Changes in sleep–wake cycle after chronic exposure to uranium in rats

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Abstract

Uranium is a heavy metal known to induce toxicity in kidneys. It is also known to enter the central nervous system, thus inducing neurophysiological effects, after exposure to relatively high concentrations. The effect of chronic uranium exposure (40 mg l⁻¹ in drinking water, for 90 days) on electroencephalographic architecture has been studied on freely moving rats using a telemetry technique. The main effects of uranium on the sleep–wake cycle were an increase in rapid eye movement sleep (REM-sleep) and theta band power during the light period, as early as Day 30 after exposure commenced. The most probable explanation for these effects is that uranium directly affects the brain. This increase in REM-sleep was previously described in human depression or models of chronically stressed rats and it may be assimilated with some protective or compensatory mechanisms.

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1. Introduction

Uranium (U) is a heavy metal found naturally in the environment. Exposure to U is most likely to occur through inhalation or oral ingestion. Once absorbed into the gastrointestinal tract or lungs, U is translocated into blood and then stored mainly in the kidneys and skeleton [20]. The kidneys are usually considered as the most sensitive target organ to U toxicity [7]. However, a chronic ingestion of water contaminated with U can also result in toxicity in the central nervous system (CNS) [6].

Uranium has numerous military and civil applications. The majority of U is used by the military; depleted uranium (DU) munitions were employed during the conflicts in Afghanistan and Iraq. Human studies focusing on DU exposure have been conducted on Gulf War veterans, leading to the description of the “Gulf War Syndrome”, with neurocognitive deficits [15]. In civil applications, states of depression or agitation have been described after industrial contamination by U compounds [13]. Animal studies have suggested that U crosses the blood–brain barrier [14] and accumulates in the CNS with a regional concentration in some cerebral structures, as the hippocampus [10,18,3]. It could be neurotoxic [12,18] after high-dose chronic exposure by drinking water or after the implantation of pellets. Another study has found that the open-field behaviour and brain lipid oxidation were modified in rats after two weeks or six months exposure to a high U concentration in drinking water [6]. Electrophysiologic changes were also observed in vitro on hippocampal slices isolated from rats implanted with U fragments for six and twelve months [19]. All these data suggest possible neurological toxicity of chronic U exposure at relatively high doses.

The key question was to elucidate the effects of U in the CNS after a chronic exposure to a lower U
concentration. To achieve this, the electroencephalographic (EEG) activity and sleep–wake profiles were measured in vivo, in freely moving rats, after chronic U ingestion at 40 mg l\(^{-1}\) through drinking water using an original telemetric method.

### 2. Methods

#### 2.1. Animals

Twenty-eight Sprague–Dawley male rats (Charles River, France), 12 weeks old, weighing 402±10 g, were used and divided into two groups of fourteen rats—control and U groups. Seven rats in each group were implanted in order to record their EEG activity. The other seven rats of each group were not implanted. Each of these seven non-implanted rats were housed with one implanted rats of the same group (exposure or control), in order to prevent isolation and depression.

The rats were housed under a 12 h/12 h light–dark cycle (light on from 8 a.m. to 8 p.m.) and a temperature of 22±1 °C. Water and food were supplied ad libitum. Body weight gain, food and water intakes were measured weekly. The study was conducted in accordance with French legislation on the protection of animals used for experimental purposes.

#### 2.2. Exposure

The rats were exposed through drinking water for 90 days. Uranium was obtained as a nitrate from Cogema (France). It contained 4.92% 235-U (DU contained 0.26% 235-U) and was diluted in mineral water to obtain a dosage of 40 mg U l\(^{-1}\) (about 1 mg d\(^{-1}\) per rat). This dose 40 mg/L was also the double of highest concentration found naturally on Earth, in the drinking water of Finland [2].

Control rats drank uncontaminated mineral water.

#### 2.3. Electrode implantation

The surgical operations took place 35 days before exposure commenced. All surgical procedures were carried out in an appropriate sterile environment. After anaesthesia with Imalgene\(^ \text{R}(150 \text{ mg kg}^{-1}, 1 \text{ IM})\), the sterile transmitter body was fixed intraperitonealy and the lead wires were passed under the skin to the EEG/EOG electrode position (three pairs and one additional lead). The first pair of differential leads was placed over somesthesic and hippocampic corteces and the second pair over visual and hippocampic corteces. These two pairs of electrodes served to record EEG. One pair of wires was placed longitudinally to the eye for EOG measurement. Finally, a lead inserted in the frontal bone served as the reference electrode. Finally, to prevent any further movement, all electrodes were cemented to the skull with adhesive (Vetbond, 3M, France) and dental cement (Hesadon, Import Dentaire, France). After a 21-day recovery period, EEG and EOG will be recorded.

#### 2.4. EEG recording and spectral analysis

EEG/EOG activities were recorded by a telemetric system (Data Sciences International, USA) and data collected by an acquisition system (Somnologica software, Resmed, France). With this system, all rats (seven controls and seven exposed) could be recorded at the same time. It was the time necessary to turn-on telemetric implants and start computer system. The end of EEG/EOG recordings was the following day at 8:00, just before the light-on. In these conditions, all rats were recorded during 23.5 h. For each rat, this session of 23.5 h of recording was performed during control period (i.e. two sessions of recording between Day-14 and Day 0, with one session per week) and then during exposure period (i.e. 14 sessions of recording between Day 0 and Day 90, with one session per week). The investigator doing the recordings and analysing the data on sleep–wake cycle was blinded to the particular treatment condition. Scoring was manual and performed by a trained observer, assigning sleep stages to 10-s periods along a time line of 23 h and 30 min. Three sleep stages from the EEG and EOG were distinguished: wakefulness (W), slow wave sleep (SWS) and rapid eye movement sleep (REM-sleep), as described earlier [21]. EEG spectral power was analysed off line using 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.

#### 2.5. Uranium measurement

At the end of exposure, the rats were anaesthetised by intraperitoneal injection of 400 ml kg\(^{-1}\) sodium pentobarbital and exsanguinated in order to try to prevent tissue contamination from blood. The brain and kidneys were removed, weighed and mineralised. Uranium content was measured by Kinetic Phosphorescence Analysis (KPA, Chemcheck, USA).

#### 2.6. Statistical analysis

The data were submitted to the overall analysis of variance (ANOVA, single factor). When a significant difference between groups (U or control) was obtained, the results were compared using the Student–Newman–Keuls post hoc test. Differences were considered to be significant if \(p<0.05\) or \(p<0.01\).
3. Results

3.1. Health parameters

Body weight, food intake and water consumption were similar in the control and U-exposed rats throughout the experiment (data not shown).

3.2. EEG activity and sleep study

During the control period, i.e. before exposure commenced, EEG activity and the amount of each wake–sleep phase were similar in the U and control groups.

After 90 days of U exposure, no patent abnormalities, such as peak-waves, were observed.

No significant differences occurred in the amount of wakefulness (W) or slow-wave sleep (SWS) between exposed and control rats during the 90-day period (Fig. 1). In return, in exposed rats, analysed over a 23 1/2-h period, rapid eye movement (REM) sleep was affected, with a significant increase in the REM-sleep amounts from 60.9±5.5 to 89.9±4.3 min for 30 days exposure period, rapid eye movement (REM) sleep was affected, with a significant increase in the REM-sleep amounts from 60.9±5.5 to 89.9±4.3 min for 30 days exposure

<table>
<thead>
<tr>
<th>Days post-exposure</th>
<th>Number of episodes</th>
<th>Mean duration of episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Uranium</td>
<td>Control Uranium</td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>62.0±4.0</td>
<td>56.0±4.8</td>
</tr>
<tr>
<td>D30</td>
<td>55.7±2.7</td>
<td>65.4±4.7</td>
</tr>
<tr>
<td>D60</td>
<td>54.6±2.2</td>
<td>62.4±4.6</td>
</tr>
<tr>
<td>D90</td>
<td>54.7±3.4</td>
<td>69.3±6.2</td>
</tr>
</tbody>
</table>

Mean durations of episodes are in seconds; data are expressed as mean±SEM; n=7 for each group of rats.

**significantly different from control (p<0.01).

Fig. 1. Wakefulness, slow wave sleep (SWS) and rapid eye movement sleep (REM-sleep) amounts in rats chronically exposed to uranium (40 mg l⁻¹ in drinking water). Data are presented for the 23 1/2 h of the recording period. Amounts are in minutes. Data are expressed as mean±SEM; n=7 for each group of rats; * significantly different from control (p<0.05).

Fig. 2. Rapid eye movement sleep (REM-sleep) amounts of rats chronically exposed to uranium (40 mg l⁻¹ in drinking water). Data are presented per cumulated amounts on 2-h periods for the 23 1/2 h of the recording. Amounts are in minutes. Data are expressed as mean±SEM; n=7 for each group of rats; ** significantly different from control (p<0.01); * significantly different from control (p<0.05).
3.3 EEG power analysis

After 30 days exposure, the theta band power (theta: 4 to 7.99 Hz) of exposed rats increased significantly during the light period compared with the control [from 4.59±0.67 to 7.45±0.75 μV²; F(1–12)=8.057; p=0.02] (Fig. 3). At 60 days exposure, this effect was insignificant (from 4.59±1.02 to 6.80±0.85 μV²; p=0.13) (Fig. 3). No significant effect on the theta band was found during the dark period (Fig. 3).

The integrated EEG powers of other frequency bands (delta: 0.5 to 3.99 Hz; alpha: 8 to 11.99 Hz; sigma: 12 to 13.99 Hz and beta: 14 to 24.99 Hz) were not modified significantly during 90 days U exposure for all sleep stages during the light or dark periods (data not shown).

3.4 Uranium measurements

After exposure over a 90-day period, amounts of U increased significantly in the brain (from 75.4±0.6 to 86.1±4.1 ng; p=0.01) and kidneys (from 101.9±4.0 to 579.0±8.5 ng; p=0.0001) compared with control.

4. Discussion

The data reported here show that chronic U exposure induces a significant increase in REM-sleep as early as Day 30, mainly during the light period. Indeed, these data indicate that the spectral analysis of EEG traces were not perturbed, except for the theta band that increased during the light period, 30 days after exposure commenced. This study confirms that EEG activity is a particularly sensitive parameter in detecting perturbations to the CNS [4].

To our knowledge, the marked increase in REM-sleep in vivo mainly during the light period after U exposure is an original finding that has not been described previously. It has been shown that U induced electrophysiological changes in vitro on hippocampus slices after the implantation of U pellets in rats [19]. This effect occurred late, six and twelve months after implantation, however. The effect observed here occurred as early as Day 30 after exposure commenced and with lower U concentration. This increase in REM-sleep occurred mainly during the light period, i.e. during the rats’ sleeping period. This result suggested that the architecture of circadian rhythms was not altered by chronic exposure to U. Moreover, the increase in REM-sleep was accompanied by an increase in the theta band power. The theta rhythm derives from the synchronous firing of neurons located within the various cell layers of the hippocampus. The generation of hippocampal theta rhythm depends on the pacemaker activity of cholinergic inputs from the media septal and Broca’s diagonal band nuclei of the basal forebrain. A link between the theta rhythm and behaviours has been demonstrated, and notably with REM-sleep [22]. So, theta rhythm is one bio-electrical activity that
characterised REM-sleep. This increase in theta band power after U-exposure is consequently in accordance with an increase in REM-sleep amount during the light period. The significant increase of REM-sleep in uranium-exposed rats was accompanied by a non significant decrease of Wakefulness. This apparent paradox can be explained by the relative proportion of REM-sleep versus Wakefulness which is very small. The mechanisms by which U causes these neurophysiological perturbations are unknown. The increase in REM-sleep amount was not due to altered health parameters. The exposed rats looked healthy throughout the experiment. Their food and water intakes, body weight gain and general behaviour were very similar to those of the control rats. As uranium is a well-known nephrotoxic compound [7], one possible explanation for this increase in REM-sleep was an indirect effect of U via its nephrotoxicity. To our knowledge, no correlation has ever been established between kidney injury and an increase in REM-sleep. In our experiment, at the time of sacrifice, i.e. after a 90-day exposure period, the amounts of U in the kidneys of exposed rats were 0.58 µg in both kidneys or 0.18 µg U g⁻¹ kidneys. This concentration is far below the lowest concentration described as nephrotoxic, i.e. 1.2 µg U g⁻¹ kidneys [7]. Our concentration of U cannot be considered as nephrotoxic. There are arguments in favour of a direct effect of U on the CNS. Under our experimental conditions, U concentration in the whole brain of exposed rats was increased after 90 days exposure. Previous studies have also demonstrated that U concentrate in some cerebral structures, such as hippocampus, striatum or hypothalamus after chronic exposure [10,18]. Some of these cerebral structures are implicated in the REM-sleep and this local concentration of U could induce the neurophysiological effects observed. These effects could be chemical, since uranium is a heavy metal and other heavy metals are known to affect the CNS [17,11]. These effects could also come from the radiological properties of 235-U, however.

Surprisingly, chronic U exposure modifies one sleep state only, specifically the REM-sleep, and more surprisingly an increase in REM-sleep is observed. This phenomenon is rarely described in the literature. Other heavy metals, such as methyl-mercury, are also known to produce an increase in REM-sleep [1]. In prenatally stressed rats or in genetic rat depression models, increases in REM-sleep amount and sleep fragmentation were observed [8,9]. An increase in REM-sleep has also been reported in humans, depression is associated with two main types of sleep abnormalities: an increase in REM-sleep amount and sleep fragmentation [23]. Alterations in the sleep cycle observed after U exposure were very similar to those observed in animal depression models or in depressed patients. These data indicate that an increase of REM-sleep is strongly associated with depressive-like behaviour. Depression could be one explanation for REM-sleep increases. Further studies to examine the potential neurocognitive effects of U and the link with REM-sleep increases seem to be warranted. However, at the present time, the neurophysiological mechanisms underlying the REM-sleep increases are only very partially understood. A large variety of neuronal structures and substances, including neurotransmitters, peptides and some substances of a lipidic nature, are known for their involvement in REM-sleep. More interestingly, a role for the hypothalamic-pituitary axis in sleep regulation has been suggested and notably a role of glucocorticoids in the modulation of REM-sleep [5]. It has been proposed that glucocorticoids protect the organism during stress by counteracting rather than enhancing a stress-activated defence reaction, thereby preventing them from causing damage by overshooting [16]. We can hypothesise that after a chronic exposure to U and/or stress, the increase in REM-sleep plays the same role for the brain that glucocorticoids play for the organism, and subsequently an increase in REM-sleep may be assimilated to some protective or compensatory mechanism. After 90 days exposure, the increase in REM-sleep began to be insignificant. This result suggests that the length of exposure plays a role in the electrophysiological perturbations. In this hypothesis, compensatory mechanisms, i.e. the increase in REM-sleep or other phenomena, would operate during short-term exposure and fail after 90 days. These findings are in agreement with previous data already suggesting a link between uranium-induced effects and the length of exposure [6].

In conclusion, this study shows for the first time that chronic U exposure induced an increase in the amount of REM-sleep during the light period as early as Day 30 after exposure commenced. A direct effect of U on the CNS seems a likely explanation for these neurophysiological perturbations, as it accumulated in the brain. Further studies should be performed in order to elucidate and understand the effects of U on the CNS, especially at a depressive-like behaviour level. Finally, further investigations are also warranted to determine if U could induce similar phenomena at even lower exposure levels.

References