Circadian-dependent effect of melatonin on dopaminergic D_2 antagonist-induced hypokinesia and agonist-induced stereotypies in rats

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Abstract

Although a melatonin/dopamine relationship has been well established in nonmotor systems wherein dopamine and melatonin share an antagonist relationship, less clear is the role melatonin may play in extrapyramidal dopaminergic function. Therefore, the purpose of the present experiments was to examine the relationship between melatonin and the dopaminergic D_2 receptor system and behavior. Hypokinesia was induced in male Sprague–Dawley rats with fluphenazine (D_2 antagonist, 0.4 mg/kg ip) and stereotypies with apomorphine (D_2 agonist, 0.6 mg/kg sc) during the light (1200 h) and dark (2200 h) phases. As expected, fluphenazine induced severe hypokinesia during the light phase (482 ± 176 s); however, unexpectedly, fluphenazine-induced hypokinesia during the dark was almost nonexistent (25 ± 6 s). Furthermore, melatonin treatment (30 mg/kg ip) produced a strong interaction with fluphenazine in that it reduced fluphenazine-induced hypokinesia by nearly 80% in the light (112 ± 45 s) but paradoxically increased the minimal fluphenazine-induced hypokinesia in the dark by more than 60% (70 ± 17 s). Melatonin also reduced apomorphine-induced stereotypies by nearly 40% in the light but had no effect in the dark. Taken together, these data show (1) a strong and unexpected nocturnal effect of fluphenazine on hypokinesia and (2) provide support for an antagonistic melatonin/dopaminergic interaction in the context of motor behavior and D_2 receptor function which appears to be critically dependent on the light/dark status of the dopaminergic system.
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1. Introduction

Melatonin is an indoleamine neurohormone rhythmically synthesized and secreted, primarily by the pineal gland. With little exception, the acrophase of melatonin occurs during the dark, and the nadir during the light in both humans and animals regardless of circadian activity pattern. It is the unique absence and presence of melatonin that serves as a reliable neuroendocrine marker for the environmental photoperiod. As such, melatonin shares an intimate relationship with the onset of locomotor activity in animals. Contingent on time of administration, exogenous melatonin has been shown to phase advance or phase delay running wheel activity in rodents kept in constant conditions (Benloucif and Dubocovich, 1996). The phase-related shifts are thought to occur via the activation of G-protein-coupled melatonin receptors (Reppert, 1997) identified in the main oscillator of the mammalian system, the suprachiasmatic nucleus (SCN; Hunt et al., 2001; Dubocovich et al., 1996).

There is growing evidence that melatonin may play yet another role in the modulation of motor behavior apart from the well-established circadian-related phase shifts regulated by the SCN. In animals entrained to a light/dark cycle, pharmacological doses of melatonin have been shown to decrease spontaneous locomotor activity in rodents (Chuang and Lin, 1994; Sugden, 1983), birds (Murakami et al., 2001), monkeys (Zhdanova et al., 1998) and humans (Kunz and Bes, 2001). Corroborating an inhibitory effect of melatonin on spontaneous locomotor behavior was the early report by Hata and Kita (1987) in which an increase in exploratory movement was found after pinealectomy in

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several strains of mice. At present, the mechanism of action by which melatonin exerts an inhibitory effect on spontaneous locomotor behavior is unknown.

One system that might be affected by melatonin and which would be expected to mediate motor function is the extrapyramidal system. Within the basal ganglia, several neurotransmitters are responsible for the proper initiation and execution of motor behavior (i.e., dopamine, glutamate, GABA and acetylcholine); however, the dopamine D_2 receptor system is especially important. Dysregulation within this system has been implicated in such motor pathology as Parkinson's disease (Joel and Weiner, 2000), Tourette syndrome (Muller-Vahl et al., 2000) and tardive dyskinesia (Silvestri et al., 2000). Although an antagonist melatonin/dopamine relationship has been well established in the nonmotor systems in the mammalian retina and hypothalamus (Tosini and Dirden, 2002; Jaliffa et al., 2000), only a limited number of studies have investigated a melatonin/dopamine interaction within the striatum and, to even a lesser extent, behavioral outcomes. This may be due in part to the early in vitro findings that melatonin treatment at physiological concentrations (pM range) had no effect on rodent dopamine release evoked by electrical stimulation or calcium-dependent release in rabbit striatum (Zisapel et al., 1982; Dubocovich, 1983). More recently, and using pharmacological doses, a decrease in mouse striatal dopamine was reported after treatment with melatonin. Additionally, after an endogenous circadian rhythm for striatal dopamine was identified (high dopamine levels during the light and a sharp nadir during the early dark), it was found that pinealectomy served to dampen the rhythm while melatonin treatment served to restore it (Khaldy et al., 2002).

Melatonin may directly modulate striatal dopaminergic receptor function. For example, exogenous melatonin in rodent drinking water increased the affinity of D_2 striatal receptor binding to the D_2 antagonist, YM-09151-2 (Hamdi, 1998). This increase in affinity served to decrease the Kd of the antagonist by 50%. Another line of evidence in support of direct melatonin modulation of D_2 receptor function is that iontophoretic treatment with melatonin decreased neuronal firing in rat striatal neurons and this effect was reversed by treatment with a D_2, but not a D_1, antagonist (Escames et al., 1996). Although limited, in the aggregate, the data provide several promising lines of evidence in support of a melatonin/dopaminergic interaction in striatal biochemical and D_2 receptor function. At present, little is known about the behavioral implications.

The purpose of these experiments was to identify the behavioral correlates during pharmacological treatment with melatonin in the presence of the D_2 antagonist, fluphenazine, the classic neuroleptic (Experiment 1), and the D_2 agonist, apomorphine (Experiment 2). These drugs were specifically chosen based on their known pharmacological and behavioral effects: fluphenazine, for its high affinity for D_2 sites resulting in marked hypokinesia (Chen and Weiss, 1993), and apomorphine, for its ability to potentiate dopamine release (at high doses, binding postsynaptically) thus inducing stereotyped behaviors (sniffing, rearing, head bobbing, licking and biting; Tamminga and Gerlach, 1987). Additionally, because of the distinctive circadian profile of melatonin, and the recently described circadian rhythm for striatal dopamine (Khaldy et al., 2002), it was of utmost importance to investigate the effect of melatonin on D_2 antagonist and agonist behavioral outcomes in the light when melatonin is negligible and during the dark when melatonin is at acrophase.

2. Method

2.1. Animals

The animals used in Experiment 1 (D_2 antagonist) were male Sprague–Dawley albino rats ($n = 11$, 450–500 g) reared and maintained in the Department of Psychology animal colony at the University of Texas at El Paso. In Experiment 2 (D_2 agonist), male Sprague–Dawley albino rats ($n = 60$, 250–280 g) purchased from Harlan (Indianapolis, IN, USA) were used. All animals were kept in a 12L/12D cycle (lights on at 0600 h) and housed under controlled conditions (22 °C) with food and water provided ad libitum. Upon arrival to the colony, the animals purchased from Harlan were given a 14-day acclimation period prior to testing. The handling, care and use of the animals were in accordance with institutional guidelines set forth by the National Institute of Health and approved by the Institutional Animal Care and Use Committee at the University of Texas at El Paso.

2.2. Drugs

In Experiment 1, fluphenazine dihydrochloride and melatonin (Sigma, St. Louis, MO) were made up for injection immediately prior to use. Fluphenazine (FLU: 0.4 mg/kg ip) was dissolved in saline and melatonin (MEL: 30 mg/kg ip; dose based on previous behavioral studies: Raghavendra et al., 2001; Chuang and Lin, 1994; Sugden, 1983) was dissolved in propylene glycol and saline (1:1) with saline serving as the control vehicle for FLU and propylene glycol and saline (1:1) for MEL. FLU was injected at 0800 h during the light and 1800 h during the dark, both 4 h prior to bar testing in the light and dark based on previous experimentation in our laboratory showing peak hypokinesia at 4 h postinjection. MEL was injected 20 min prior to bar testing based on its short half-life which has been reported between 15 and 30 min (Gibbs and Vriend, 1981). All injections in the dark were administered under dim red light. The drug conditions included the following eight treatment groups ($n = 11$ in each group): VEH + VEH (VEH), VEH + MEL (MEL), VEH + FLU (FLU) and FLU + MEL in both the light and dark. According to a repeated-measures design, each animal received all treatment conditions with 3 days between treatments. Order

of treatment was assigned to each animal based on an 8×8 Latin square design which was used to control for possible order of treatment effects.

In Experiment 2, MEL and apomorphine hydrochloride (Sigma) solutions were made up immediately prior to injections. MEL (10 mg/kg ip) was prepared in a 1:1 solution of propylene glycol and saline and apomorphine (APO: 0.6 mg/kg sc) was prepared in saline. In both the light and dark, MEL was administered 20 min and APO immediately prior to testing. In the dark, all injections were administered under dim red light. According to a between-subjects design, rats were randomly assigned to one of the following six drug conditions ($n = 10$ in each group): VEH + VEH (VEH) (light or dark), VEH + APO (APO; light or dark) and APO + MEL (light or dark). A MEL-alone group was not included based on previous experimentation in our laboratory showing no effect of MEL on stereotypies (behaviorally, MEL-alone group was identical to control animals on stereotypies scores). Additionally, because it has been well established that MEL treatment alone serves to decrease spontaneous locomotor behavior and because the animal model here focused on stereotypies (increases in behavior), it was not prudent to include a MEL-alone group based on the nature of the dependent variable.

2.3. Behavioral testing

2.3.1. Hypokinesia

In Experiment 1, hypokinesia was quantified via the bar test devised by Moss et al. (1989). Briefly, the rat was placed on a Plexiglas apparatus with the front paws placed on a horizontal bar (6 mm thick, 5 cm wide and 9 cm in height) and the hind paws placed on the base. The horizontal bar was connected to a micro switch whereby the weight of the animal triggered a computerized timer. The amount of time the rat remained on the bar was recorded in seconds. The degree of hypokinesia was measured by how long it took the animal to remove itself from the bar. A maximum of 1500 s was allowed. The rats were tested in the light at 1200 h when endogenous melatonin was known to be negligible. During the dark, animals were tested under dim red light at 2200 h when melatonin has been shown to be at acrophase in the Sprague–Dawley rat (Hernandez et al., 1990).

2.3.2. Stereotypies

In Experiment 2, stereotypies were quantified by placing each rat in a rectangular Plexiglas observation chamber (30×22 cm) 1 h prior to testing. Stereotypy behaviors were quantified by the method of Tieppo et al. (2000) with few modifications. Briefly, stereotypies were quantified every 10 min for 60 min beginning immediately following the APO injection. The scoring system of Tieppo et al. (2000) was as follows: 0 = asleep or still; 1 = active; 2 = predominately active but with bursts of stereotyped sniffing and rearing; 3 = constant stereotyped activity, including sniffing, rearing or head bobbing, but locomotor still present; 4 = constant

stereotyped activity maintained at one location; 5 = constant stereotyped activity but with bursts of licking and/or gnawing and biting; 6 = continual licking of cage grids; and 7 = continual biting of cage grids. Because the chambers used in the present experiment did not contain biting grids, the score of 6 was given when continual licking was observed and the score of 7 was omitted from the scale. As in Experiment 1, the rats were tested at 1200 h during the light and at 2200 h during the dark. In the dark, all behavioral quantifications were performed under dim red light. To establish the reliability of stereotypy scoring, two observers (primary and secondary) rated behavior in both the light and dark.

2.4. Statistical analysis

All statistical analyses were conducted using SPSS (10.0 for Windows-SPSS, Chicago, IL). In Experiment 1, bar times were analyzed using a (2) Phase (light/dark) × (2) FLU (VEH/FLU) × (2) MEL (VEH/MEL) repeated measures three-way analysis of variance (ANOVA). In Experiment 2, stereotypy scores were first assessed for interrater reliability using Pearson's product moment correlation coefficient. Overall stereotypy behaviors (cumulative score for all time points) were analyzed using a 2 (Phase: light/dark) × 3 (Drug: VEH, APO, APO + MEL) between-subjects two-way ANOVA. For the light and dark time course analysis, two separate 3 (Drug: VEH, APO and APO + MEL) × (6) (Time: 10, 20, 30, 40, 50 and 60 min post-APO injection) mixed-design ANOVAs were used, whereby time served as the within-subjects variable and drug served as the between-subjects variable. For all analysis, and when appropriate, the Bonferroni correction was used for post hoc comparisons with P values $\leq .05$ considered statistically significant.

3. Results

3.1. Experiment 1 (melatonin and fluphenazine)

The effects of melatonin on fluphenazine-induced hypokinesia in the light and the dark are shown in Fig. 1. The Phase × MEL × FLU ANOVA showed a significant main effect for phase, $F(1,10) = 6.04$, $P < .05$, and FLU, $F(1,10) = 8.84$, $P < .05$. Significant interactions were Phase × FLU, $F(1,10) = 6.45$, $P < .05$, Phase × MEL, $F(1,10) = 4.98$, $P < .05$ and Phase × FLU × MEL, $F(1,10) = 5.08$, $P < .05$. Of interest was the Phase × FLU interaction which can be attributed to the unexpected finding that FLU did not induce a substantial hypokinesia during the dark (25.42 ± 5.54 s) as compared to the light (481.83 ± 176.26 s). More important was the significant three-way interaction. This interaction can be attributed to the differential effect of FLU and MEL in the light and dark. Paradoxically, MEL treatment served to block the effect
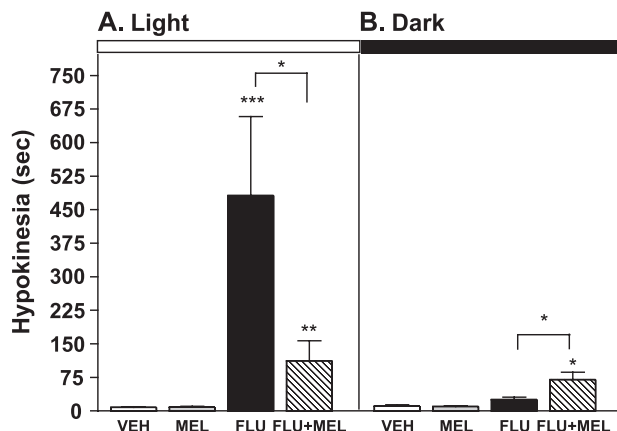
Fig. 1. Hypokinesia as measured by the bar test during the light (1200 h) (A) and the dark (2200 h) (B) in control (VEH: open bars), melatonin (MEL: grey bars), fluphenazine (FLU: black bars) and fluphenazine + melatonin (FLU + MEL: hatched bars) groups. Rats were kept in a 12L/12D cycle (lights on at 0600 h). Each bar represents the mean ± S.E.M. of 11 rats. FLU (0.4 mg/kg ip) was injected 4 h prior to testing and MEL (30 mg/kg ip) 20 min prior to testing in both the light and the dark. $***P < .001$ vs. VEH and MEL; $**P < .01$ vs. VEH and MEL; *(light) $P < .05$, FLU vs. FLU + MEL; *(dark) $P < .05$ vs. VEH, MEL and FLU.

of FLU during the light and potentiate the effect of FLU during the dark. Additionally, no effect of treatment order was found.

3.2. Experiment 2 (melatonin and apomorphine)

3.2.1. Interrater reliability

The correlation between stereotypy scoring for Rater 1 and Rater 2 in the light and dark was $r(58) = .99$, $P < .001$, showing near-perfect interrater reliability. Consequently, the observations from the secondary rater were used only to establish reliability.

3.2.2. Overall stereotypy

The effect of melatonin on apomorphine-induced stereotypies in the light and dark is shown in Fig. 2. The Phase × Drug between-subjects ANOVA conducted on overall stereotypy scores showed a significant main effect for phase, $F(1,54) = 63.60$, $P < .001$, drug, $F(2,54) = 204.38$, $P < .001$, and a significant interaction for Phase × Drug, $F(2,54) = 9.49$, $P < .001$. Of main interest was the Phase × Drug interaction. This interaction can be attributed to the differential effect of MEL on APO-induced stereotypies in the light and dark. MEL significantly decreased APO-induced stereotypies in the light ($P < .05$) but not in the dark ($P = .88$).

3.3. Time course for stereotypies

As well as investigating overall stereotypy scores, it was of interest to examine the time course of the effects of melatonin treatment on apomorphine induced stereotypies. In the light, the Drug × Time mixed ANOVA revealed
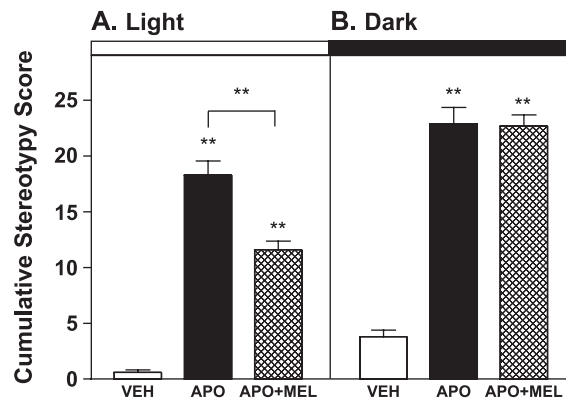

Fig. 2. Overall stereotypy behavior (cumulative mean score) in the light (1200 h) (A) and the dark (2200 h) (B) in control (VEH: open bars), apomorphine (APO: black bars) and apomorphine + melatonin (APO + MEL: hatched bars) groups. Rats were kept in a 12L/12D cycle (lights on at 0600 h). Each bar represents the mean ± S.E.M. of 10 rats. MEL (30 mg/kg ip) was injected 20 min and APO (0.6 mg/kg sc) immediately prior to testing in both the light and dark. Stereotypies were quantified every 10 min for 60 min. $**P < .01$ vs. VEH; APO vs. APO + MEL.
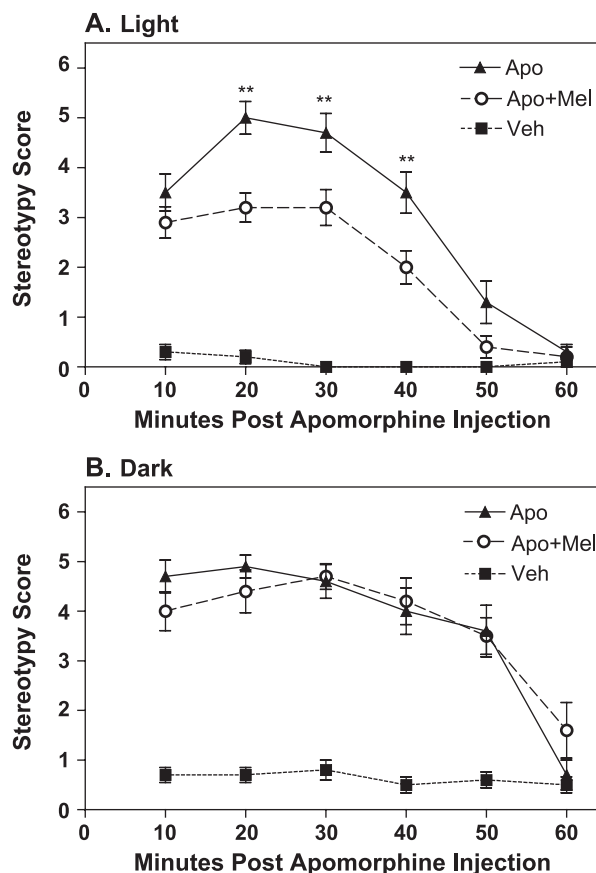

Fig. 3. Time course of stereotypy behavior in the light (1200 h) (A) and the dark (2200 h) (B) in control (VEH: ■), apomorphine (APO: ▲) and apomorphine + melatonin (APO + MEL: ○) groups. Rats were kept in a 12L/12D cycle (lights on at 0600 h). Each point represents the mean ± S.E.M. of 10 rats. (A and B) MEL (30 mg/kg ip) was injected 20 min and APO (0.6 mg/kg sc) immediately prior to testing in both the light and dark. Stereotypies were quantified every 10 min for 60 min. $**P < .01$ vs. APO + MEL.
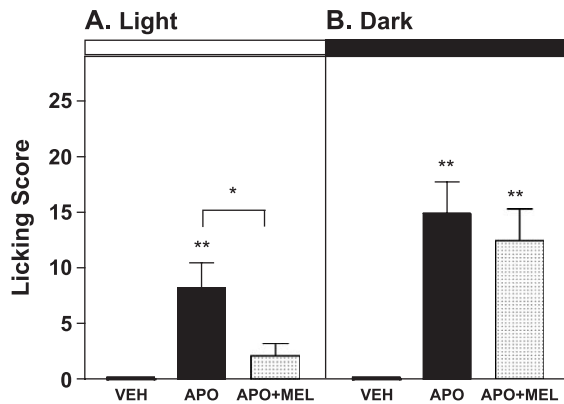
Fig. 4. Licking behavior in the light (1200 h) (A) and the dark (2200 h) (B) in control (VEH: open bars), apomorphine (APO: black bars) and apomorphine + melatonin (APO + MEL: hatched bars) groups. Rats were kept in a 12L/12D cycle (lights on at 0600 h). Each bar represents the mean ± S.E.M. of 10 rats. MEL (30 mg/kg ip) was injected 20 min and APO (0.6 mg/kg sc) immediately prior to testing in both the light and dark. Stereotypies were quantified every 10 min for 60 min. $**P < .01$ vs. CON; $*P < .05$ APO vs. APO + MEL.

significant main effects for drug, $F_{(2,27)} = 96.21$, $P < .001$, time, $F_{(5,135)} = 51.85$, $P < .001$, and a significant Drug × Time interaction, $F_{(10,135)} = 9.37$, $P < .01$. As seen in Fig. 3A, at 20 ($P < .001$), 30 ($P < .001$) and 40 min ($P < .005$) post-APO injection, MEL significantly decreased stereotypy behavior during the light.

In the dark, the $3 \times (6)$ mixed ANOVA showed significant main effects for drug, $F_{(2,27)} = 98.90$, $P < .001$, time, $F_{(5,135)} = 24.84$, $P < .001$, and a significant Drug × Time interaction, $F_{(10,135)} = 5.94$, $P < .001$. Important to note, the main effects and interactions were solely attributed to the difference between the control group and the other two groups combined (Fig. 3B). MEL did not counteract the effect of APO at any time point in the dark.

3.4. Licking scores

Animals showed less APO-induced licking in the light than in the dark. Therefore, it was of special interest to further investigate the effects of MEL on this circadian-dependent behavior. A 2×3 between-subjects ANOVA was conducted on licking scores showing significant main effects for phase, $F_{(1,54)} = 13.05$, $P < .005$ and drug, $F_{(2,54)} = 18.22$, $P < .001$. The Phase × Drug interaction was also significant, $F_{(2,54)} = 3.72$, $P < .05$. As can be seen in Fig. 4, licking scores were significantly reduced by melatonin treatment during the light but not in the dark.

4. Discussion

We have demonstrated here that acute pharmacological treatment with melatonin attenuated hypokinesia induced by the D$_2$ antagonist, fluphenazine. Melatonin blocked the effect of the classic neuroleptic by nearly 80%. To our knowledge, this is the first report of an ameliorating effect of melatonin during D$_2$ antagonist-induced hypokinesia. However, this effect was present only during the light phase. In the dark, melatonin served to potentiate the effect of the D$_2$ antagonist. The potentiating effect of melatonin on hypokinesia during the dark must be interpreted in light of the fact that fluphenazine did not produce a substantial behavioral effect in the dark. That is to say, melatonin served to counteract the lack of effect of the D$_2$ antagonist. Fluphenazine's ability to induce hypokinesia was reduced by 95% under dark conditions. Recently, a similar phenomenon was reported with use of haloperidol in rats whereby a maximum effect of the neuroleptic was shown at midlight and the minimum effect at midnight (Viyoch et al., 2001). The effect of haloperidol was dampened by nearly 50% during dark conditions. Taken together, these findings demonstrate the importance of the light/dark cycle in the susceptibility of extrapyramidal effects with use of drugs that target D$_2$ receptor function.

Melatonin also had an ameliorating effect on stereotypies induced by the D$_2$ agonist, apomorphine. In the case of the agonist, melatonin decreased hyperactivity by nearly 40%. Interestingly, while the antagonist (fluphenazine) alone did not show a substantial behavioral effect during the dark, the agonist, apomorphine, did induce strong behavioral effects during the dark. And, in fact, apomorphine-induced stereotypies were found to be more pronounced during the dark. These findings suggest an important differential effect in the light and dark within the D$_2$ receptor system itself. Although, theoretically, these two D$_2$ ligands work to affect the same receptor system, failure to find similar circadian fluctuation in the light and dark may be representative of their distinct behavioral and pharmacological profiles. Apomorphine with a half-life of 30 min (Gancher, 1995) induces robust but short-acting behavioral effects within minutes of administration. Consistent with others (Cho et al., 1991; Tieppo et al., 1997), stereotypies in the present study were completely absent 60 min postapomorphine injection. In contrast, 4 h was necessary to induce the robust behavioral effect of fluphenazine as found in the light phase. With a half-life of 0.62 days (Cohen et al., 1992), the behavioral effect of fluphenazine was noted up to 7 h postinjection (data not shown). It is not out of the realm of speculation that melatonin, with its distinct circadian rhythm, may serve as the biochemical signal for endogenous receptor rhythms. This line of thought is supported by chronobiological studies showing dopamine receptor binding to follow ultradian (Naber et al., 1980), circadian (Planeta et al., 1994) and seasonal rhythms (Naber et al., 1981). If melatonin modulates dopaminergic responses by providing a biochemical signal for endogenous receptor rhythms, it could be that dopaminergic ligands showing quick-acting pharmacological effects might be less likely to be affected by a circadian or long ultradian change in receptor rhythmicity.

With regard to stereotypies, melatonin was particularly effective in reducing the oral–facial behavior of licking

which is representative of one of the maximal effects of drug-induced stereotypies (Tieppo et al., 2000). Our results are consistent with a previous report using chronic reserpine treatment in rats as an animal model of tardive dyskinesia where it was found that melatonin reversed vacuous chewing movements (Raghavendra et al., 2001). Tardive dyskinesia, associated with prolonged use of neuroleptics in schizophrenic populations, is characterized by involuntary movements manifesting mainly in oral and peri–oral regions (Fernandez and Freeman, 2003). Our data support others who have suggested that melatonin may be an effective therapeutic agent in the management of tardive dyskinesia (Shamir et al., 2001). Shamir et al. (2001) proposed that melatonin, as it has been found to activate striatal central-type benzodiazepine GABA(A) receptors, works in this capacity to reduce oral and peri–oral movements. We offer an alternative explanation based on the finding that the physical action of licking increases neuronal firing in rodent striatum (Mittler et al., 1994). As noted earlier, melatonin decreases neuronal firing in the rat striatum (Castillo-Romero et al., 1992, 1995). It could be that melatonin's attenuation of oral–facial behavior was due, at least in part, to modulation of neuronal firing and a not a direct interaction with D_2 function. Whatever the case may be, melatonin's modulation of extrapyramidal effects are sufficiently important to investigate, given the possible clinical implications.

These data build on existing in vitro evidence and provide in vivo support for a melatonin/dopamine relationship within the confines of D_2 dopaminergic function in the extrapyramidal system. If not working as a biochemical marker for circadian receptor function, it seems plausible that melatonin's effect on motor behavior may be generated, at least in part, within the extrapyramidal system and may be receptor mediated. For instance, Paredes et al. (1999) has shown that local administration of melatonin in the rat nucleus accumbens decreased locomotor behavior in a dose-dependent manner, suggesting the possibility of local melatonin receptors. Additionally, with regards to the present experiment, because high pharmacological doses of melatonin were used, it would be expected that low-affinity melatonin receptors sites would be involved. However, because evidence is poor and lacking for the existence of melatonin receptors within the basal ganglia (Zisapel et al., 1988; Morgan et al., 1994; Poirel et al., 2003), the contributions local melatonin receptors may play in the modulation of motor behavior remain unclear. Based on the previous report that melatonin increased the affinity and not the density of D_2 striatal receptors after in vitro application of a D_2 antagonist (Hamdi, 1998), it appears most likely that melatonin's modulation of motor behavior may be via modulation of D_2 receptor binding. Because little is known about the molecular and cellular relationships between melatonin and dopaminergic function in the milieu of the extrapyramidal system, we cannot rule out that the effect of melatonin on D_2 function may have originated via

modulation of other important extrapyramidal neurochemical events. For example, Tenn and Niles (1997) have shown that the melatonergic agonist, S-20098, activated striatal central-type benzodiazepine GABA(A) receptors, and Paredes et al. (1999) found local melatonin administration to increase extracellular acetylcholine in the nucleus accumbens, suggesting melatonin interacts within the GABAergic and cholinergic systems.

Whatever its route of action, the salient feature of melatonin's effect may be the magnitude rather than direction of change in its systemic concentration. This is supported by the fact that its modulatory effects were consistently greater in the light phase, when the change in circulating concentrations of melatonin due to injection over systemic levels of secretion would be greatest. This is also consistent with the fact that a change in circulating melatonin, whether low in diurnal animals or high in nocturnal animals, signals an increase in motor activity and arousal.

In summary, we have documented marked changes in kinetic and stereotyped motor behavior attributable to the interaction between melatonin and drugs that act on the dopamine D_2 receptor. Melatonin was highly effective in blocking the effects of fluphenazine and apomorphine. However, melatonin's ameliorating effect was shown only in the light. As documented in other biological systems, the present data support an antagonist relationship between melatonin and D_2 dopaminergic function within the extrapyramidal system and motor-related behavior. Importantly, however, the effect of melatonin on motor behavior in the presence of a D_2 antagonist and agonist depends critically on the light/dark status of the dopaminergic system.

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