$ or $p < 0.01$.

RESULTS

Health indicators

Body weight and drinking water and food consumption were monitored at the end of U exposure (at 10 weeks of age in pups). Neither weight nor diets differed between the U-exposed (10 or 40 mg.L^{-1}) and the control rats (data not shown).

Behavioral tasks

Open-field activity

The locomotor and exploratory behaviors of rats were assessed by the total number of lines crossed and the total number of rearing movements in the open field. Fig. 1 shows the results for both indicators.

Rats exposed to the high U dose (40 mg.L^{-1}) for 10 weeks after birth crossed significantly fewer lines than the controls during the 10 last minutes of the test (-19.3% at 15 min and -18.5% at 20 min) (Fig. 1A). This effect was also observed in rats exposed to the low U dose (10 mg.L^{-1}) after 5 (-21.6%) and 15 (-19.4%) min (Fig. 1A).

The total number of rearing movements in the open field did not differ significantly in rats exposed to 10 or 40 mg.L^{-1} and control animals (Fig. 1B).

Elevated plus-maze

The anxiety-like behavior of rats was assessed in the elevated plus-maze by the number of entries into closed arms and the time spent in closed arms. The latter indicator is shown in Fig. 2.

The number of closed-arm entries did not differ between rats exposed to either U dose and controls (data not shown). However, rats exposed to 40 mg.L^{-1} of U, but

Fig. 1. Total activity of rats exposed to U from birth for 10 weeks. Panel A shows the number of lines crossed, reflecting locomotor activity. The number of rearing is presented in panel B and reflects exploratory activity. The data are presented as mean $\pm$ S.E.M.; $n = 12$ for each group; # $p < 0.05$, significant difference between U10 group and control; * $p < 0.05$, significant difference between U40 group and control.

not those exposed to 10 mg.L^{-1} , spent significantly more time in closed arms than the controls (17.3 ± 1.5 sec, versus 12.6 ± 1.5 sec, $p < 0.05$) (Fig. 2).

Rotarod test

The rats' motor coordination and practice effect were assessed by the time spent on the rod. The results are shown in Fig. 3.

Motor coordination at 5 rpm during the first trial did not differ significantly in rats exposed to U at 10 or 40 mg.L^{-1} compared with controls (Fig. 3).

The time on the rod increased significantly over the trials at 5 rpm for the control rats (Fig. 3), but not for the U-exposed rats (Fig. 3). During the third trial, the time spent on the rotarod decreased significantly in the rats

Fig. 2. Anxiety like-behavior of rats exposed to U from birth for 10 weeks. The time spent in closed arms of the elevated plus-maze is expressed in seconds and reflects the anxiety level. The data are presented as mean $\pm$ S.E.M.; $n = 12$ for each group; * $p < 0.05$, significant difference from control.

Fig. 3. Rotarod task of rats exposed to U from birth for 10 weeks. Motor coordination and learning ability (practice effect) are expressed in seconds. The data are presented as mean $\pm$ S.E.M.; $n = 12$ for each group; # $p < 0.05$ and ## $p < 0.01$, significant difference from trial 1; * $p < 0.05$, significant difference between U10 or U40 groups and control.

exposed to U at 10 and 40 mg.L⁻¹ (respectively, -51.4% and -55.9%, $p < 0.05$) compared to controls (Fig. 3)

Porsolt test

Immobility time in the Porsolt test evaluates depression-like behavior. This indicator is shown in Fig. 4.

At 10 weeks of age, the immobility time recorded during the last 5 min of the test was significantly lower in the group exposed to 40 mg.L⁻¹ compared with the control group (-50.8%, $p < 0.05$) (Fig. 4). No significant difference was observed in the group exposed at a dose of 10 mg.L⁻¹. This result indicates that exposure to U at 40 mg.L⁻¹ reduced depressive-like behavior.

Porsolt test: depressive like-behavior

Fig. 4. Depression-like behavior of rats exposed to U from birth for 10 weeks. Time of immobility is expressed in seconds and reflects the depression level. The data are presented as mean $\pm$ S.E.M.; $n = 12$ for each group; * $p < 0.05$, significant difference from control.

Oxidative damage indicator

Lipid peroxidation levels in the hippocampus of rats exposed to U at 40 mg.L⁻¹ for 10 weeks after birth increased significantly compared to control animals ($100.3 \pm 9.3 \mu\text{M}$ versus $74.1 \pm 7.1 \mu\text{M}$, $p < 0.05$). No significant increase was observed in rats exposed to U at 10 mg.L⁻¹ ($91.2 \pm 9.2 \mu\text{M}$ versus $74.1 \pm 7.1 \mu\text{M}$).

Protein carbonyl concentrations in the hippocampus of rats exposed to U at 40 mg.L⁻¹ for 10 weeks since birth increased significantly compared to control animals ($0.488 \pm 0.044 \text{ nmol/mg prot.}$ versus $0.206 \pm 0.039 \text{ nmol/mg prot.}$, $p < 0.05$). Again, no significant increase was observed for the group exposed to U at 10 mg.L⁻¹ (0.269 ± 0.037 versus $0.206 \pm 0.039 \text{ nmol/mg prot.}$).

Antioxidant enzymes

Exposure to U at 40 mg.L⁻¹ induced a significant increase (+122.5%, $p < 0.05$) in the rats' SOD activity, compared to that of the controls (Fig. 5). GPx activity was also higher (+13.6%, $p < 0.05$) in the hippocampus of these rats (Fig. 5). Catalase activity, however, was not significantly modified in rats exposed to U at 40 mg.L⁻¹ compared to control rats (Fig. 5).

Exposure to U at 10 mg.L⁻¹ for 10 weeks did not result in any difference with controls in SOD, GPx, or catalase activities (Fig. 5).

Uranium in tissues

U levels in the whole brain were significantly higher in rats exposed to U (40 mg.L⁻¹ group: $2.49 \pm 0.43 \text{ ng.g}^{-1}$; 10 mg.L⁻¹ group: $2.02 \pm 0.61 \text{ ng.g}^{-1}$; $p < 0.05$; $n = 6$ per group) compared with controls ($1.07 \pm 0.05 \text{ ng.g}^{-1}$; $n = 6$).

Fig. 5. Enzymatic activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in hippocampus of rats exposed to U from birth for 10 weeks. Activities are expressed as a percentage of activity in the controls. The data are presented as mean $\pm$ S.E.M.; $n = 8$ for each group; * and # $p < 0.05$, significant difference between U40 group and control group or U 10 group, respectively.

Fig. 6. Correlation and linear regression for protein carbonyl concentration and uranium levels in hippocampus of rats exposed to U at 40 or 10 mg.L⁻¹ from birth for 10 weeks. Carbonyl protein concentration is expressed in nmol.mg⁻¹ protein. The uranium level is expressed in ng.g⁻¹.

The U level in the hippocampus was significantly higher after exposure to U in the 40 mg.L⁻¹ group (2.79 ± 0.13 ng.g⁻¹; $n = 6$) compared to the control (1.22 ± 0.22 ng.g⁻¹; $n = 6$). No significant difference was observed between the 10-mg U.L⁻¹ group (1.91 ± 0.32 ng.g⁻¹; $n = 6$) and the control group.

Protein carbonyl concentrations were directly correlated with uranium levels in the hippocampus (Fig. 6).

DISCUSSION

Our results clearly show that U exposure from birth to 10 weeks, during a critical period of perinatal brain development, induces behavioral alterations in rats. This is a sensitive period for exposure to low levels of environmental contaminants (Eriksson *et al.*, 2010). The higher levels of lipid peroxidation in the hippocampus are indirect evidence of oxidative damage that might play a role in these behavioral changes.

This study investigated the potential adverse effects of U on the behavior of rats exposed from birth. The first indicator we studied is total activity. Activity levels measured in an open-field test have been widely used in behavioral studies (Prut and Belzung, 2003). The open-field procedure involves estimating primary motor activity, evaluated from the number of line crossings, and exploratory activity, evaluated from the number of rearing movements onto the hind limbs. Here we observed significantly less locomotor activity in rats exposed to U at 10 or 40 mg.L⁻¹. Previously published results are sim-

ilar. In mice exposed to U during gestation and lactation, the open-field activity was about 20% lower than in controls (Briner and Abboud, 2002). This reduced activity was reported at a dosage of 75 mg U.L⁻¹ in drinking water and appeared earlier in mice aged 3 weeks. The opposite result, *i.e.* hyperactivity, has been described in adult rats exposed to U. Locomotor activity and rearing movements increased by 100% in an open field over a 5-min observation period in adult rats exposed to 150 mg U.L⁻¹ for either 2 weeks or 6 months (Briner and Murray, 2005). Similarly, a 25% increase in locomotor activity and a 35% increase in rearing movements have been reported over a 5-15 min observation period in an open field in rats exposed to repeated U inhalation (30 min inhalation at 197 mg.m⁻³, 4 days a week for 3 weeks) (Monleau *et al.*, 2005). These differences in motor activity were unexpected. It seems likely that the detection of hypo- or hyperactivity in rats depends on the time exposure starts and the uranium dose used.

In the rotarod task, the U-exposed rats showed significant impairment in their practice effect in terms of synchronized walking. This deficit appears to indicate an impairment in adaptive ability. For motor coordination itself during the trial, none of the U-exposed rats (at either 10 or 40 mg.L⁻¹) showed any significant deficit in motor coordination compared with control animals. Moreover, the animals walked normally, showing no muscular weakness. The lack of effect on motor coordination of U-exposed rats in the rotarod is consistent with data from the literature showing that low-dose exposure to other heavy

metals does not produce gross motor deficits (Goulet *et al.*, 2003). One possibility is that the rotarod task was not sensitive enough to detect weak impairment of motor behavior caused by heavy metals.

In the elevated plus-maze test, our study suggests that exposure to U produces an increase of anxiety-like behavior at the highest dose used. This result is not consistent with previous data showing no significant effect of U exposure at a dose of 40 mg.L⁻¹ on anxiety-like behavior in adult rats (Houpert *et al.*, 2005). We should bear in mind that the rat's biochemical system is not complete until 4 weeks after birth (Giorgi *et al.*, 1987). This might explain the differences in some behavioral effects detected in rats exposed from birth or at adult age.

We also analyzed the effects of U on depressive behavior, a topic never addressed in previous studies. For this purpose, we used the forced swimming task, generally considered to test an animal model of depression (Porsolt, 2000). Data from this task reveals that animals exposed to the higher U dose showed less immobility. These unexpected findings suggest decreased susceptibility to depressive-like behavior. An interaction on the molecular level has been demonstrated between corticoreleasing factor and serotonin receptors in the presence of anxiety-like behavior (Magalhaes *et al.*, 2010) and could cause abnormal brain signals that could have an effect on depressive-like behavior. This reduction in depressive-like behavior might thus be related, directly or indirectly, to the increase of anxiety. Previously, it has been suggested that the increased rapid-eye-movement sleep observed in adult rats exposed to U might be linked to depression (Lestaevel *et al.*, 2005). Depressive syndromes have been reported in Gulf War veterans (McDiarmid *et al.*, 2002). Other heavy metals induce also depressive-like behaviors in mouse offspring (Onishchenko *et al.*, 2007; Martinez *et al.*, 2008). It has been suggested that impairment in hippocampal function may be involved in the etiology of depression (Warner-Schmidt and Duman, 2006). Further in-depth studies are needed into the potential effects of developmental U exposure on emotional behavior. Finally, while the hippocampus is important in many of the behaviors examined, so are other regions, such as the amygdal body or striatum, and they should be examined.

Several causes might explain the behavioral effects we observed. A correlation between lipid peroxidation — a marker of oxidative stress measured by the thiobarbituric acid (TBA) assay — and locomotor activity has previously been demonstrated after ingestion of U in adult animals (150 mg.L⁻¹) for 2 weeks (Briner and Murray, 2005). Here we quantified lipid peroxidation

by the direct determination of hydroperoxides, which was significantly higher in animals exposed to the higher U dose. In the same experimental conditions, the protein carbonyl concentration also rose significantly. After U exposure at 40 mg.L⁻¹ it was correlated with higher uranium levels in the hippocampus than after U exposure at 10 mg.L⁻¹. These results might suggest that oxidative stress plays a key role in behavior impairment. Several previous studies have shown that oxidative stress can provoke anxiety-like behavior in rodents. For example, the consumption of a highly palatable diet enriched with sucrose increased protein oxidation in the frontal cortex of rats and induced anxiety-like behavior in the dark/light choice (Souza *et al.*, 2007). A deletion of the p66Shc longevity gene in mice, which results in lower levels of oxidative stress and an extended life span, decreased anxiety-related behavior (Berry *et al.*, 2007). These data suggest an association between oxidative stress and elevated plus-maze performance, but not necessarily a cause-and-effect relation. Antioxidative defense mechanisms can be activated to counteract oxidative stress. Here, SOD and GPX activity were significantly higher in the hippocampus of rats exposed to U (40 mg.L⁻¹) for 10 weeks after their birth than in controls. Similar results have been observed in the brain of rats exposed to U at adult age (Linares *et al.*, 2007; Lestaevel *et al.*, 2009). The accumulation of U in the hippocampus suggests that U has a direct neurotoxic effect on the central nervous system. It has previously been shown that U may be toxic to this cerebral structure (Pellmar *et al.*, 1999b). Finally, the lack of any significant effect on oxidative stress by exposure to 10 mg.L⁻¹ of U suggests in the context of this study that oxidative stress is not the only causative factor involved in these behavioral disturbances.

In conclusion, our data indicate that exposure to U from birth to 10 weeks induces behavioral and neurochemical consequences. Some precautions should be taken regarding chronic exposure to this contaminants at environmental doses. Finally, since males are less resistant than females to U-mediated toxicity (Briner and Murray, 2005), this study focused only on male pups, but further experiments on females might also be useful.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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