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Pharmacology, Biochemistry and Behavior 72 (2002) 669–679

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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EEG spectra, behavioral states and motor activity in rats exposed to acetylcholinesterase inhibitor chlorpyrifos

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Received 6 July 2001; received in revised form 15 January 2002; accepted 17 January 2002

Abstract

Exposure to organophosphates (OP) has been associated with sleep disorders such as insomnia and "excessive dreaming." The central mechanisms of these effects are not well understood. OPs inhibit acetylcholinesterase (AChE) activity, leading to a hyperactivity of the brain cholinergic systems that are involved in sleep regulation. We studied alterations in the EEG, behavioral states, motor activity and core temperature in rats orally administered with 10 or 40 mg/kg of the OP insecticide chlorpyrifos (CHP). Occipital EEG, motor activity and core temperature were recorded with telemetric transmitters. Behavioral sleep–wake states were visually scored. Both doses of CHP produced alterations of the EEG (decrease in power of σ/β and increase in slow θ and fast γ bands) characteristic of arousal. EEG alterations were consistent with behavioral changes such as an increase in wakefulness and a decrease in sleep. Waking immobility was a prevalent behavior. We did not detect any overt signs of CHP toxicity, such as an abnormal posture or gait, suggesting that reduced locomotion can be a result of central effects of CHP (such as activation of cholinergic motor inhibitory system) rather than peripheral (such as an impairment of neuromuscular function). Changes in the EEG and behavior occurred independently of the decrease in core temperature. Increased wakefulness together with reduced motor activity after exposure to CHP seems to be a result of hyperactivity in brain cholinergic neuronal networks.

Keywords: Sleep–wake; Spindles; Waking immobility; θ rhythm; γ activity; Core temperature; Organophosphate

1. Introduction

Chlorpyrifos (CHP) is a broad-spectrum organophosphate (OP) insecticide that has been widely used in agricultural, commercial and domestic applications. Like many OPs, CHP irreversibly inhibits acetylcholinesterase (AChE), the hydrolytic enzyme of acetylcholine (Ach). Systemic administration of this compound globally increases Ach level at cholinergic terminals, leading to a hyperactivity in cholinergic pathways and a variety of pathophysiological changes (Gallo and Lawruk, 1991).

The two major groups of brain cholinergic neurons, one within the pontomesencephalic tegmentum that projects into the thalamocortical relay system and the pontine reticular formation and the other within the basal forebrain that receives input from the brainstem reticular formation and projects in turn upon the cerebral cortex and thalamus, are critically involved in sleep–wake states regulation (Steriade et al., 1990; Jones, 1993). Stimulation of the brainstem (Steriade et al., 1990; Vertes, 1990; Siegel, 1994; Datta and Siwek,1997; Kinney et al., 1998) or the basal forebrain (Buzsaki et al., 1988; Cape et al., 2000) cholinergic neurons activates arousal state or REM sleep, thus leading to two major manifestations of sleep alteration: insomnia and somnolence. Likewise, the clinical symptoms caused by OPs often include sleep disorder such as insomnia, nightmares, excessive dreaming (Burchfiel and Duffy, 1982; Jamal, 1997) and even narcolepsy (Metcalf and Holmes, 1969). However, central mechanisms of OP-induced dysfunctions have not been consistently investigated. Moreover, little is known on how OPs affect the EEG (Burchfiel and Duffy, 1982; Gralewicz and Socko, 1997; Gralewicz et al., 1990; 1991) and the state of vigilance (Deurveilher et al., 1999) in humans and experimental animals. Clearly, there is a need to

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0091-3057/02/$ – see front matter
PII: S0091-3057(02)00737-2

understand the central effects of OP exposure, particularly those associated with sleep disorder, since it is frequently observed in humans exposed to OP insecticides.

In our previous study (Timofeeva and Gordon, 2001), we found that rats exposed to 25 mg/kg of CHP exhibited consistent alterations in the EEG power spectra that are characteristic of an arousal state. The present study was designed to compare the alterations in spectral EEG to the sleep–wake states following exposure to a relatively low and high dose of CHP.

2. Materials and methods

2.1. Subjects

We used female Sprague–Dawley rats obtained from Charles River Laboratories (Raleigh, NC) at 90 days of age. The animal care, surgery and experimental protocol were in accordance with NIH and institutional ACUC guidelines. Animals were housed individually in acrylic cages lined with wood shavings and kept in standard conditions at an ambient temperature of 22 °C, relative humidity of 50% and a 12:12-h light/dark photoperiod. Rats were permitted free access to food and water at all times.

2.2. Surgery

The animals were surgically implanted with radio transmitters (Data Sciences International, St. Paul, MN, models TA10ETA-F40) according to the procedure described by Timofeeva and Gordon (2001) and Livezey (1997) to provide telemetric monitoring of cortical EEG, core temperature and motor activity. The rats were anesthetized with an intramuscular injection of a cocktail (2.5 ml/kg) consisting of ketamine, rompun and acetopromazine (2.5, 2.3 and 0.25 mg/ml, respectively). The transmitter body was placed inside the abdomen and the two lead wires were threaded out subcutaneously to the incision in the cranium. The transmitter body was stitched to the abdominal muscles.

Following the abdominal surgery, the rat was placed in a stereotaxic apparatus. The tips (1.0 mm in diameter) of the EEG leads (stainless-steel coiled wire with silastic insulation) were exposed by stripping approximately 4 mm of the insulation from the ends. A few coils of the wire were placed in the skull holes ($\sim$1.5 mm in diameter) drilled 3 mm anterior to the lambda and 3 mm right to the midline for the recording electrode (occipital position) and 4–5 mm anterior to the bregma and 2 mm left to the midline for the reference electrode. The electrodes were sealed in the holes with a small amount of the acrylic cement and the incision was closed with silk. A general penicillin antibiotic was administered (30,000 units im) immediately after surgery and the rats were allowed at least 2 weeks of recovery.

2.3. Protocol

Rats were gavaged with a single oral dose of 10 ($n=6$) or 40 ($n=6$) mg/kg of CHP or corn oil ($n=8$) at 14:00 h. Intragastric administration with gavage needle assured that the entire dose was consumed properly. Telemetry data were collected from each animal for 24 h before (Day 1) and after (Day 2) the treatments. All data were averaged hourly and compared at the same time within each group to eliminate variations due to circadian rhythm (Gündel and Witthöft, 1983; Cacot et al., 1995).

2.4. Data acquisition

Radio transmitters allowed EEG, core temperature and motor activity monitoring in undisturbed, freely moving rats while housed in their home cages. Telemetry data were detected by a receiver board placed beneath the rat's cage. The outputs of the transmitter (10 min in duration) for temperature and motor activity were monitored at 5-min intervals. The rat's motor activity was qualitatively assessed from the change in position of the transmitter in relation to the antennae in the receiver board and the speed with which movement occurred. This technique provided a relative measure of the rat's activity in dimensions of units per 10 min. EEG epochs (20-s duration) were stored digitally at 10-min intervals. EEG signals were sampled at a rate of 500/s.

2.5. Spectral analysis of the EEG

The EEG epochs, which contained artifacts, i.e. waves of unusual amplitude, morphology and pattern, were eliminated from the study by visual examination. Power spectra of artifact-free segments were computed using Fast Fourier Transforms. EEG spectral amplitudes were measured at 0.5-Hz intervals within a frequency range of 0.1–50 Hz. The digitizing and Fast Fourier Transform routines were supplied by Data Sciences International. The power spectra data were exported to a graphics program Prism 3.0 (GraphPad Software, San Diego, CA) for further analysis. We averaged four to six EEG samples collected hourly and analyzed averaged samples, which were acquired in the following time periods: from 12:00 to 23:00 h (Days 1 and 2 that include 2 h before and 9 h after treatment) and for animals exposed to higher dose from 1:00 to 2:00 h, from 4:00 to 5:00 h and from 7:00 to 8:00 h (Days 1 and 2 that include 12, 15 and 18 h after treatment, respectively) and from 13:00 to 14:00 h (Days 2 and 3 that include 24 h after treatment). One-hour averages of the spectral profiles were used to compute absolute and relative amplitudes and determine peak frequency in each frequency band. Mean power spectra were divided into eight classical frequency bands: δ (0.1–3.5 Hz), slow θ (4.0–6.5 Hz), fast θ (7.0–8.5 Hz), α/σ (9.0–14.0 Hz), β (14.5–30.0 Hz) and γ (30.5–50.0 Hz). We divided β and γ ranges into two additional bands, β-1 (14.5–

24.0 Hz), β-2 (24.5–30.0 Hz), γ-1 (30.5–35.0 Hz) and γ-2 (30.5–50 Hz), because our previous investigations demonstrated that changes in γ-1 were transitional between changes in β-2 and γ-2 and there was a strong reciprocal relationship between γ-2 and β-2 manifestations. Total amplitudes were estimated by summing the absolute amplitudes within each frequency band in the range of 0.1–24.0 and 24.5–50.0 Hz. Relative powers were calculated by dividing the absolute amplitude within a given frequency range by corresponding measures of total amplitude.

2.6. Behavioral evaluation

Home cage behavioral observations were performed continuously between 14:00 and 18:00 h (light photoperiod, 4 h after the treatments). The following categories of behavior were distinguished during the observation: exploring, eating, drinking, grooming, chewing, quiet waking (reclining on the cage floor with eyes opened) and sleeping (lying on the cage floor with eyes closed). The time spent in each behavioral category was visually scored in 60-min epochs. Behavioral observations were performed on 10 animals implanted with radiotelemetric transmitters (five of which received 10 mg/kg of CHP and another five received 40 mg/kg of CHP) and on additional 15 rats without transmitters. Six out of the 15 additional rats were gavaged with corn oil and served as behavioral control, and the rest of them received 10 or 40 mg/kg of CHP. Because behavioral changes were similar in rats exposed to similar dose of CHP, these data were pooled together and averaged hourly. The time spent in each behavioral state after exposure to 0, 10 or 40 mg/kg of CHP was compared.

2.7. Statistical evaluation

Transformation from absolute to relative amplitudes resulted in a normal distribution of the EEG amplitudes. The hypothesis of statistical differences between mean relative powers in each EEG band, mean core temperature and motor activity within groups as a function of treatment and time was tested by means of two-factor analysis of variance (ANOVA). In addition, a bidirectional *t* tests were applied to assess the significance of mean EEG relative amplitude differences in each hour of the studied period and of mean time spent in each behavioral state. A P value of $< .05$ was accepted as criteria of statistical significance.

3. Results

3.1. Changes in EEG

There was no significant difference in EEG parameters (Fig. 1) between Days 1 and 2 in rats treated with corn oil

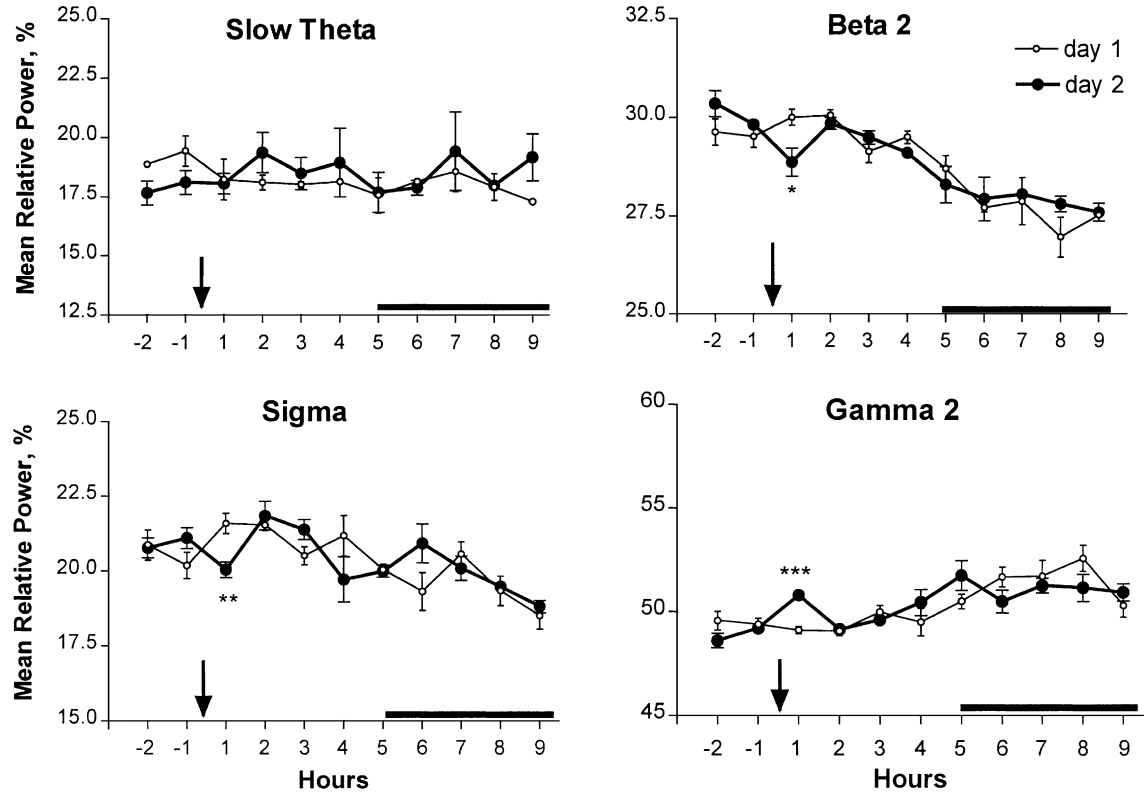

Fig. 1. Effect of corn oil gavage, time and 24-h period on EEG power variations in the main EEG bands ($n = 8$). Day 1 (before treatment) and Day 2 (treatment day) at the same clock time. Arrows indicate time of treatment. The two-way ANOVA was significant only for the factor 'Time' in σ ($P < .0001$), β ($P < .0001$) and γ bands ($P < .0001$). * $P < .01$, ** $P < .001$, *** $P < .0001$, two-tailed *t* test. Horizontal bars indicate phase of darkness.

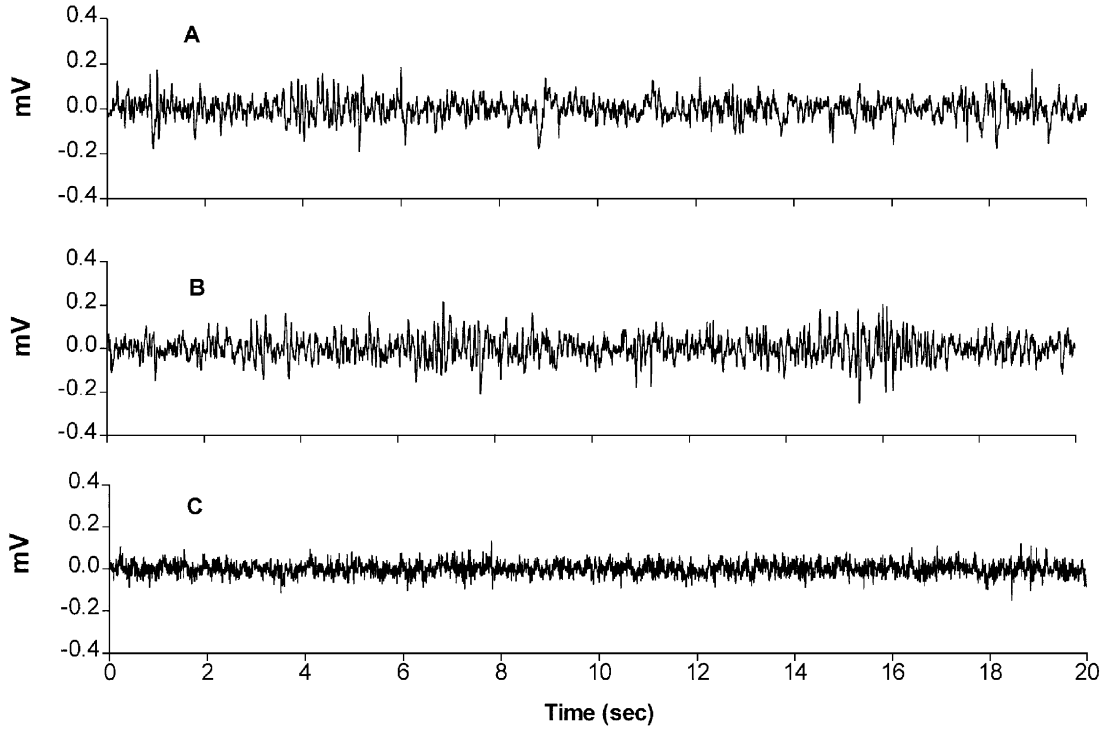

Fig. 2. Changes in the occipital EEG of the representative rat after exposure to 40 mg/kg of CHP. (A) EEG recorded 30 min before treatment. (B) EEG recorded at the same clock time as in C on Day 1. (C) EEG manifestation 2.5 h after treatment (Day 2).

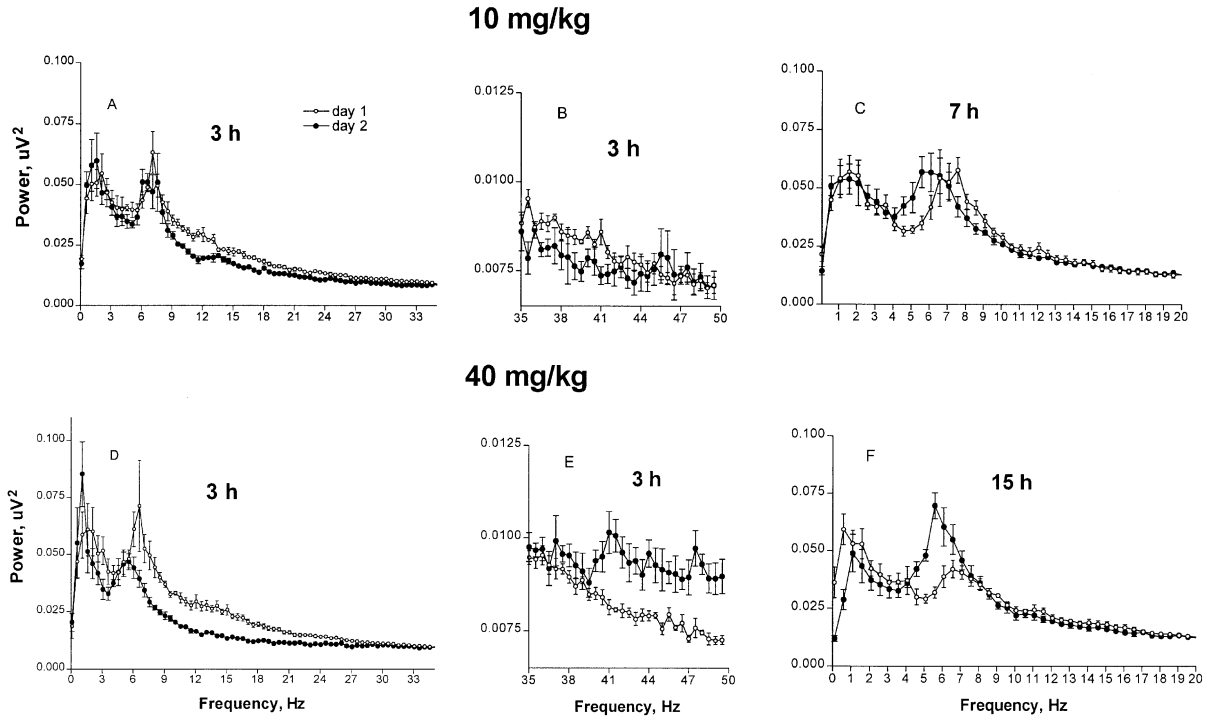

Fig. 3. Mean EEG spectral profiles ($n=6$). Day 1 (before treatment) and Day 2 (after treatment) at the same clock time. (A, B, D and E) 3 h after exposure to CHP. Note in A and D the profound decrease of power in the range 8–33 Hz with higher magnitude after 40 mg/kg. (B and E) The significant increase of absolute power in frequency faster than 38 Hz occurred only after the higher dose. (C) The changes in all bands except slow θ fully recovered 7 h after exposure, while shifts in θ reached maximum. Note leftward shift with peak frequency in 5.5 Hz. (F) 15 h after exposure. Note that changes are similar to C but of higher magnitude.

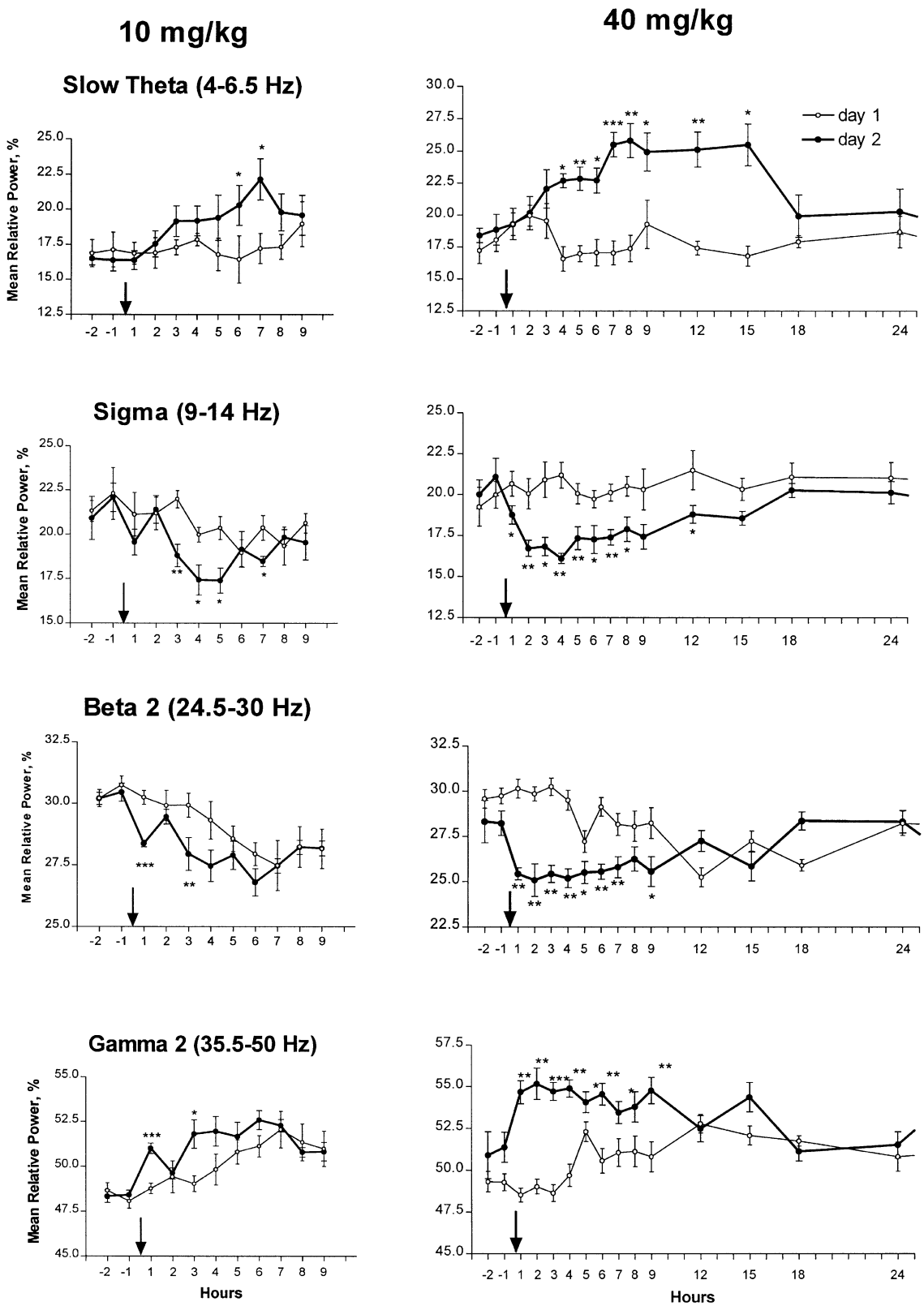

Fig. 4. Effect of CHP on mean relative power in the slow θ, σ, β-2 and γ-2 bands during the first 9 (10 mg/kg, $n=6$) and 24 (40 mg/kg, $n=6$) h postexposure. The two-way ANOVA was significant for the factor 'Treatment' in slow θ ($P<.002$, $P<.0001$), σ ($P<.0005$, $P<.0001$), β-2 ($P<.0001$, $P<.0001$) and γ-2 ($P<.0001$, $P<.0001$) after exposure to 10 and 40 mg/kg of CHP and for the factor 'Time' in σ ($P<.0005$, $P=.009$), β-2 ($P<.0001$, $P<.0001$) and γ-2 ($P<.0001$, $P<.0001$) after exposure to 10 and 40 mg/kg. A significant interaction (Treatment × Time) was found only after exposure to 40 mg/kg in slow θ ($P<.0001$), β-2 ($P<.0001$), σ ($P<.01$) and γ-2 ($P<.0001$). * $P<.01$, ** $P<.001$, *** $P<.0001$, two-tailed t test. Days 1 and 2 at the same clock time. Horizontal bars indicate phase of darkness, and arrows indicate time of treatment.

except at the first hour after treatment. Transient changes in σ, β and γ bands during the first hour after gavage can be a result of animal disturbance during handling. The two-way ANOVA did not detect a treatment effect. However, it revealed a highly significant effect of clock time on variation of EEG relative power in σ, β and γ bands. Significant alterations in the EEG were found after administration of both the low and high doses of CHP (Figs. 2–4). The changes were qualitatively similar between the two doses, but the effects started earlier, lasted longer and were larger in magnitude after 40 mg/kg of CHP.

Visual examination of raw EEG samples revealed a dramatic reduction in the incidence of sleep spindles (σ rhythm, 7–14 Hz), a response that was more profound after treatment with 40 mg/kg of CHP. We also detected an increase in γ activity (>35 Hz) that was more intensive after the higher dose of CHP (Fig. 2).

Alterations in spectral morphology (Fig. 3) after 10 mg/kg of CHP occurred mainly 3–4 h after the treatment characterized by a drop in the 8–33 Hz range (Fig. 3A) and 6–8 h after treatment characterized by an elevation in the slow θ (Fig. 3C). Peak frequency in the slow θ range shifted leftward from 6.5 to 5.5 Hz. Exposure to 40 mg/kg of CHP caused a drop in amplitude in the 7–33 Hz range (Fig. 3D). However, the drop occurred during the first hour, lasted at least 12 h and was higher in magnitude. Increase in the slow θ band was observed 4 h after exposure to 40 mg/kg of CHP. It reached maximum at 7 h and remained at this level at least to 15 h posttreatment (Fig. 3F). Peak frequency in the slow θ after 40 mg/kg also shifted leftward to 5.5 Hz. This shift persisted throughout the time when the slow θ was increased. Spectral profile did not reveal significant alterations in absolute power of the γ-2 activity after exposure to the low dose. However, after the high dose, there was a dramatic increase in the fast γ (Fig. 3B and E).

Changes in relative power after both doses were also qualitatively similar (Fig. 4). Alterations were significantly more powerful and lasted longer after the higher dose of CHP. Changes were observed in all EEG bands except δ. Alterations in the slow θ band started and recovered later than in the other EEG bands after 10 and 40 mg/kg of CHP. Increase in the slow θ was observed 3 h after both treatments and reached statistically significant level 6 h after a exposure to the low and 4 h after exposure to the high dose of CHP. Maximal shifts in the slow θ amplitude occurred ~7 h post-exposure. However, in the low dose group, the slow θ returned to baseline values during the next 2 h. In the high dose group, the slow θ remained at the maximal level for at least 8 h and abruptly returned to a baseline during the next 3 h.

Both doses of CHP produced a decrease in relative power of the σ, β-1, β-2 and γ-1 and a reciprocal increase in the γ-2. These changes were larger in magnitude following exposure to 40 mg/kg. There was a transient, full recovery in all bands during the second hour after exposure to 10 mg/kg of CHP. This was followed by a second drop in σ/β and increase in γ-2 lasting for the next 3–4 h. Relative power in the range 8–50 Hz recovered within 12–15 h after exposure to 40 mg/kg of CHP.

3.2. Changes in behavior

Exposure to CHP resulted in consistent and dramatic alterations of the behavioral states (Fig. 5). Changes in behavior were manifested mainly by a significant reduction of the time spent in sleeping postures and by a marked increase of the time spent in waking states. Among waking activities, quiet waking and grooming

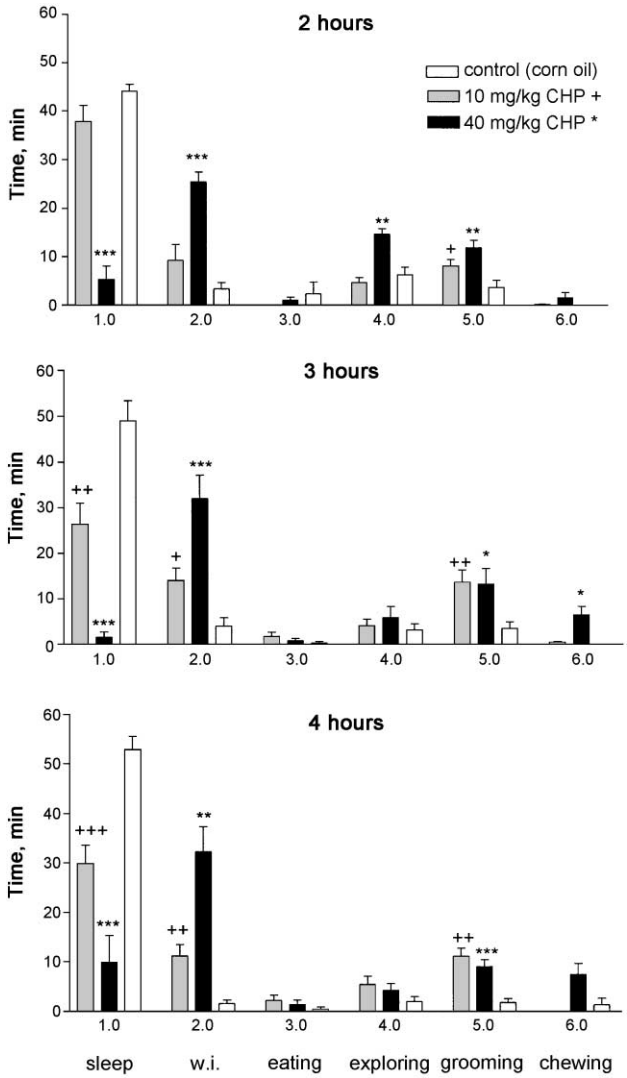

Fig. 5. Changes in the behavioral states after exposure to 0, 10 and 40 mg/kg of CHP. Note that 2 h after the treatment animals exposed to 0 ($n=4$) and 10 ($n=8$) mg/kg exhibit similar behavioral pattern, while animals administered with 40 mg/kg ($n=5$) demonstrate dramatic decrease of time spent in sleeping states, significant increase in waking immobility (w.i.), exploring and grooming. Three and four hours after the treatment, a significant reduction in sleep time was observed in animals exposed to 10 mg/kg ($n=11$) together with increased waking immobility and grooming. Animals exposed to 40 mg/kg ($n=8$) exhibited a further increase in waking immobility, while their exploring activity returned to control values ($n=6$). $^{*,+}P<.01$, $^{**,++}P<.001$, $^{***,+++}P<.0001$, two-tailed t test.

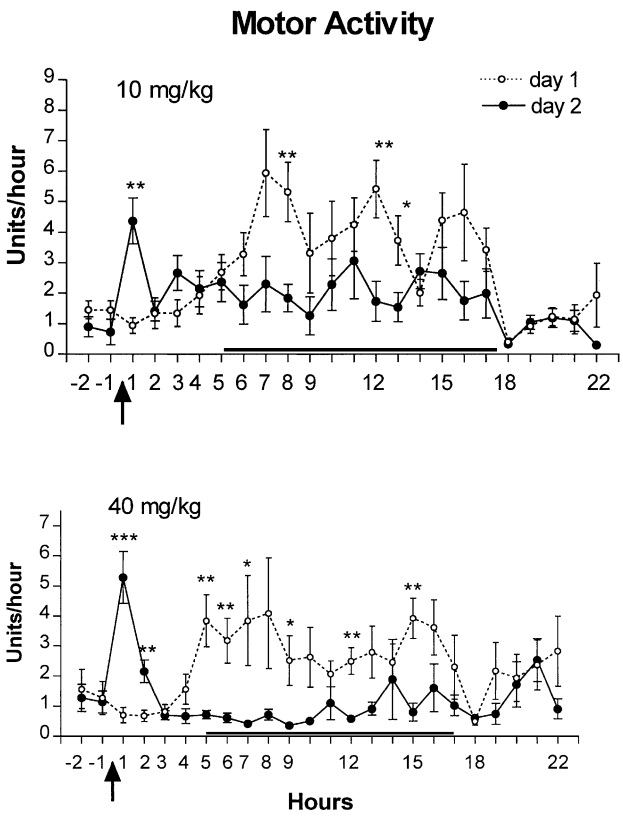

Fig. 6. Effect of CHP on motor activity ($n=6$). The two-way ANOVA was highly significant after both doses for the factor of 'Treatment' ($P<.0001$) and 'Time' ($P<.0001$). Significant interaction (Treatment × Time) found after the smaller dose ($P<.0001$). Other signs as in Fig. 2.

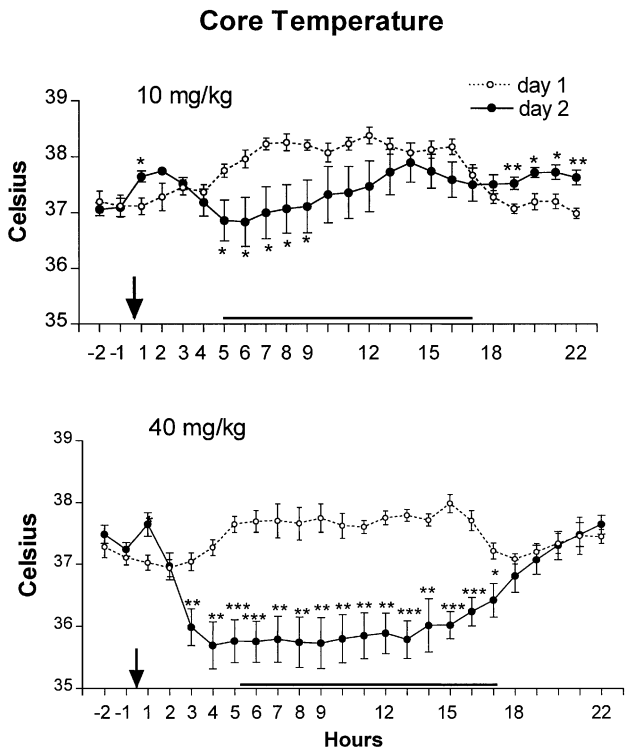

Fig. 7. Effect of CHP on core temperature ($n=6$). The two-way ANOVA was highly significant after exposure to both doses for the factor 'Treatment' ($P<.0001$) and 'Time' ($P<.0001$) with significant interaction ($P<.001$). Other signs as in Fig. 2.

were most frequently observed. Quiet waking prevailed in animals exposed to 40 mg/kg. Because handling produced an immediate arousal expressed as moving, exploring and grooming in all three dose groups, we did not estimate behavioral activity during the first hour after treatment. In rats exposed to 0 and 10 mg/kg of CHP, the handling effect disappeared in 20–30 min and animals returned to a typical for the daytime behavioral activity. These two groups showed similar behavioral pattern 2 h after treatment characterized by predominance of sleeping states. However, 3 and 4 h after treatment, rats exposed to 10 mg/kg of CHP showed a decrease in sleeping states and an increase in waking activities (quiet waking and grooming) compared to corn oil-treated animals.

Animals exposed to the higher dose of CHP demonstrated a dramatic reduction of sleeping postures during the second hour. After handling, their activity did not return to the behavioral pattern typical for the daytime period. Their arousal activity such as exploring and grooming initiated by handling continued, though to a lesser extent during the second hour, and gradually shifted to a marked waking immobility. It is important to mention that among waking activities manifested during the second hour, there was a significant increase in exploring in addition to quietness and grooming. However, exploring activity returned to control levels 3–4 h after treatment, while time spent in waking immobility increased. Quiet waking in animals exposed to 40 mg/kg of CHP was periodically interrupted by grooming and chewing the bedding material. Thus, in animals exposed to the higher dose of CHP, sleeping was suppressed and waking immobility prevailed among other waking activities. In addition, a transient increase in exploring (during the second h after the treatment) was observed only in rats exposed to 40 mg/kg of CHP. No overt signs of toxicity such as lacrimation, tremor or abnormal posture/gait were observed after the two doses of CHP.

3.3. Changes in motor activity and core temperature

Both doses of CHP produced a significant reduction in motor activity at night when animals normally exhibit a high level of motor activity (Fig. 6). A transient increase in motor activity during the first hour after exposure can express an arousal effect caused by handling. However, there was also an increase in motor activity during the second hour after exposure to 40 mg/kg and during the third hour after exposure to 10 mg/kg of CHP, although after 10 mg/kg an increase in motor activity did not reach a statistically significant level.

Both doses of CHP caused a reduction in core temperature (Fig. 7). However, the EEG and behavioral alterations

preceded the changes in core temperature. The hypothermic effect after the higher dose occurred faster, was larger and persisted longer compared to the lower dose of CHP. A transient mild elevation in core temperature observed during the first hour after the drug administration can be a result of animal disturbance caused by handling. No significant changes in motor activity and core temperature were observed in rats treated with corn oil except for first hour after treatment.

4. Discussion

The data demonstrate that 10 and 40 mg/kg of CHP elicit profound alterations in the EEG, sleep–waking states and motor activity that are not directly related to the CHP-induced changes in core temperature. In rats treated with corn oil, the same parameters varied significantly only in relation to clock time, supporting the hypothesis of their circadian evolution (Gündel and Witthöft, 1983; Cacot et al., 1995). Effects of CHP at doses 40 and 10 mg/kg were qualitatively similar to those produced by 25 mg/kg (Timofeeva and Gordon, 2001), except that changes in δ EEG activity did not reach statistically significant level in the present study. Most of the changes caused by CHP were dose dependent.

Visual inspection of raw EEG epochs revealed a significant reduction in incidence of sleep spindles after exposure to CHP. Taking in consideration that the α activity is not well represented in the rat, EEG drop in the range of 7–14 Hz can be attributed to a significant suppression of the σ rhythm or spindling. Cholinergic inhibition of the thalamic spindle pacemaker (Steriade and Llinas, 1988; Steriade et al., 1990; von Krosigk et al., 1993; Buzsaki et al., 1988) as a result of Ach accumulation after exposure to CHP can be behind the drop of the σ activity. A reduction in spindling is strongly associated with brain activated state and arousal (Steriade et al., 1990; Buzsaki et al., 1988). After exposure to 10 mg/kg of CHP, a decrease in the σ was observed at two time points. A reduction during the first hour can be a result of an arousal caused by handling. The second drop in the σ occurred spontaneously without an external animal disturbance at the third and the fourth hour and can be a result of accumulation of Ach caused by CHP. At these time points, animals also exhibited a significant decrease in the sleep states and an increase in the waking activity. After exposure to 40 mg/kg, a decline in the σ continued during the first 4 h in parallel with the behavioral changes. We suggest that the higher dose of CHP affected brain function already during the first hour, because there was no recovery to a normal activity after handling as it was after the lower dose. In addition, shifts of relative power in the σ–γ-2 range were higher in magnitude already during the first hour after exposure to 40 mg/kg of CHP.

A reduction in the 14–33-Hz EEG rhythmic activity observed after exposure to CHP can be associated with the decrease in the sleep states (Bauer, 1993; Neidermeyer, 1993) and the decreased spindling (Maloney et al., 1997). An increase in the γ-2 activity can be a result of activation of the basal forebrain cholinergic neurons (Buzsaki et al., 1988; Cape and Jones, 2000; Mann et al., 2000) and the brainstem thalamocortical cholinergic projections (Steriade et al., 1991), which are involved in genesis of such high-frequency activity.

The θ rhythm (4–10 Hz) is commonly associated with the hippocampal formation because it is most prominent in this area. However, it has also been found in the entorhinal, cingulate (Holsheimer, 1982; Borst et al., 1987; Dickson and Alonso, 1997), occipital and other cortical areas (Maloney et al., 1997). A modulating role of the basal forebrain cholinergic cells in the cortical θ activity was reported (Holsheimer, 1982; Borst et al., 1987; Mann et al., 2000). Several reports bring evidence that the θ waves recorded from the occipital cortex may also be volume conducted from a generator located in the dorsal hippocampus (Bland and Whishaw, 1976; Winson, 1976). Timo-Iaria et al. (1970) and Sano et al. (1973) observed a synchronous occurrence of the θ waves in the occipital cortex and in the hippocampus during REM sleep. Thus, literature reports demonstrate that the θ activity in the occipital cortex may reflect to a certain extent the hippocampal θ activity. It is generally agreed that cholinergic cells in the medial septum and the vertical limb of diagonal band of Broca act as pacemakers of the θ activity in the hippocampus (Petsche et al., 1962; Vinogradova et al., 1980; Brazhnik and Fox, 1999). Numerous observations have shown that there are two types of θ activity: the slow θ (4–6 Hz), which is activated during waking immobility and grooming (Leung et al., 1982; Bringmann, 1995), and the fast θ (7–10 Hz), which is found during walking, voluntary movements (Vanderwolf, 1975; Vanderwolf et al., 1975; Leung et al., 1982). Both types of θ activity were recorded during REM sleep (Vandervolf et al., 1977). It has also been shown that the slow θ is regulated by cholinergic mechanisms (Kramis et al., 1975; Vanedrvolf et al., 1977; Stewart and Fox, 1989). We found that CHP produced a dose-dependent increase in the slow θ power, which is consistent with increased waking immobility and grooming activity observed in the exposed animals. Peak frequency after both doses shifted leftward to the same point of 5.5 Hz, demonstrating that it depended on the drug's identity rather than on its dose. Increase in the slow θ with a peak frequency at 5.5 Hz has been reported in the literature after administration of cholinergic drugs such as pilocarpine (Yamamoto, 1998), oxotremorine (Timofeeva and Gordon, 2001), carbachol (Bringmann, 1995) and AChE inhibitors (Yamamoto, 1998). Unlike the literature reports, significant alterations in the slow θ after exposure to CHP occurred after a conspicuous delay when changes in the other bands fully or partially recovered. The mechanism of such delay in the slow θ alterations is unclear. Interestingly, that power in the slow θ did not show significant changes in response to handling.

Cholinergic stimulation usually results in an inhibition of the slow wave sleep and a decrease of the related δ EEG activity (Buzsaki et al., 1988; Riekkinen et al., 1991). However, we did not observe a decrease in the δ power although sleep states were significantly reduced in the rats exposed to CHP. Bringmann (1995) reported that cholinergic agonist carbachol produce a significant decrease of the δ power in the frontal but not in the occipital region. He also found that the δ activity was prominent in the occipital cortex not only during the slow wave sleep but also during quiet waking. In our previous study, we detected a transient increase in the δ range after exposure to 25 mg/kg of CHP (Timofeeva and Gordon, 2001). However, in the present study, changes in δ did not reach statistically significant values.

Both doses of CHP caused a significant reduction of motor activity. This effect of CHP has been consistently observed in our studies with doses 25 mg/kg and higher (Gordon and Padnos, 2000; Timofeeva and Gordon, 2001). A reduction in motor activity was detected by radio transmitters only during period of darkness when animals are normally active. However, our present behavioral observations suggest that a decrease in motor activity preceded the period of darkness. It happened approximately 3 h after exposure to 40 mg/kg of CHP and 4 h after exposure to 10 mg/kg of CHP. Exposed animals exhibited a dramatic reduction in sleep states and a concomitant increase in waking. Yet, it was mainly quiet waking marked by a reclining, normally sleeping posture with eyes opened. Similar behavioral changes have been reported by Cape et al. (2000) after application of neurotensin into the basal forebrain. They also reported that quiet wake state caused by neurotensin-induced activation of cholinergic basal forebrain neurons was characterized by low electromiographic activity. Muscle hypotonia normally occurs during sleep and is mediated by an active sleep-specific motor inhibitory system (Chase and Morales, 1990). There is a consensus that the structures from which inhibitory drive emanates are located in the lower brainstem, with a cholinoceptive trigger zone situated in the dorsolateral pontine tegmentum. It is possible that in abnormal conditions, such as exposure to cholinergic drugs, the motor inhibitory system, which is regulated mainly by cholinergic mechanisms (Chase and Morales, 1990; Vertes, 1990; Wu et al., 1999), can be activated during the state of wakefulness. This can produce unusual combination of waking and immobility. It is pertinent to mention that muscle atonia can be triggered in waking conditions by athletic activity or emotions (laughter, anger and fear) of sudden onset and in narcoleptics (Guilleminault, 1994; Wu et al., 1999). Because no abnormality in posture or gait was detected in the CHP-treated rats, we suggest that the decreased motor activity was not due to neuromuscular dysfunction but likely was a result of the central cholinergic effects of CHP.

It is well known that exposure to cholinergic drugs can cause epileptic activity, including limbic status epilepticus (Turski et al., 1989; Sparenborg et al., 1993; Rutecki and Yang, 1998). We can exclude the possibility of the development of limbic seizures in our animals on the basis of behavioral observations and EEG data. During long-lasting states of waking immobility or periodically occurring grooming and chewing, exposed rats were always able to respond adequately to external stimuli such as noise, light and touch. In addition, EEG monitoring during at least 24 h after exposure did not detect epileptic activity in any of the animals. It appears that the doses of CHP used in our study were not sufficient enough to produce seizure activity.

Numerous observations indicate that cholinergic stimulation can initiate and prolong REM sleep (Berkowitz et al., 1990; Vertes, 1990; Riemann et al., 1994; Cape et al., 2000; Schredl et al., 2000). Our experimental design did not allow us to investigate changes in REM sleep. However, available behavioral observations and the EEG alterations, such as a reduction of sleep spindles, suggest that REM sleep was not significantly elevated probably because of antagonizing effect of dramatically increased waking at least during the first 4 h after CHP exposure. Deurveilher et al. (1999) reported that administration of diisopropilfluorophosphate, an irreversible inhibitor of AChE, increased waking at the expense of the slow wave sleep but not of REM sleep. They suggested that effects on waking would be more rapid and more consistent than those on REM sleep because brain-activating structures are more widely distributed than those involved in REM sleep generation.

Both doses of CHP produced a significant reduction in core temperature. According to our previous investigation, this is a central effect of CHP causing a tail vasodilatation and a reduction of motor activity (Gordon, 1994). Decrease in core temperature started later than the EEG and behavioral alterations, indicating that these changes were relatively independent.

Thus, our study revealed that acute exposure to orally administered AChE inhibitor CHP at doses as low as 10 mg/kg result in significant alterations of the EEG manifestations, which are characteristic of cortical arousal. This is consistent with parallel changes in vigilance states such as a dramatic decrease in sleep and a concomitant increase in waking activity. Significant inhibition of locomotion does not seem to be a result of disturbance in neuromuscular function but rather a sequence of central cholinergic affect of CHP.

Acknowledgments

The authors thank Drs. J. Victor Nadler and William Wilson for comments on the manuscript.

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