

## Melatonin administration impairs visuo-spatial performance and inhibits neocortical long-term potentiation in rats

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### Abstract

Melatonin has been shown to inhibit long-term potentiation (LTP) in hippocampal slices of rats. Since LTP may be one of the main mechanisms by which memory traces are encoded and stored in the central nervous system, it is possible that melatonin could modulate cognitive performance by interfering with the cellular and/or molecular mechanisms involved in LTP. We investigated in rats the effects of intraperitoneally-administered melatonin (0.1, 1 and 10 mg/kg), its saline-ethanol solvent, or saline alone, on the acquisition of visuo-spatial memory as well as on the ability of the cerebral cortex to develop LTP *in vivo*. Visuo-spatial performance was assessed daily in rats, for 10 days, in an 8-arm radial maze, 30 min after they received a single daily dose of melatonin. Visual cortex LTP was determined in sodium pentobarbital anesthetized rats (65 mg/kg i.p.), by potentiating transcallosal evoked responses with a tetanizing train (312 Hz, 500 ms duration) 30 min after administration of a single dose of melatonin. Results showed that melatonin impaired visuo-spatial performance in rats, as revealed by the greater number of errors committed and time spent to solve the task in the radial maze. Melatonin also prevented the induction of neocortical LTP. It is concluded that melatonin, at the doses utilized in this study, could alter some forms of neocortical plasticity involved in short- and long-term visuo-spatial memories in rats.

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### 1. Introduction

Melatonin, the main hormone secreted by the pineal gland, mediates a variety of cellular, neuroendocrine and physiological processes; besides, melatonin displays through different mechanisms a protective role against damage caused by free radicals (Reiter, 1995). It has been reported that melatonin may reduce cognitive processing in healthy humans, as revealed by decreased performance on response and reaction time scores for a two-choice visual task and for auditory tasks (Rogers et al., 1998), as well as by impaired performance in a psychomotor digit-symbol substitution test (Naguib and Samarkandi, 1999). Although the sites and mechanisms of action by which melatonin induced these neurobehavioral effects are not entirely

known, it has been suggested that the neurobehavioral deficits induced by melatonin could be the results of decreased levels of psychomotor vigilance, as revealed by increased reaction time scores evaluating alertness during sustained attention (Graw et al., 2001) or even by the hormone's hypothermic properties causing a slowing down of cerebral processing speed (Slotten and Krekling, 1996), rather than by a direct effect of melatonin on neurons that are relevant to cognitive processes. Animal studies on this matter have not provided a conclusive answer to these questions. In fact, it has been reported that melatonin affects passive and active avoidance learning (Kovacs et al., 1974) and induces short-term memory deficits in rats (Shaji and Kulkarni, 1998), however, other reports showed that melatonin facilitates short-term memory in an olfactory social memory test (Argyriou et al., 1998). Concerning the possibility that melatonin could directly affect neuronal circuitries involved in cognitive processes, it has been shown that melatonin inhibits long-

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term potentiation (LTP) in hippocampal slices of rodents (Collins and Davies, 1997; Wang et al., 2005), attenuates the population spike triggered by low-frequency stimulation in the mouse hippocampus (Hogan et al., 2001; El-Sherif et al., 2003), and depresses synaptic potentiation (wind-up) in the spinal cord dorsal horn of rats (Laurido et al., 2002; Noseda et al., 2004; Mondaca et al., 2004a). As a whole, these data suggest that melatonin could modulate specific forms of plasticity in central nervous system neurons. Since LTP may be one of the main synaptic mechanism by which memory traces are encoded and stored in the hippocampus, cerebral cortex and other regions in the central nervous system (Martin et al., 2000), it is possible that melatonin could alter cognitive performance by interfering with the cellular and/or molecular mechanisms involved in LTP.

This study was designed to investigate the effects of intra-peritoneally-administered melatonin on the acquisition of visuo-spatial memory in rats as well as on the ability of the cerebral cortex to develop LTP *in vivo*.

## 2. Materials and methods

All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, 1985) and the protocols approved by the Bioethical Committee for the Experimental Study on Animals of the Institute of Nutrition and Food Technology (INTA). Male and female young healthy Long–Evans rats from our laboratory were housed four per cage and separated by sex at weaning, with free access to food and water under controlled laboratory conditions (a 12-h light/dark cycle with lights off at 10:00 a.m.). During the light cycle, light intensity was maintained at 300 lx as measured at the level of the cage floor.

### 2.1. Visuo-spatial memory test

Visuo-spatial memory was evaluated in 40 rats by employing an 8-arm radial Olton maze (Olton, 1978), according to a methodology described elsewhere (Burgos et al., 2005; Soto-Moyano et al., 2005). It consisted of eight equally spaced plexiglas arms (70-cm-long, 8-cm-wide) extending from a central octagonal hub (34-cm-across). The maze was placed 92 cm from the floor in a room with white walls. During the adaptation and testing sessions, all the arms of the maze were baited with rice puffs. Spatial cues external to the maze were provided by the experimenter itself, together with different articles for dressing placed on different hangers fixed on the walls of the room; the position of these articles and the position of the experimenter never changed during the 10-day of maze testing in each group of rats. To test animals in this maze, motivation for food is required. Food motivation was induced by keeping animals on a restricted diet (8 g/day/rat) from day 50 of age until a 15% body weight deficit was obtained. Thereafter, at 58, 59 and 60 days of age, each animal was submitted to an adaptation period which consisted in placing the rat in the center of the maze to explore and run to the end of the arms and consume the bait. From days 61 to 70 of age the animals were submitted to the visuo-spatial memory test (one assay daily,

10 days of testing). In each daily assay, at 12:00 h, 2 h after lights off and 30 min before beginning memory testing, rats received a single dose (1 ml/kg body weight) of 0.1, 1.0 or 10 mg/kg i.p. melatonin dissolved in saline containing 5% ethanol ( $N=8$  rats for each dose of melatonin), the ethanolic solvent alone ( $N=8$  rats), or saline ( $N=8$  rats), and then were placed in the central platform where they could freely run the maze until they obtained the 8 baits of food. Melatonin, its solvent or saline was injected 30 min before testing, since it has been shown that the peak concentration of the hormone in the brain is reached 30 min after the systemical administration of exogenous melatonin (Crespi et al., 1994). The time required to solve the task (with a cut-off time of 10 min) and the number of errors (entry to already visited arms) were measured as significant parameters for memory evaluation.

### 2.2. Cortical LTP determination

Cerebral cortex LTP was induced in the right occipital cortex of 40 rats of 60–70 days of age. Rats were weighed, anesthetized with 65 mg/kg i.p. of sodium pentobarbital and placed in a stereotaxic apparatus. A single dose of 1.5 mg/kg of d-tubocurarine was injected i.m. and adequate ventilation was maintained by means of a respirator pump. d-Tubocurarine does not penetrate the normal intact brain–blood barrier and is therefore devoid of effects in the central nervous system when administered systemically. LTP was induced in the occipital cortex according to the method reported elsewhere (Racine et al., 1994; Mondaca et al., 2004b; Soto-Moyano et al., 2005).

After exposure of the occipital lobe of both cerebral hemispheres, electrical stimulation of the corpus callosum (CC) was carried out by means of a bipolar electrode that penetrates through the left visual cortex at the de Groot coordinates  $A=0.0$  mm,  $L=3.5$  mm, according to the atlas of Pellegrino et al. (1967). The stimulating electrode consisted of two braided 100- $\mu$ m-diameter wires with a 1.0-mm tip separation; one tip of the electrode was located over the CC and the other penetrated the CC until the de Groot coordinate  $V=3.0$  mm. Transcallosal evoked responses (TERs) were recorded from the right visual cortex with a surface monopolar silver ball electrode, 0.5 mm diameter, located on the right primary visual area at similar surface de Groot coordinates to those utilized for transcortical stimulation of the CC. The test stimuli consisted of 100  $\mu$ s duration square-wave pulses generated by a Tektronix 161 stimulator in conjunction with a Grass SIU-5 stimulus isolation unit. The stimulating voltages were measured directly with an oscilloscope and the stimulating currents were conventionally measured by inserting a 1 k-ohm resistor in the circuit. In each experiment the impedance of the electrode saline junction was determined as the quotient between the mean stimulating voltage and the mean current intensity measured. Thus, stimulating parameters (voltage, current intensity and electrode impedance) were quite similar in all rats. Recordings were amplified by a Grass P-511 preamplifier (0.8–1000 Hz bandwidth) and displayed on a Philips PM 3365A digital oscilloscope. In all the experiments body temperature and expired CO<sub>2</sub> were monitored and remained within normal limits. Before beginning each

experiment, a full input–output series was performed at a stimulus intensity of 300–1100  $\mu\text{A}$ , and test stimuli with a stimulation intensity yielding responses with peak-to-peak amplitude of 50% of the maximum were used for the remainder of the experiment. After a 30-min stabilization period, a 5-min control period of basal responses (30 averaged responses) was recorded. Afterwards, rats received a single dose (1 ml/kg body weight) of 0.1, 1.0 or 10 mg/kg i.p. melatonin dissolved in saline containing 5% ethanol ( $N=8$  rats for each dose of melatonin), the ethanolic solvent alone ( $N=8$  rats), or saline ( $N=8$  rats). The tetanizing stimulus, applied 30 min after melatonin or solvent administration, consisted in a single train of 100  $\mu\text{s}$  duration of square-wave pulses at 312 Hz and 500 ms duration, whose intensity was 50% higher than that of the test stimuli. Basal responses evoked in the rat cerebral cortex by contralateral stimulation of the corpus callosum begin with an early downward surface positive deflection (P) followed by a late upward surface negative wave (N). P–N latency and P–N peak-to-peak amplitude were measured using time and voltage cursors provided in the digital oscilloscope. Slope was determined as the amplitude/time ratio on the nearest sample to the 10% and the 90% level between cursors set on peaks P and N. The efficacy of the tetanizing train to potentiate cortical evoked responses was evaluated by measuring both the peak-to-peak amplitude and the maximal slope increases. The results were similar, so amplitudes were used for analyses of the experiments, according to a procedure reported elsewhere (Racine et al., 1994; Mondaca et al., 2004b; Soto-Moyano et al., 2005). At the end of the electrophysiological experiments, the animals were sacrificed with an overdose of sodium pentobarbital.

### 2.3. Statistics

All statistical analyses were performed with the Instat 3.0 software (GraphPad Software, Inc., San Diego, CA, USA). Data are reported as means  $\pm$  S.E.M. For intergroup comparisons a one-way ANOVA was performed, followed by the Student–Neuman–Keuls multiple comparisons test at a probability level of 0.05.

### 3. Results

Fig. 1A (left panel) shows the number of errors performed by rats in ten assays in the radial maze. Rats receiving melatonin committed a significantly greater number of errors than control rats receiving either the ethanol-saline solvent or saline, with 0.1 mg/kg melatonin increasing the number of errors in block 1–2, 1.0 mg/kg melatonin increasing the number of errors in blocks 1–2 and 3–4, and 10 mg/kg melatonin increasing the number of errors in all 2-assay blocks. Fig. 1A (right panel) shows the total number of errors committed by rats for the 10 days of testing. It can be observed that all the melatonin groups exhibited an increased total number of errors when compared to the solvent- or saline-injected control groups, but without showing a dose–response relationship. Fig. 1B (left panel) shows that melatonin treated rats spent significantly more time to solve the task than solvent injected rats in each 2-assay

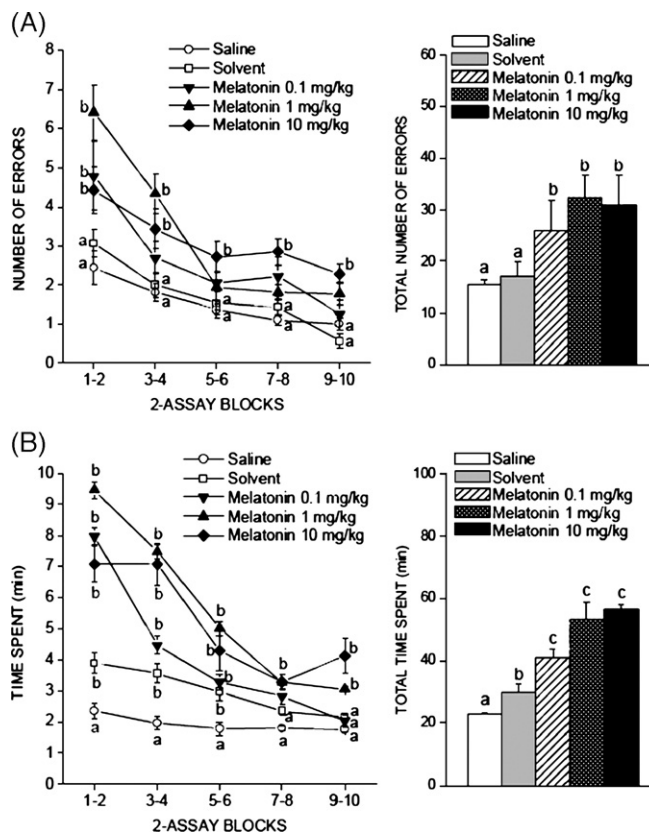


Fig. 1. Effect of 0.1, 1 and 10 mg/kg of intraperitoneal melatonin on visuo-spatial memory of rats. Left panels show the number of errors (upper panel) and the time spent (lower panel) to solve the task in the radial maze, 30 min after administration of melatonin, its solvent, or saline, during 10 consecutive days of testing. Values are the means  $\pm$  S.E.M. of scores obtained from 8 rats per group, recorded during ten consecutive days of testing (one assay daily) and grouped in 2-assay blocks. In each block, the values were calculated as the mean of the two scores composing the block. For each 2-assay block, values with the same letter are not significantly different, while different letters indicate a statistically significant ( $P < 0.05$ ) intergroup difference (one-way ANOVA followed by the Student–Newman–Keuls multiple comparison test). Right panels show the means  $\pm$  S.E.M. of the total number of errors (upper panel) and of the total time spent (lower panel) for the 10 days of testing (5 blocks of 2 days each). Values with the same letter are not significantly different, while different letters indicate a statistically significant ( $P < 0.05$ ) intergroup difference (one-way ANOVA followed by the Student–Newman–Keuls multiple comparison test).

block, 0.1 mg/kg melatonin increasing the time spent in blocks 1–2, 3–4 and 5–6, while 1.0 and 10 mg/kg melatonin increased the time spent in all 2-assay blocks. A similar trend is observed when considering the total time spent for the 10 days of testing (Fig. 1B, right panel), but without showing a dose–response relationship. Administration of the ethanolic solvent resulted in a moderate but significant greater time spent for task solving compared to saline administration (Fig. 1, left and right panels). Observation of the overt behavior of melatonin-treated rats while performing the task revealed no detectable changes in mobility and exploratory ability of the animals.

Fig. 2A shows representative examples of averaged TERs recorded from one control and one melatonin injected rat, before and 30 min after the application of the tetanizing train. After application of the tetanizing train the transcallosal



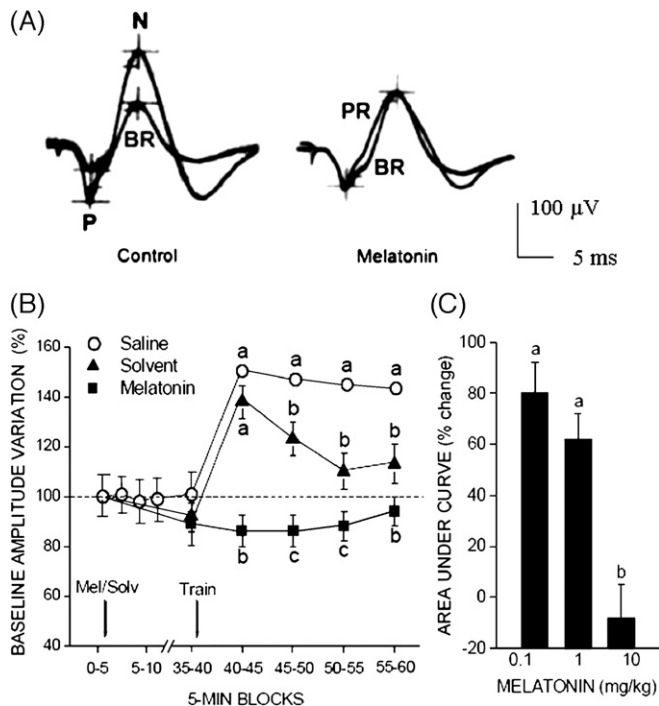


Fig. 2. (A) Representative example of the average of 10 successive pre-train basal responses (BR) and 10 successive post-train responses (PR) evoked in occipital cortex of one control and one melatonin-treated (10 mg/kg i.p.) rat by contralateral stimulation of the corpus callosum at 0.1 Hz. Positive (P) and negative (N) waves used to measure peak-to-peak amplitude and calibration bars are indicated. (B) Time-course of the effect of intraperitoneal melatonin (10 mg/kg i.p.), its solvent, or saline (left bar), on long-term potentiation in the visual cortex of rats. Ordinates: peak-to-peak amplitude variation (% of previous baseline value) of transcallosal evoked responses after application of a single tetanizing train of electrical pulses (right bar). Values are means  $\pm$  SEM, grouped in 5-min blocks. For each 5-min block, values with the same letter are not significantly different, while different letters indicate statistically significant ( $P < 0.05$ ) intergroup difference (one-way ANOVA followed by the Student–Newman–Keuls multiple comparison test). (C) Global effect of 0.1, 1 and 10 mg/kg of intraperitoneal melatonin on long-term potentiation in the visual cortex of rats, as evaluated by the area under the time–course curves. Values are means  $\pm$  SEM of the percentage change of the area under the curves; different letters indicate statistically significant ( $P < 0.05$ ) intergroup difference (one-way ANOVA followed by the Student–Newman–Keuls multiple comparison test).

responses of control rats had higher peak-to-peak amplitude compared to pre-train basal responses, suggesting the development of LTP; in contrast, no change in amplitude of the transcallosal response was observed after application of the tetanizing train to the melatonin injected rat. Fig. 2B shows the time-course of LTP in rats after i.p. administration of either 10 mg/kg of melatonin, the saline-ethanol solvent or saline. Administration of melatonin 10 mg/kg did not change the peak-to-peak amplitude of cortically evoked responses by its self, but prevented the induction of LTP by the tetanizing stimulation, as compared to saline injected controls. In contrast, animals receiving the solvent of melatonin exhibited about 40% increase of peak-to-peak amplitude of TERs 5–10 min after application of the tetanizing stimulus, a potentiating effect nearly similar to that observed in the saline group. Potentiation was still present 10–15 min after the ethanol solvent administration, but there-

after decreased to values below those of the saline control group. Fig. 2C shows the global effect of 0.1, 1.0 or 10 mg/kg melatonin i.p. over the 30 min period of testing, as evaluated by the area under the time–course curves (the integral was calculated using the Origin 6.0 software, Microcal Software, Inc., Northampton, MA, USA). Melatonin prevented the induction of neocortical LTP in a dose-dependent manner, the larger dose preventing completely LTP development.

#### 4. Discussion

After systemic administration, melatonin crosses rapidly the blood–brain barrier and is distributed to the cerebrospinal fluid and throughout the different regions of the brain (Vitte et al., 1988). Reported data showed that intraperitoneal administration of melatonin to anesthetized rats during the light phase, at similar doses to those used in the present study (0.1, 1 and 10 mg/kg), resulted in melatonin plasma levels of around 50-, 500- and 5000-fold the normal diurnal plasma melatonin concentration respectively (Kato et al., 2001); this is about 5, 50, and 500 times the normal nocturnal melatonin plasma level, since plasma melatonin increases in rats by about a 10-fold factor during the darkness (Klante et al., 1999). Interestingly, a dose of 10 mg of oral melatonin resulted in 50 times the normal nocturnal levels in humans (Dollins et al., 1993).

Exogenous melatonin administration impaired the visuo-spatial performance of rats in the radial maze, as revealed by the greater number of errors committed and the time spent to solve the task. Since the 8-arm radial maze is classically viewed as a test for evaluation of working memory, our results suggest that melatonin, administered 30 min before testing, impaired the working memory of rats as revealed by their deficient performance in the radial maze compared to the rats receiving the saline-ethanol solvent or saline alone. However, it must be considered that rat performance in the radial maze improves on successive assays indicating the involvement not only of short-term memory but also of some type of long-term memory. In fact, after 9–10 days of assays, solvent-injected rats performed significantly better than on days 1–2 of testing (Fig. 1, left upper and lower panels). On the basis of these observations, it is apparent that melatonin administration could also impair some long-term memory processes, as revealed by the significantly higher number of errors committed in task solving during the first 2 days of assays (with 0.1 mg/kg melatonin), the first 4 days of assays using 1 mg/kg melatonin and the whole assay period when using the larger dose of 10 mg/kg melatonin. A rather similar trend is observed when considering the time spent to solve the task. Although the effect of melatonin on the number of errors and time spent in task solving seems to be dose-independent, at least when considering the global effect of melatonin over the total period of testing, the evolution of the effects indicate a differential duration of the memory deficit induced by the daily administration of the hormone related to the dose of melatonin used, the higher the dose the longer the duration of the deficit. This suggests that some adaptive processes take place in the rat brain allowing functional recovery to

occur after small, repetitive doses of melatonin. This may be related to the reported ability of melatonin receptors to undergo desensitization or internalization (Gerdin et al., 2003). Despite the fact that ethanol solvent- and saline-injected rats committed closely similar numbers of errors in the radial maze, rats receiving the ethanolic solvent spent significantly more time than the saline-injected controls to solve the task, which could be ascribed to the well-known hypnotic effect of ethanol and related deficits in exploratory activity.

Hypothetically, melatonin could affect the visuo-spatial performance of rats by influencing their sleep–wake behaviour. In this respect there is evidence that its acute systemic administration at a dose of 10 mg/kg i.p. did not influence sleep architecture nor sleep electroencephalogram (Langebartels et al., 2001). Nevertheless, melatonin was administered in these experiments at the middle of the light phase, while in our study the hormone was injected 2 h after lights off. Recent data have shown that melatonin shortened time to onset of sleep when administered during darkness (Wang et al., 2003a), an effect that seems to be mediated by interaction of the hormone with brain GABA<sub>A</sub> receptors (Wang et al., 2003b). In addition, it has been shown that microinjection of melatonin into the medial preoptic area, a common site of action of hypnotic compounds, causes increased sleep in rat (Mendelson, 2002). The mechanisms by which melatonin influences the sleep–wake behaviour could be related to modulatory influence of the hormone on the GABA<sub>A</sub> receptor complex. For instance, melatonin has been reported to increase GABA concentration in the rat hypothalamus (Rosenstein and Cardinali, 1986) and tritiated diazepam binding affinity in the rat forebrain (Niles et al., 1987). A second possibility to explain the inhibitory effect of exogenously administered melatonin on visuo-spatial learning could be that melatonin can inhibit the accumulation of cyclic AMP in neurons of the central nervous system via Gi protein and thereby the phosphorylation of the transcription factor cyclic AMP response element binding protein (for review see von Gall et al., 2002). This signaling pathway is thought to be crucially involved in some neuroplastic changes underlying memory formation (Lonze and Ginty, 2002; Izquierdo et al., 2002). A third but less likely possibility is that melatonin could reduce the performance of rats in the radial maze by decreasing their motivation for food, since both acute (Raghavendra and Kulkarni, 2000) and chronic (Wolden-Hanson et al., 2000) melatonin treatments have been shown to not affect food consumption in the rat.

Control rats receiving saline exhibited about a 50% increase of peak-to-peak amplitude of TERs after tetanizing stimulation, an effect that was maintained for at least 30 min. This is consistent with reported data showing that LTP occurs in a number of different forebrain sites, including the neocortex (Martin et al., 2000), and that *in vivo* cortical LTP lasts for more than 60 min in the adult anesthetized rat (Mondaca et al., 2004b; Soto-Moyano et al., 2005). Results also show that 10 mg/kg i.p. melatonin completely prevented the induction of neocortical LTP by the tetanizing stimulation. The ability of melatonin to inhibit LTP has already been demonstrated in hippocampal slices of rodents (Collins and Davies, 1997; Wang et al., 2005).

Melatonin seems to exert no direct interactions with NMDA receptors, at least in hippocampal formation, as it fails to inhibit depolarization evoked by NMDA on CA1 neurons (Collins and Davies, 1997). Melatonin-induced cortical LTP inhibition could be the result of an intracellular interference of the hormone with GABA receptors in the brain (Rosenstein and Cardinali, 1986; Niles et al., 1987). In fact, both GABA<sub>A</sub> and GABA<sub>B</sub> receptor-dependent mechanisms have been shown to be involved in LTP modulation (Izquierdo and Medina, 1995; Remondes and Schuman, 2003), which may be related to the inhibitory effect of melatonin on neocortical LTP reported here. Another possibility is that melatonin could affect neocortical LTP by inhibiting PKA-dependent neuroplasticity downstream of MT<sub>2</sub> receptor activation. In this regard, it has recently been reported that this pathway mediates the inhibitory effect of melatonin on LTP recorded from hippocampal slices; this effect of melatonin was prevented by the MT<sub>2</sub> receptor antagonist luzindole and did not appear in mice deficient in MT<sub>2</sub> receptors (Wang et al., 2005). A third possibility is that melatonin-induced cortical LTP inhibition could be the result of the intracellular interference of the hormone with the NMDA receptor-dependent nitric oxide generating pathways. As already shown, nitric oxide has been shown to play a key role in LTP induction (Zorumski and Izumi, 1998) and, furthermore, it has been reported that melatonin can inhibit the calmodulin-dependent neuronal nitric oxide synthase activity (Leon et al., 2000) and neuronal NADPH-diaphorase/nitric oxide synthase activity (Chang et al., 2000) in central neurons of rats. In addition, it has been proposed that melatonin could directly react with nitric oxide (Turjanski et al., 2001). Finally, melatonin can inhibit cyclic GMP levels downstream nitric oxide signaling, via the soluble guanylyl cyclase pathway (Petit et al., 1999). All these actions of melatonin on NMDA receptor-dependent signaling pathways are in the correct direction to induce a depression of LTP and related neuronal plastic changes. Furthermore, they are in agreement with reports showing that inhibition of nitric oxide synthesis impairs spatial learning in the rat (Holscher et al., 1996; Prendergast et al., 1997). It has been reported that melatonin could produce cell hyperpolarization through binding to membrane receptors, at least in pituitary cells (Vanecek, 1998). However, the possibility that melatonin could prevent the development of cortical LTP by reducing the excitability of cortical neurons receiving callosal connections seems to be unlikely on the basis that melatonin, even at the larger dose used here, did not affect by itself latencies or peak-to-peak amplitudes of TERs. Interestingly, rats receiving the saline-ethanol solvent to dissolve melatonin developed full cortical LTP after tetanization, but they were unable to maintain this potentiation for more than 15 min. This effect is different from that produced by melatonin, which totally prevented neocortical LTP induction, and it is possibly related to the already reported partial inhibitory effect produced by low doses of ethanol on hippocampal LTP (Blitzer et al., 1990).

Numerous studies have examined the relationship between learning and hippocampal LTP, which is viewed as a synaptic model of memory. In this respect, it has been reported that learning can induce LTP in the neocortex (Riout-Pedotti et al.,

2000) and that drugs that modulate LTP can also interact with spatial learning (Villarreal et al., 2002). Since in the present study melatonin prevented the development of cortical LTP and impaired visuo-spatial memory, it is tempting to speculate that a causal relationship between these two observations exists. However, the possibility that melatonin could impair visuo-spatial performance by acting on brain regions other than the neocortex cannot be discarded. For example, hippocampal LTP has been reported to be importantly involved in visuo-spatial learning (Tang et al., 1999), and melatonin has been shown to depress LTP elicited in hippocampal slices (Collins and Davies, 1997; Wang et al., 2005). Finally, it is also possible that depression of LTP and decreased visuo-spatial learning could just represent parallel phenomena and further investigations involving pharmacological manipulation using melatonin analogs and inhibitors would be necessary to clarify this question.

Melatonin has been reported to have a dominant role in the generation and regulation of several behavioral rhythms in mammals, including circadian rhythms of central neurons and photoperiodic regulation of seasonal cycles, such as feeding and reproductive behaviors, as well as thermoregulation and hibernation. Although it is likely that the ability of melatonin to modulate neuronal excitability may account for many of its physiological actions of melatonin on the central nervous system, it is also conceivable that magnification of these effects when higher, pharmacological doses of the hormone are administered, could result in altered function of some central neurons such as those involved in long-term synaptic potentiation and the related processes of memory formation.

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