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Original Article

Cerebral cortex and hippocampus respond differently after post-natal exposure to uranium

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ABSTRACT — The central nervous system (CNS) is known to be sensitive to pollutants during its development. Uranium (U) is a heavy metal that occurs naturally in the environment as a component of the earth's crust, and populations may therefore be chronically exposed to U through drinking water and food. Previous studies have shown that the CNS is a target of U in rats exposed in adulthood. We assessed the effects of U on behavior and cholinergic system of rats exposed from birth for 10 weeks at 10 $mg.L^{-1}$ or 40 $mg.L^{-1}$. For behavioral analysis, the sleep/wake cycle (recorded by telemetry), the object recognition memory and the spatial working memory (Y-maze) were evaluated. Acetylcholine (ACh) and acetylcholinesterase (AChE) levels were evaluated in the entorhinal cortex and hippocampus. At 40 $mg.L^{-1}$, U exposure impaired object recognition memory (-20%), but neither spatial working memory nor the sleep/wake cycle was impaired. A significant decrease was observed in both the ACh concentration (-14%) and AChE activity (-14%) in the entorhinal cortex, but not in the hippocampus. Any significant effect on behaviour and cholinergic system was observed at 10 mg $U.L^{-1}$. These results demonstrate that early exposure to U during postnatal life induces a structure cerebral-dependant cholinergic response and modifies such memory process in rats. This exposure to U early in life could have potential delayed effects in adulthood.

Key words: Acetylcholinesterase, Heavy metal, Cerebral cortex, Hippocampus, Behavior, Sleep

INTRODUCTION

Heavy metals can induce and exacerbate developmental neurotoxic effects when present during the critical period of brain development, *i.e.,* just before birth in humans and during the first 2-3 weeks after birth in rodents (Antonio and Leret, 2000; Desi *et al*., 1998; Leret *et al*., 2003). Uranium (U) is naturally present in the environment as a component of the earth's crust. Because its concentrations vary highly according to the location and the type of rocks (Cuney, 2009), the uranium content in drinking water also varies greatly (in microgram-per-liter range in USA. and France for example until milligram-per-liter range in some US states and southern Finland) (Jurgens *et al*., 2010; Orloff *et al*., 2004; Juntunen, 1991). Concentrations of uranium in private drilled wells can reach more than 100-1,000 times those given in the current World Health Organisation (WHO) guideline of 30 $\mu g.L^{-1}$. Populations can be chronically exposed to U through drinking water or food (ATSDR, 1999). Studies have demonstrated that U crosses the blood-brain barrier (Lemercier *et al*., 2003) and that the brain is one of its targets (Lestaevel *et al*., 2005a). Uranium exposure in adult rats induces changes in behavior, *i.e.* grooming and rearing troubles (Briner and Murray, 2005). In previous papers, we have also demonstrated that U can impair acetylcholinesterase (AChE) activity in adult rats (Bussy *et al*., 2006; Bensoussan *et al*., 2009).

In the central nervous system (CNS), the cholinergic system is still not completely specified at birth, probably because its projections are widespread. The cortex and hippocampus are rich in cholinergic neurons (Mesulam *et*

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al., 1983). The cholinergic system modulates neurons that release various neurotransmitters, so its disturbances may induce direct or indirect effects on the CNS. For example, acetylcholine (ACh) neurotransmission induces the changeover from non-rapid eye movement sleep to rapid eye movement (REM) sleep or wakefulness (Steriade, 2004). Much evidence indicates that the cholinergic system of the entorhinal cortex is critically involved in object recognition memory (Tinsley *et al*., 2011). The suggestion has been made also that cholinergic projections to the hippocampus are important in regulating aspects of hippocampal physiology that are central to its normal function in spatial working memory (Markowska *et al*.,1995).

As the immature brain is known to be more susceptible to pollutants than the adult brain (Castoldi *et al*., 2001), the aim of this study was to assess the effects of U on the entorhinal cortex and hippocampus of rats after exposure from birth for ten weeks. Accordingly, the cholinergic system experiments were conducted on these two cerebral structures. We observed also the sleep-wake cycle with electroencephalogram (EEG) recordings and performed tests on spatial working memory and object recognition memory, controlled by the entorhinal cortex and hippocampus. .

MATERIALS AND METHODS

Animals

Twelve litters of rats were used for the control group, 12 litters for the U 10 mg.L^{-1} group and 12 litters for the U 40 mg.L^{-1} group. One hundred thirty eight Sprague-Dawley male rat pups (Charles River, L'Arbresle, France) were divided into four groups: the EEG study group (n = 36, with two subgroups: U 40 mg.L^{-1} n = 18 and control, n = 18), the behavioral study group (n = 36, with three subgroups: U 10 mg.L^{-1}, n = 12; U 40 mg.L^{-1}, n = 12 and control n = 12), the neurochemical study (n = 30, U 10 mg.L^{-1}, n = 10; U 40 mg.L^{-1}, n = 10 and control, n = 10) and the time-course U concentration group (n = 36, with two subgroups: U 40 mg.L^{-1}, n = 6 per time and control, n = 6 per time, with three time of exposure: 3, 20 and 40 weeks). Each assessment (EEG, behavior, neurochemistry) used one animal per litter. For "EEG animals", 9 rats in each group were implanted with a telemetric implant to record their EEG activity. The other 9 rats in each group were not implanted. Each non-implanted rat was housed with one implanted rat from the same group (exposure or control) to prevent isolation and depression.

Rats were housed under a 12 hr/12 hr light-dark cycle (light on from 8 a.m. to 8 p.m.) at a constant temperature (22 ± 1°C). Water and food were supplied *ad libitum.* Body weight and food and water consumption were measured weekly. The study was conducted in accordance with French legislation and European legal requirements (Decree 86/609/EEC) for the protection of animals used for experimental purposes. Scientists certified by the French Ministry of Agriculture performed all procedures in animals.

Exposure

Uranyl nitrate was dissolved in mineral water (Table 1) at 10 mg U.L^{-1} or 40 mg U.L^{-1} (AREVA, Pierrelatte, France). The higher U concentration chosen corresponds to twice the highest concentration found in underground water (Juntunen, 1991). The specific activity of U is 1.4 x 10^{-4} Bq/g, and its isotopic composition is ^{234}U = 0.0055%, ^{235}U = 0.255%, and ^{238}U = 99.74%. The dams were exposed to U at the birth of pups (Day 0) (Wappelhorst *et al*., 2002), such that the pups' exposure to U started *via* the mother's milk at the birth and continued after weaning (at 21 days old) *via* drinking water, until they reached 10 weeks of age. Dams of control animals drank mineral water, as did the control pups after weaning. Health parameters, *i.e.* body weight, water consumption, and food intake, were measured at the end of U exposure (at 10 weeks of age in pups).

Sleep-wake cycle

The EEG activity was recorded in freely moving rats by a telemetric system (Data Sciences International, Saint Paul, MN, USA), as previously described (Lestaevel *et al*., 2005b). Briefly, after anesthesia with Imalgene (100 mg/kg, IM), the transmitter was fixed intraperitoneally and the lead wires were passed under the skin to

Table 1. Mineral contents of the mineral water used for the experiments.

Mineral contents	Concentration (mg.L^{-1})
Ca	78
Mg	24
Na	5
K	1
Bicarbonates	357
Chlorures	4.5
Nitrates	3.8
Silice	13.5
Sulfates	10
Uranium	0.00142 *

* Dosage made at laboratory by ICP-MS

the skull where EEG electrodes were placed. After a one-week recovery period, the EEG of each rat was recorded during a session of 23.5 hr, at 10 weeks of age. A trained observer, masked to the particular treatment condition, scored the EEGs manually, assigning three sleep stages: wakefulness (W), slow-wave sleep (SWS), and rapid-eye movement sleep (REM sleep), as described earlier (Rechtschaffen and Kales, 1968). EEG spectral power was analyzed offline with Somnologica software. EEG traces were subjected to a routine fast Fourier transformation (256 points; 50% overlap). The daily spectra were averaged in 10-s epochs and divided into five contiguous bands.

Behavioral tests

At 10 weeks of age, the rats were tested in a two-object recognition task. Each animal was placed into the open field with two identical objects for 3 min (first session). One hour later, the rat was returned to the open field and allowed to explore two objects, one identical to those presented at the first session (familiar object) and the other different (novel object), for an additional 3-min period (second session). The time spent exploring each object was measured in both sessions (Thompson *et al*., 2005).

The next day, spatial working memory was assessed in a Y-maze made with three arms (70 cm in length, 50 cm in height, 10 cm wide at the bottom, and 20 cm wide at the top) that converged at equal angles. Each rat was placed at the center of the maze and was allowed to move freely through the maze for a ten-minute test session. The sequence and number of arm visits were manually recorded. Alternation was defined as consecutive entry into each of the three different arms (Pothion *et al*., 2004).

Brain sampling

After the 10 weeks of postbirth exposure, rats were anesthetized by inhalation (TEM anesthesia, Pessac, France) of 95% air/5% isoflurane (Florène®, Abbott France, Rungis, France) and intracardiac puncture performed to remove blood. After decapitation, the brain was quickly removed and placed on ice. The entorhinal cortex and hippocampus were microdissected out, according to the procedure described by Glowinski and Iversen (1966), and then weighed and stored at -80°C until use.

The behavioral study group was used for tissue analysis (n = 5-6 per subgroup). Rats were perfused transcardially with 4% ice-cold paraformaldehyde followed by 1 × PBS; brains were post-fixed in 4% paraformaldehyde overnight and then transferred to 20% sucrose until they sank to the bottom of the tubes. Brains were frozen in O.C.T compound (A.O. USA) and 16 µm sections were made in the coronal planes using a cryostat. Sections were thaw-mounted on probe-on plus charged slides.

Cresyl violet staining

Cresyl violet was used to stain tissue sections for histological examination and measurement of neuronal loss. Nissl histology of rat brain and the presence and absence of dead and injured neurons were analyzed on microscope slides mounted 16 µm thick brain sections. Sections derived from all investigated rats were defatted in ascending alcohols (70-100%), hydrated in descending alcohols (95-70%), washed in acetate buffer pH 5.0 and subsequently stained with a 0.25% cresyl violet for approximately 10 min. Section were then washed with distilled water and dehydrated in graded ethanol. Images were viewed with a light microscope.

Acetylcholine level and acetylcholinesterase activity

ACh concentrations and AChE activity in the entorhinal cortex and hippocampus were measured with the Amplex® Red Acetylcholine/Acetylcholinesterase Assay Kit (A12217, Molecular Probes, Invitrogen, Cergy Pontoise, France) as previously described (Bensoussan *et al*., 2009).

Uranium concentrations

Tissue samples were prepared by adding 8 ml of ultrapure 70% nitric acid and 2 ml of hydrogen peroxide and then mineralizing them in a 1000 W microwave (Ethos Touch; Milestone microwave laboratory systems; Italy), with a 20-min ramp to 180°C and then 10 min at 180°C. Brain U content from mineralized entorhinal cortex or hippocampus samples was determined with an inductively coupled plasma mass spectrometer (ICPMS-VGPQ, EXCELL, ThermoElectron, France) with bismuth (1 µg/L) as the internal standard. The ICPMS limit of detection for U is 10^{-4} µg/L. Values were expressed as ng U/g (wet brain) tissue and presented as means ± S.E.M.

Statistical analysis

Results are expressed as means ± S.E.M. The statistical analysis routinely used unpaired Student's t-tests to compare the data between groups. Differences were considered statistically significant when $p < 0.05$: *, $p < 0.01$: **.

RESULTS

Neurobehavioral study

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 and 40 mg.L^{-1}) and the control rats (Table 2).

Sleep-wake cycle analysis

The sleep-wake cycle and the EEG power analysis have been performed only on rats exposed to U at 40 mg.L^{-1} *versus* control.

The percentage of time spent in wakefulness, SWS, and REM sleep did not differ significantly between the exposed and control rats (Table 2). The 10-weeks of U exposure did not modify the amount of time spent in these 3 stages.

The number of episodes and the mean duration of each stage were also determined and similarly did not differ significantly between the two groups (data not shown).

EEG power analysis

The power spectra of the EEG frequency bands (delta: 0.5 to 3.99 Hz; theta: 4 to 7.99 Hz; alpha: 8 to 11.99 Hz; sigma: 12 to 13.99 Hz and beta: 14 to 24.99 Hz) were not significantly different in U40-exposed compared with control rat pups (data not shown). These results are thus consistent with the sleep-wake cycle analysis.

Object recognition memory

Fig. 1 shows the results of the two-object recognition task: percentage of time spent exploring the left or right object during the first session and percentage of time spent exploring the new object during the second session. In the first session, the global time spent on the two objects (left and right objects) was equal for control and U-exposed rats (data not shown). Furthermore, the lack of difference between the groups in the time spent on each object indicates that the attention of control and U-exposed rats was similar for the objects.

During the second session, the control rats spent significantly more time exploring the new object, *versus* the familiar one ($p < 0.05$), while the U40-exposed rats spent an equal amount of time on each (data not shown), and the time spent exploring the new object differed significantly between the U40-exposed group (56.8 ± 0.2%) and the controls (70.8 ± 0.1%) ($p < 0.05$). These results indicate a loss of recognition memory induced by U exposure.

Spatial working memory

At the age of 10 weeks, we measured the number of arm entries and the percentage of alternation in the Y-maze in both control pups and those exposed to U at 10 or 40 mg.L^{-1} from birth for 10 weeks. The number of arm entries did not differ between the three groups (Table 2) and thus indicated that U exposure had no significant effect on locomotor activity. The percentage of alternation exceeded 50% and did not differ significantly between the U-exposed and control rats (Table 2). This result shows that U exposure did not impair spatial working memory.

Table 2. Several indicators measured in control rats and rats exposed to U at 10 or 40 mg/L^{-1} from birth for 10 weeks.

		Control	U 10	U 40
Health indicators	Weight (g)	434.4 ± 10.3	435.8 ± 9.2	409.4 ± 11.1
(n = 12/group)	Food (g/day/rat)	28.9 ± 5.1	26.9 ± 0.4	24.8 ± 0.7
	Water (ml/day/rat)	29.5 ± 2.9	26.2 ± 1.0	24.4 ± 3.0
Sleep-wake cycle	W (%)	54.1 ± 2.8	N.D.	56.0 ± 1.5
(n = 9/group)	SWS (%)	38.0 ± 2.6	N.D.	35.6 ± 1.0
	REM-S (%)	7.9 ± 0.8	N.D.	8.5 ± 0.6
Y-maze	Number of entries	35.1 ± 1.2	36.7 ± 2.8	36.7 ± 1.3
(n = 12/group)	Alternation (%)	69.6 ± 2.3	66.2 ± 1.5	66.0 ± 2.3

The rats' body weight and food and water consumption (health indicators) were measured at the end of the experiment; the sleep-wake cycle was measured as the percentage of time spent awake (W), or in slow wave sleep (SWS) or rapid-eye-movement sleep (REM-S) for 23.5 hr at 10 weeks of age; the number of arm entries and the alternation of arms in the Y-maze are reported; uranium concentrations were measured in the entorhinal cortex and hippocampus. The determination of health indicators has been made on "behavioural study group".

Results are expressed as mean ± S.E.M.

U10 : group U 10 mg.L-1; U40: group U 40 mg.L-1.

N.D.: not determined

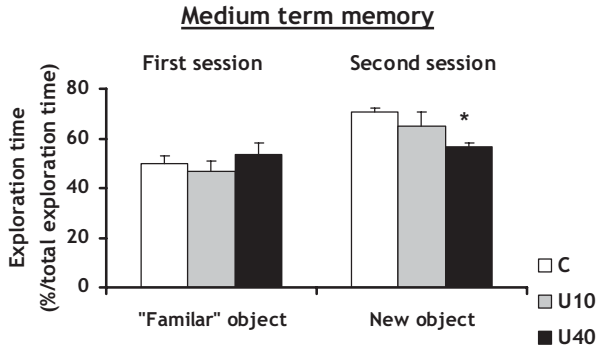

Fig. 1. Figure 1 shows the results of the object recognition task in control rats and rats exposed to U at 10 or 40 mg.L^{-1} from birth for 10 weeks. The time spent exploring one of the two identical objects (left or right objects) for the first session and the time spent on the new objects in the second session are represented as percentage of total exploration time. Results are expressed as mean ± S.E.M (n = 12, C = Control group, U10 = uranium 10 mg.L^{-1} group, U40= uranium 40mg.L^{-1} group). * p < 0.05 significantly different from control value.

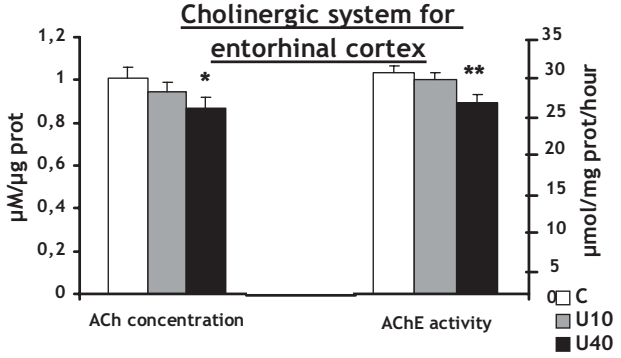

Fig. 2. Figure 2 depicts the results of the assays of ACh and AChE activity for entorhinal cortex in control rats and rats exposed to U at 10 or 40 mg.L^{-1} from birth for 10 weeks. Results are expressed as mean ± S.E.M. (n = 10, C = Control group, U10 = uranium 10 mg.L^{-1} group, U40 = uranium 40 mg.L^{-1} group). ACh concentrations were expressed in µM/µg protein and AChE activity in µmol/mg protein/hour. *p < 0.05; **p < 0.01 significantly different from control value.

Cholinergic system study

Acetylcholine concentration and acetylcholinesterase activity in the cerebral cortex

The ACh concentration and AChE activity in the entorhinal cortex after 10 weeks of U exposure are presented in Fig. 2. Rats exposed to U at 40 mg.L^{-1} had an ACh concentration 14% lower than that in control rats (0.87 ± 0.05 µM/µg prot compared with 1.01 ± 0.05 µM/µg prot, $p < 0.05$). AChE activity was also 14% lower in U40-exposed rats (26.3 ± 1.2 µmol/mg prot/hr, compared to 30.5 ± 0.9 µmol/mg prot/hr, $p < 0.01$). No significant effect was observed on ACh concentration and AChE activity for rats exposed to U at 10 mg.L^{-1}.

Acetylcholine concentration and acetylcholinesterase activity in the hippocampus

The ACh concentration in the hippocampus was not significantly different in exposed and control rats (U10: 0.38 ± 0.02 µM/µg prot, U40: 0.40 ± 0.02 µM/µg prot, compared with 0.37 ± 0.01 µM/µg prot for control). Nor did U exposure significantly modify AChE activity (U10: 11.4 ± 0.2 µmol/mg prot/hr, U40: 11.2 ± 0.4 µmol/mg prot/hr, *versus* controls, 11.2 ± 0.5 µmol/mg prot/hr).

Histology study

Nissl staining was performed to examine the extent of neuronal cell death induced by U in the cortex and hippocampus of the rat brain exposed from birth. Nissl staining identifies all structures, particularly the nucleus and nucleic acids, which appear violet. This method allowed clear identification of dead neuronal cells (i.e., those with large or small condensed, fragmented and dark nuclei and apoptotic bodies). No vacuolization, neuronal loss, and tissue breakdown were seen in the cortex and the hippocampus (data not shown) of rats exposed to U at 10 or 40 mg.L^{-1} as compared to control animals.

Uranium concentrations

The U concentration in the entorhinal cortex was significantly higher in the rats exposed to U from the birth during 10 weeks compared with control rats (+63%, p < 0.01) (Table 3). No significant accumulation of U was observed at 3, 20 and 40 weeks compared to control (Table 3).

On the other hand, U concentrations in the hippocampus did not differ significantly between the rats exposed to U during 10 weeks since the birth and control rats (U: 1.91 ± 0.32 ng.g^{-1} *versus* Control: 1.22 ± 0.22 ng.g^{-1}), that is, no significant levels of U accumulated in this cerebral structure of the exposed rats.

DISCUSSION

Developing animals are the most sensitive to the effects of U. A study has demonstrated that gestational day 10 (neural tube formation) appears to be the most vulner-

Table 3. The time-course of uranium concentration in the entorhinal cortex in control rats and rats exposed to U at 40 mg.L^{-1} from birth for 3, 10, 20 or 40 weeks.

		3 weeks	10 weeks	20 weeks	40 weeks
U concentration	Control (ng/g)	9.9 ± 0.3	0.65 ± 0.06	1.42 ± 0.15	0.89 ± 0.10
(n = 5-6/group)	U 40 (ng/g)	10.6 ± 0.4	1.06 ± 0.05**	1.95 ± 0.26	0.91 ± 0.11

The determination of the uranium concentration in the group "10 weeks" has been made on some rats of the "neurochemical study group".
Results are expressed as mean ± S.E.M. n = 5-6 per group. ** $p < 0.01$ significantly different from control value.

able time for U exposure (Domingo, 2001).and the window of susceptibility of developing brain to uranium appears to be the pre-natal period (Briner, 2006). But in rodents, the first few weeks following birth are also crucial for brain development, since neurons are built, spread out and become interconnected during this stage. The neurotoxic activity of heavy metals during this neuronal maturation can induce severe cognitive impairments, as already shown for lead (Reddy *et al*., 2007). We demonstrated for the first time in this study that U can induce effects on the entorhinal cortex, not hippocampus, of rats exposed from the post-natal period.

Physiological and pharmacological studies indicate that neurotransmitters play a critical role in the regulation of cognitive processes (Levin *et al*., 1990; Nieoullon and Coquerel, 2003). The cholinergic system is widely hypothesized to play a prominent role in mechanisms of memory and attention (Blokland, 1995; Voytko, 1996; Hasselmo and Stern, 2006). This study showed that ACh concentration and AChE activity were both lower in the entorhinal cortex of U-exposed pups at 10 weeks of age than in the control group. This difference could be due to reduced choline transport, but it might also reflect a reduction in mitochondrial activity that results in a decreased amount of acetyl-CoA available for choline acetyltransferase (Tripathi and Srivastava, 2008). Decreases in ACh or AChE have also been noticed after U exposure of adult rats (1.5 months, 40 mg/L U *via* drinking water) (Bensoussan *et al*., 2009).

The effects of U on the ACh system that are reported here were correlated with U accumulation in the entorhinal cortex. The uranium levels in the cortex of rats exposed to uranium were significantly higher than those of the control rats but lower than those in the hippocampus of the control rats. This discrepancy could be explained by a heterogeneous distribution of U in cerebral structures (Paquet *et al*., 2006; Houpert *et al*., 2007). After exposure, U not accumulated significantly in the hippocampus and had no significant effect on either ACh concentration or AChE activity. The sensitivity of the hippocampus to U thus seems to differ from that of the cortex, and U exposure affected ACh and AChE activity in the latter more strongly than in the former. These results are consistent with our previous work on adult rats (Bensoussan *et al*., 2009). This is particularly important given that cholinergic projections to the cortex are primarily focused, compared with the much more diffused cholinergic projections that reach the hippocampus. The hippocampus seems to be able to adjust to U exposure. It remains to be seen how U disturbs the different components of the cholinergic system. The model we used here, *i.e.,* postnatal exposure, might have abnormal afferents due to U-induced disturbances in differentiation and neuronal migration.

In this study, U exposure of rats exposed from birth for 10 weeks did not perturb the sleep-wake cycle and the spatial working memory. The lack of effect of U on working spatial memory could result from the fact that the hippocampus is the substrate for this memory (Olton *et al*., 1979), where U not significantly accumulated and no disturbed cholinergic system. Here, we found also that object recognition memory was significantly poorer in animals exposed to U since birth. The hippocampus and the entorhinal cortex play different roles in object recognition memory. While the entorhinal cortex is involved in object recognition once it is necessary to representing basic information about familiarity or novelty of an object, the hippocampus is involved in object memorization by encoding information about the experience of object. The entorhinal cortex codes object recognition decays fast and is not sufficient for maintaining information about object during longer retention intervals, while the hippocampus, by coding object memory, maintains strong novel object preference after long but not short delays (Hammond *et al*., 2004). Cholinergic transmission within entorhinal cortex seems to be important for the object recognition memory (Winters and Bussey, 2005). The cholinergic input to entorhinal cortex may facilitate object encoding and/or consolidation processes involved in storage of the memory trace shortly following acquisition. These results suggest that U can alter or not mem-

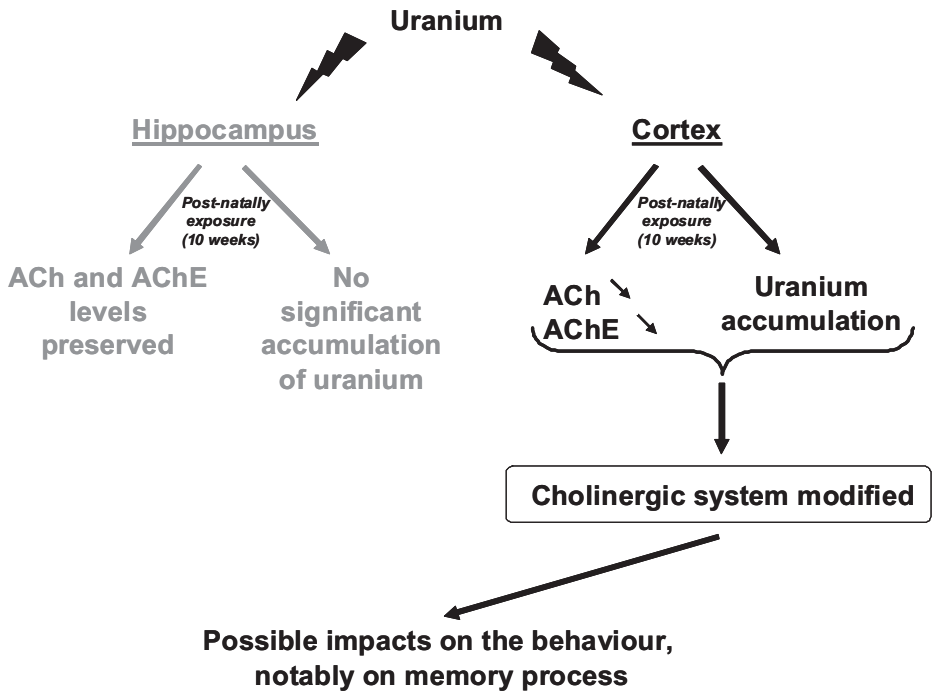

Fig. 3. Figure 3 shows the different response of the hippocampus and entorhinal cortex to U exposure. Exposure to uranyl nitrate (for 10 weeks from birth at 40 mg.L^{-1}) induces no alterations in the hippocampus of ACh and AChE levels. In the cortex, exposure to U affects cholinergic system balance, which could participate to behavioural effects.

ory processes during the brain development of rodents, depending of the cerebral structure involved. However, the exact neurobiological basis of U impairment of neurobehavioral function remains unclear, even if cholinergic system could play a role.

Behavioral studies sometimes, but not always, show similar results between rats exposed during the postnatal period and rats exposed in adulthood. Like us, Houpert *et al*. (2005) did not observe significant effects on the sleep-wake cycle after adult rats were exposed to U for 1.5 months. Object recognition memory was perturbed only when exposure to U started at birth: no significant effects were observed in animals exposed to U only in adulthood (Houpert *et al*., 2005). Spatial working memory did not differ significantly in this study between the rats exposed to U since birth and the control group. However, working memory can be decreased in rats that undergo chronic U exposure in adulthood at a high dose (40 mg/kg/day), or acute exposure (1 mg/kg in IM injection) or exposure by inhalation (Albina *et al*., 2005; Monleau *et al*., 2005; Barber *et al*., 2007). All these results indicate that exposure to U during development or adulthood can induce different behavioral effects.

In conclusion, U may induce some cholinergic changes (decrease of ACh concentration and AChE activity) in rats exposed from birth for 10 weeks at 40 mg.L^{-1}, without histopathological effect. This U-induced cholinergic response was cerebral structure-dependant and may contribute to the sensitivity of the entorhinal cortex to U. Other neurotransmitters, such as glutamate and GABA, could be also a target of U exposure. It would be interesting to consider their role after U exposure during the critical period of development. These effects on brain come along to some behavioral disturbances, and notably a loss of object recognition memory, where the entorhinal cortex plays an important role (Fig. 3). Finally, the time course of adverse neurotoxic effects might be very long and not restricted to childhood. Potential delayed effects in rats exposed to U early in life must be examined in the future.

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