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Ciproxifan improves working memory through increased prefrontal cortex neural activity in sleep-restricted mice

F. Chauveau [a], K. Laudereau [a], P.A. Libourel [b], D. Gervasoni [b], J. Thomasson [a], B. Poly [a], C. Pierard [a], D. Beracochea [c,*]

[a] IRBA (Armed Biomedical Research Institute) BP73, F-91223 Bretigny-sur-Orge Cedex, France
[b] Centre de Recherche en Neurosciences de Lyon, INSERM U1028, CNRS UMR 5292, Université Claude Bernard Lyon 1, France
[c] INCIA (Institute of Cognitive and Integrative Neurosciences of Aquitaine), UMR CNRS 5287, University of Bordeaux1, F-33400 Talence, France

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ABSTRACT

Histamine receptor type 3 (H3) antagonists are promising awakening drugs for treatment of sleep disorders. However, few works have tried to identify their cognitive effects after sleep restriction and their impact on associated neural networks. To that aim, Bl/6J male mice were submitted to acute sleep restriction in a shaker apparatus that prevents sleep by transient (20–40 ms) up and down movements. Number of stimulations (2–4), and delay between 2 stimulations (100–200 ms) were randomized. Each sequence of stimulation was also randomly administered (10–30 s interval) for 20 consecutive hours during light (8 h) and dark (12 h) phases. Immediately after 20 h-sleep restriction, mice were injected with H3 antagonist (ciproxifan 3 mg/kg ip) and submitted 30-min later to a working memory (WM) task using spatial spontaneous alternation behaviour. After behavioural testing, brains were perfused for Fos immunohistochemistry to assess neuronal brain activation in the dorsal dentate gyrus (dDG) and the prefrontal cortex. Results showed that sleep restriction decreased slow wave sleep (from 35.8 ± 1.4% to 9.2 ± 2.7%, $p < 0.001$) and was followed by sleep rebound (58.2 ± 5.9%, $p < 0.05$). Sleep restriction did not modify anxiety-like reactivity and significantly decreased WM at long (30 s) but not short (5 s) inter-trial intervals. Whereas sleep restriction failed to significantly modify immunopositive cells in vehicles, ciproxifan administration prevented WM deficits in sleep restricted mice through significant increases of Fos labelling in prelimbic, infralimbic and cingulate 2 cortex.

In conclusion, ciproxifan at 3 mg/kg enhanced WM in sleep restricted mice through specific modulation of prefrontal cortex areas.

1. Introduction

Ciproxifan (cyclopropyl 4-(3-(1H-imidazol-4-yl)propyloxy) phenyl ketone) is an extremely potent histamine H_3 receptor inverse agonist/antagonist. The histamine H_3 receptor is an inhibitory autoreceptor located on histaminergic nerve terminals, and is involved in modulating the release of histamine in the brain. Histamine has an excitatory effect in the brain via H_1 receptors in the cerebral cortex, and so drugs such as ciproxifan which block the H_3 receptor and consequently allow more histamine to be released have an alertness-promoting effect (Le et al., 2008; Ligneau et al., 1998; Parmentier et al., 2002; Vanni-Mercier et al., 2003). Interestingly, H3 receptors are also found on cholinergic and dopaminergic neurons, and therefore modulate the release of several neurotransmitters. Therefore, histaminergic neurons are involved in cognitive processes such as memory mainly via the interaction with the cholinergic system and/or the activation of H1 and H2 receptors of the cerebral cortex or the hippocampus. Antagonists of H3 receptors such as ciproxifan or thioperamide produces wakefulness in animal studies and also produces cognitive and memory enhancing effects mainly the consolidation phase of memory processes (Alvarez and Banzan, 2008; Benetti and Izquierdo, 2013; Bonini et al., 2013; Da Silveira et al., 2013; Köhler et al., 2011). It has been also found that thioperamide could enhance spontaneous alternation involving spatial working memory (Vohora et al., 2005) and counteract the memory impairments induced by anticholinergic compounds such as scopolamine (Bernaerts et al., 2004; Komater et al., 2005; Miyazaki et al., 1995; Orsetti et al., 2001; Xu et al., 2009).

* Corresponding author.
E-mail address: daniel.beracochea@u-bordeaux1.fr (D. Beracochea).

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From a functional viewpoint, specific neurocognitive domains including executive attention, working memory and higher cognitive functions are particularly vulnerable to sleep loss (Durmer and Dinges, 2005; Mu et al., 2005). More specifically, sleep loss, even moderate, compromises the function of neuronal circuits critical to sub second attention processes during working memory tasks (Smith et al., 2002). The associated decrease in brain activity during working memory tasks appears to be a function of the individual sleep restriction vulnerability. Moreover, executive functioning is largely dependent on activity in the prefrontal cortex and affected negatively by one night sleep restriction (Chee et al., 2006; Chuah et al., 2006; Killgore et al., 2006; Leenaars et al., 2012; Libedinsky et al., 2011; Nilsson et al., 2005). Hippocampus-dependent memory and plasticity are also impaired after sleep restriction (Guan et al., 2004; Hagewoud et al., 2009; Hairston et al., 2005; McDermott et al., 2003; Romcy-Pereira and Pavlides, 2004; Tung et al., 2005; Vecsey et al., 2009).

Survey of the studies has thus clearly established a wakening impact of H3 antagonist in sleep-restricted subjects (Iannone et al., 2010; James et al., 2011; Wallace et al., 2011) or a cognitive enhancing effects in non sleep-restricted subjects. However, to the best of our knowledge, there is no study having as yet investigated the effect of H3 receptor antagonist on working memory after sleep restriction and related brain neural activity. Hence, the original aims of our study have been to investigate in sleep-restricted mice, the effects of ciproxifan on spatial working memory with subsequent evaluation of the neural activity level in various cerebral areas known to be involved in working memory such as the prefrontal cortex and hippocampus (Beracochea and Jaffard, 1985; Pierard et al., 2007; Vandesquille et al., 2013).

2. Materials and methods

2.1. Animals

Subjects were male mice of the C57Bl/6J inbred strain obtained from Charles Rivers (France). They were either 3 months at the time of experiments, and housed individually with continuous access to food and water, on a 12 h light–dark cycle (light onset: 8 am; offset: 8 pm) in a temperature-controlled room. All test procedures were conducted during the light phase of the cycle. Experiments were performed respecting the new European Communities Council Guidelines (Directive 2010/63/EU). Protocols were approved by an independent ethical board (authorization number: IMASSA_Chauveau1101).

2.2. Apparatus for alternation task

Behavioural tests were conducted in a grey Plexiglas T-maze. Stem and arms were 35 cm long, 15 cm wide and 10 cm high. The starting box (10 cm × 12 cm) and each goal-arm were separated from the central alley by a vertical sliding door, with opening and closing monitored by a computer (Imetronic, France). Photoelectric cells allow recording of both the choice of the goal-arm (left or right) and the latency (in sec) that elapsed between the opening of the start-box and the closing of the goal-box. The T-maze was located at the centre of a room with various allocentric cues (white or black or striped card boards) located on the wall 1 m above the apparatus. A white noise (30 dB) and controlled lighting (30 lux) were also provided in the behavioural testing room.

2.3. Behavioural procedure

The behavioural task used to test working memory (WM) is based on spontaneous alternation behaviour (SA), which does not require the use of food reinforcement to emerge. SA is the innate tendency of rodents whereby over a series of trials run in a T-maze, they alternate at each successive trial the choice of the goal arm (except for the first trial). From trial to trial, accurate performance at a given trial (N) requires for subjects to be able to discriminate the specific target trial N-1 from the interfering trial N-2. Thus, the target information required for successful performance varies from trial to trial, so that the subject is not only required to keep temporarily in short-term memory specific information, but also to reset it over successive runs. The resetting mechanisms and cognitive flexibility required to alternate over successive runs are major components of working memory processes. Working memory is a component of the sequential alternation task, since SA rates are dependent on the length of the inter trial delay interval, and/or the place of the trial in the series. Indeed, repetitive testing constitutes a potent source of proactive interference. Thus, the sequential alternation procedure is relevant to assess delay-dependent working memory in mice (Beracochea and Jaffard, 1985; Chauveau et al., 2005; Vandesquille et al., 2013). Animals were not food restricted and no food reinforcement was used. Animals were first submitted to a habituation phase consisting in 2 free exploration sessions 10 min each, over 2 consecutive days (one session per day, Day 1 and Day 2) in the apparatus with all doors opened. At the end of the habituation phase, all subjects were submitted to a training phase, involving a series of 6 successive trials separated by a 30 s inter-trial interval (ITI). The training phase was aimed at familiarizing the subjects with the opening and closing of the doors.

The behavioural schedule was identical for the training phase and test session. Thus, in both cases, the subject was placed in the start box at the beginning of a trial, and after a confinement period (predefined ITI) the door to the stem was opened. When the mouse entered one of the arms, the door was closed, and the chosen arm was recorded. After a 30 s confinement period in the chosen arm, the mouse was gently returned to the start-box for a second trial, identical to the first one. In the test session however, animals of independent groups were submitted to the same general procedure as in the training phase, except that the ITI was either 5 s or 30 s. The parameters recorded for the analysis of each trial were the chosen arm and the running latency (time between the start arm and the chosen arm). Alternation rates and running latency were averaged for 6 consecutive trials.

To avoid olfactory cues in the apparatus, visible traces of urine and faeces were removed from the apparatus between trials and the maze was cleaned with 5% ethanol solution.

The sleep restriction procedure was interpolated between the training phase (Day 3) and the test phase (Day 4).

2.4. Elevated plus maze

The plus-maze, which was constructed of grey Plexiglas, consisted of four arms arranged in the shape of a plus sign. Each arm was 30 cm long, 7 cm wide and elevated 38 cm above the ground. The four arms were joined at the centre by a 7-cm square platform. Two opposite arms of the plus maze were “closed” by side walls 24 cm high, but open on the top, while the remaining arms did not have side walls. Light intensity was controlled before experiment (100 lux in open arms; <10 lux in closed arms). These walls did not extend from the centre of the maze. At the beginning of each test, mice were placed on the centre of the maze and were allowed to freely explore all arms of the maze for 5 min. Behaviour was recorded by video tracking system (Viewpoint, France) allowing to measure the travelled distance (in cm) and running time (s) into both the open and closed arms. Entry and latency were recorded only when a mouse entered an arm with all four paws. Two measures of “anxiety” were taken. The first was the ratio of the time spent in the open arms divided by the total time spent in all arms of the maze (time ratio). The second was the ratio of distance into the open arms divided by the total distance in all arms (distance ratio). Thus, the smaller are these ratios, the more “anxious” is the mouse. This experiment was performed in animals being either non sleep deprived, or having been submitted to a 20 h-sleep restriction procedure, as described below. Behavioural testing occurred immediately after the end of the sleep restriction phase as for working memory experiment.

2.5. Sleep deprivation procedure

2.5.1. Apparatus and general schedule

Mice were submitted to acute sleep deprivation in a shaker apparatus (PVC cylinder, diameter 30 cm, 45 cm height) that prevent sleep by transient up and down movement (20–40 ms, 1 cm height). Number of stimulations (2–4) and delay between 2 stimulations (100–200 ms) were randomized and controlled by software (Viewpoint, France). Each sequence of stimulation were also randomly administered (10–30 s interval) for 10 (zeitgeber time: ZT15-ZT24) or 20 consecutive hours (ZT5-ZT24).

2.5.2. Validation of sleep deprivation model by polysomnographic recordings

EEG and EMG electrodes were implanted under deep anaesthesia with a mixture of ketamine and xylazine (100 mg/kg and 10 mg/kg *ip* respectively). Three stainless steel miniature screws (diameter: 520 μm) were implanted in contact with dura matter and served as frontal electrode (stereotaxic coordinate bregma as a reference: AP = +2 mm, LAT = +1 mm), parietal electrode (AP = −2 mm, LAT = −1.5 mm) and reference electrode (AP = −6 mm, LAT = +2 mm). Screws were soldered to Teflon® coated semi-rigid silver wires (330 μm in diameter) connected to a female contact (MS363, PlasticOne) that was inserted to a pedestal (E363, PlasticOne). For EMG recordings, two semi-rigid silver wires (330 μm in diameter) served as EMG electrodes and were connected to a female contact and inserted into the pedestal. Screws and pedestal were fixed to the skull with glass ionomer cement (GC FujiPlus, USA). Animals were allowed to recover during 10 days. EEG and EMG recordings were performed using Epas40 hardware (Deltamed, France) at a 256 Hz sampling rate. Mice were habituated to the shaker environnement during 24 h (day 1), day after, recordings were performed to get 24 h baseline polysomnographic recordings (day 2). On day 3, animals were sleep-deprived during 20 h consecutively (from ZT5 to ZT24). On day 4, the shaker was switched off (ZT0), animals were free to sleep *ad libitum* and sleep rebound was quantified (ZT0-ZT4).

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