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The 5-HT_{1A} receptor antagonist *p*-MPPI blocks 5-HT_{1A} autoreceptors and increases dorsal raphe unit activity in awake cats

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Abstract

The effects of the putative 5-HT_{1A} receptor antagonist 4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide (*p*-MPPI) were examined on the activity of serotonergic dorsal raphe nucleus neurons in freely moving cats. Systemic administration of *p*-MPPI produced a dose-dependent increase in firing rate. This stimulatory effect of *p*-MPPI was evident during wakefulness (when serotonergic neurons display a relatively high level of activity), but not during sleep (when serotonergic neurons display little or no spontaneous activity). *p*-MPPI also blocked the ability of the 5-HT_{1A} receptor agonist 8-hydroxy-(2-di-*n*-propylamino)tetralin (8-OH-DPAT) to inhibit serotonergic neuronal activity. This antagonism was evident both as a reversal of the neuronal inhibition produced by prior injection of 8-OH-DPAT and as a shift in the potency of 8-OH-DPAT following *p*-MPPI pretreatment. Overall, these results in behaving animals indicate that *p*-MPPI acts as an effective 5-HT_{1A} autoreceptor antagonist. The increase in firing rate produced by *p*-MPPI supports the hypothesis that autoreceptor-mediated feedback inhibition operates under physiological conditions.

Keywords: *p*-MPPI (4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide); 5-HT_{1A} receptor antagonist; 5-HT (5-hydroxytryptamine, serotonin) neuron; Dorsal raphe nucleus; 5-HT_{1A} autoreceptor blockade; (Cat)

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in many behavioral and physiological processes (Leonard, 1994) and in psychiatric disorders, such as anxiety and depression (Ågren et al., 1991; Blier and De Montigny, 1994). Special interest has been given to the 5-HT_{1A} receptor subtype. These receptors are located both presynaptically, on the cell bodies and dendrites of serotonergic neurons within the raphe nuclei, where they function as autoreceptors, and postsynaptically, in many brain regions (Hoyer et al., 1994). In recent years, the 5-HT_{1A} autoreceptors have been suggested to play a critical role in the mechanism of action of nonbenzodiazepine anxiolytic compounds, such as buspirone (Schreiber and De Vry, 1993; Remy et al., 1996), and in the delayed onset of therapeutic action of certain antidepressant drugs (Artigas et al., 1994; Blier and De Montigny, 1994).

The discharge rate of serotonergic neurons in the midbrain is controlled, in part, by presynaptic (somatodendritic) 5-HT_{1A} autoreceptors (Aghajanian and Vander-Maelen, 1986). 5-HT released from dendrites and possibly, axon terminals, within the raphe nuclei acts on these autoreceptors to inhibit neuronal activity, thus, functioning as a negative feedback mechanism. The pharmacological stimulation of these receptors by selective 5-HT_{1A} receptor agonists, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), consistently inhibits the spontaneous discharge of serotonergic neurons in both anesthetized and unanesthetized animals (Fornal et al., 1994a; Sprouse and Aghajanian, 1986). Whether 5-HT_{1A} autoreceptors exert a tonic inhibitory influence on serotonergic neuronal activity is controversial. Several studies, in anesthetized animals, have shown that systemic administration of 5-HT_{1A} receptor antagonists have little or no effect on neuronal activity, suggesting that 5-HT_{1A} autoreceptors are not tonically activated by endogenous 5-HT release (Blier et al., 1989;

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Blier et al., 1993; Forster et al., 1995). However, in awake and freely moving cats, both the nonselective 5-HT_{1A} receptor antagonist spiperone and the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY 100635) increase neuronal activity above baseline levels (Fornal et al., 1994a; Fornal et al., 1996). We believe that part of the reason for this apparent discrepancy is related to behavioral state, since neither spiperone nor WAY 100635 increase serotonergic cell firing during sleep. The neuronal activation produced by these drugs is only evident in awake animals (Fornal et al., 1994a; Fornal et al., 1996). Such results would be expected of a negative feedback system, i.e., it should be engaged when neurons are firing at a relatively high rate (as they do during wakefulness) and disengaged at lower firing rates (as seen during sleep).

The study of the functional role of 5-HT_{1A} autoreceptors has been complicated by the general unavailability of selective 5-HT_{1A} receptor antagonists. Several putative 5-HT_{1A} receptor antagonists have been shown to possess at least partial agonist properties at 5-HT_{1A} somatodendritic autoreceptors (Lanfumey et al., 1993; Sharp et al., 1993; Escandon et al., 1994; Millan et al., 1994). Similarly, in our preparation, the putative 5-HT_{1A} receptor antagonists 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione (BMY 7378), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) and (−)-propranolol inhibit neuronal activity in awake cats (Fornal et al., 1994b). Recently, we tested another putative 5-HT_{1A} receptor antagonist, (−)-pindolol, and again neuronal activity was reduced, rather than increased, following systemic drug administration (Fornal et al., 1997). Furthermore, all of these compounds proved to be ineffective in blocking 5-HT_{1A} autoreceptors, inasmuch as they failed to antagonize the inhibitory action of 8-OH-DPAT on serotonergic neuronal activity.

In the present study, we examined the effects of systemic administration of another putative selective 5-HT_{1A} receptor antagonist, 4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide (*p*-MPPI), on the spontaneous activity of serotonergic neurons in the dorsal raphe nucleus recorded in behaving cats. In addition, we assessed the antagonist activity of *p*-MPPI at 5-HT_{1A} autoreceptors by examining the ability of the drug to block the neuronal inhibition produced by 8-OH-DPAT.

2. Materials and methods

2.1. Animals

Male cats (2.7–5.2 kg) were housed individually in a temperature-controlled (22 ± 1°C) and light-controlled (lights on from 7:00 a.m. to 9:00 p.m.) room and had free access to food (commercial diet) and tap water. All cats were cared for and used in strict accordance to the Public Health Service Guide for the Care and Use of Laboratory Animals. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Princeton University.

2.2. Surgical procedures

Cats were pretreated with atropine sulfate (0.2 mg, s.c.; 30 min) and tranquilized with acepromazine maleate (0.5 mg/kg, i.m.) and ketamine hydrochloride (20 mg/kg, i.m.) for preoperative preparation. Prior to placing the animal in a stereotaxic instrument, pentobarbital sodium was administered (doses individually titrated; range = 6–18 mg/kg, i.v.) until deep anesthesia was achieved, as determined by loss of corneal and pedal reflexes. Supplemental doses of pentobarbital (~ 2 mg/kg) were administered, as needed, to maintain an adequate level of anesthesia. A microdrive, consisting of two inner stainless steel cannulas (23-gauge) separated by 1 mm, which could be lowered through two outer guide cannulas (19-gauge), was stereotaxically implanted towards the dorsal raphe nucleus at an angle of 40° posterior to the vertical. Microelectrode bundles were then lowered through both cannulas, so that their tips were positioned 1 mm above the dorsal raphe nucleus. Stereotaxic coordinates for the anterior bundle were posterior = 1.5 mm, lateral = 0 mm and horizontal = +1.0 mm (Berman, 1968). Each bundle consisted of three 32-μm and four 64-μm diameter Formvar-insulated nichrome wires. Electrodes were also implanted for recording the cortical electroencephalogram (EEG), the electrooculogram (EOG) and the nuchal electromyogram (EMG), as described previously (Fornal et al., 1994a), in order to monitor behavioral state. The leads from all electrodes were soldered to a 25-pin connector, and the entire apparatus was anchored to the skull with dental acrylic. Cats were removed from the stereotaxic unit, and a Tygon catheter (1 mm i.d., 1.7 mm o.d.) was inserted into the right external jugular vein and advanced to the vena cava. The distal end of the catheter, which had previously been attached to a three-way stopcock, was capped and cemented to the implant.

2.3. Postoperative animal care

Following surgery, all animals received an analgesic dose of butorphanol (0.2 mg/kg, s.c.), with additional doses given as needed. A topical antibiotic powder (nitrofurazone) was regularly applied to the implant incision. Cats were treated, twice daily, for up to 10 days with ampicillin (20 mg/kg, i.m. or 250 mg, p.o.). Intravenous catheter patency was maintained by weekly flushing with heparinized sterile saline (500 I.U./ml). The headpiece and surrounding tissue were cleaned at regular intervals. Cats were allowed to recover for at least two weeks before experiments were initiated. All cats were in excellent health when these trials were conducted.

2.4. Electrophysiological recording

Electrical potentials were recorded from each cat using a counterweighted low-noise cable system and 24-channel slip ring assembly. Microelectrode potentials were amplified (Grass 7P511 AC preamplifier), filtered (band-pass, 0.1–3.0 kHz) and monitored continuously on a storage oscilloscope. A second microelectrode served as an indifferent electrode. Single-unit activity was separated from background noise by means of a time–amplitude window discriminator (model DIS-1; Bak Electronics, Clarksburg, MD). The acceptance pulse output of the window discriminator was used to produce on-line records of cell discharge through a speaker and an electronic counter, and on a polygraph (Grass model 7C). Cortical EEG, EOG and EMG potentials were amplified (Grass 7P5 or 7P511 AC preamplifier), band-pass filtered (EEG, 1–35 Hz; EOG, 1–35 Hz; EMG, 30–90 Hz) and recorded continuously on the polygraph.

All experimental trials were conducted in an electrically shielded, sound-attenuating chamber ($65 \times 65 \times 95$ cm high) with a transparent Plexiglas door, which allowed for remote television monitoring of the animals. Each animal was habituated daily to the recording chamber over a one-week postoperative period. After habituation, the microdrive was slowly advanced through the dorsal raphe nucleus in discrete steps (~ 80 μm) until stable single-unit recordings characteristic of serotonergic neurons were encountered. Only recordings that displayed a signal-to-noise ratio of at least 3:1 were used for data collection in this study. Such neurons could be recorded typically for several hours and often for several days.

2.5. Neuronal identification

During quiet waking (defined in Section 2.6), individual neurons in the dorsal raphe nucleus were initially identified on-line as serotonergic according to previously established criteria (Fornal and Jacobs, 1988): (1) slow and highly regular discharge activity (~ 1–4 spike(s)/s), (2) biphasic action potentials of relatively long duration (≥ 2 ms), and (3) marked suppression of spontaneous activity during rapid-eye movement (REM) sleep. The neurochemical identity of these neurons was further established by determining the response to i.v. administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (10 μg/kg) (Sprouse and Aghajanian, 1986). Finally, localization of recording sites to the dorsal raphe nucleus was verified histologically.

2.6. Behavioral state analysis

Upon isolation of a stable regularly firing unit that was likely to be serotonergic, cats were allowed to go through at least one complete sleep/wake cycle. For the purpose of the present study, behavioral state was divided into four general categories, based on polygraphic criteria and visual observation of the animal: (1) active waking, characterized by a low-voltage, fast (desynchronized) EEG, phasic EMG bursts superimposed on high-amplitude tonic EMG, frequent (≥ 20/min) and large (≥ 400 μV) eye movement potentials and occurrence of gross body movements (e.g., walking); (2) quiet waking, characterized by a predominantly desynchronized EEG, an absence of both large eye movement potentials and phasic EMG bursts, and a lack of gross body movements (e.g., maintenance of a sitting or lying posture with head up and eyes open); (3) slow wave sleep (SWS), characterized by a large-amplitude (200–500 μV), synchronous EEG with sleep spindles present, greatly diminished tonic EMG, eyes closed, but small eye movement potentials present and a recumbent posture (usually lying on side or curled up with head down); and (4) REM sleep, characterized by a desynchronized EEG, the absence of tonic EMG, intermittent REM potentials and occasional body twitches while maintaining a recumbent sleep posture.

2.7. Drug administration

Drugs were administered remotely from an adjacent room via an infusion line connected to the venous catheter. Injections were made by loading the infusion line with the drug solution (0.1–0.25 ml/kg) and flushing the line with 2 ml of sterile saline over a 5-s period. All injections were made while cats were in a quiet, but alert behavioral state, based on direct visual observations and polygraphic monitoring. Pulse injections of an equivalent volume of sterile saline or sterile acidified water (pH = 5.5) served as a control. Sufficient time was allowed between experiments to ensure wash-out of drug, as determined by monitoring unit activity. Each drug treatment was tested in at least four cats.

To assess the effects of *p*-MPPI on spontaneous neuronal activity, the drug was administered in a cumulative fashion, at 5-min intervals, over a dose range from 10 to 750 μg/kg. In addition, the time course of the effect of a single dose of *p*-MPPI (100 μg/kg) on neuronal activity was determined. To assess the antagonist activity of *p*-MPPI at 5-HT_{1A} autoreceptors, two protocols were used. In the first, the effect of a challenge dose (10 μg/kg) of 8-OH-DPAT on serotonergic neuronal activity was determined before (control) and 5 min after pretreatment with a cumulative dose of 750 μg/kg of *p*-MPPI. Additional doses of 8-OH-DPAT were subsequently administered at 5-min intervals, to determine whether the antagonist effect of *p*-MPPI could be surmounted. In the second protocol, *p*-MPPI (100 μg/kg) or saline (control) was administered 5 min after 8-OH-DPAT (10 μg/kg), in an attempt to reverse the neuronal suppression produced by 8-OH-DPAT.

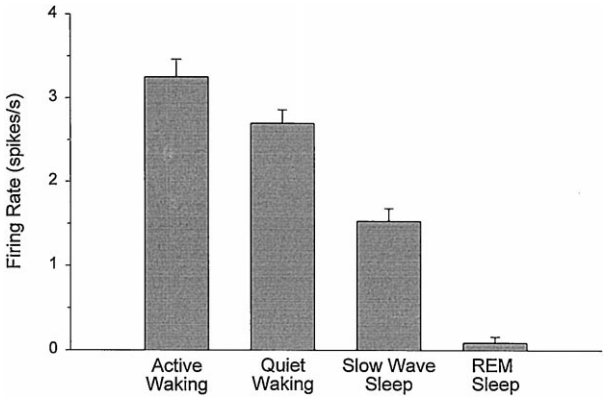

Fig. 1. Spontaneous discharge rates of serotonergic dorsal raphe nucleus neurons across the sleep/wake cycle in cats. Values are means ± S.E.M.; $n = 20$ cells. Note the pronounced decrease in neuronal activity from active waking to REM sleep. Firing rates were significantly different for each of the four behavioral states ($P < 0.05$ by Student–Newman–Keuls test).

2.8. Data collection

In the drug-response trials, firing rate data were obtained during comparable behavioral states, because the activity of serotonergic neurons varies directly with the level of behavioral arousal (Fornal and Jacobs, 1988). For the sleep/wake sessions and the drug duration and state-dependency trials, firing rates were calculated from six consecutive 10-s samples. Firing rates in the drug trials were calculated from consecutive 10-s samples beginning 1 min before the drug injection and continuing for 5 min after the injection. Changes in firing rates, expressed as a percentage of baseline, were determined by comparing the discharge rate taken over a 1-min period during the time of peak drug effect to the baseline rate obtained during the 1-min period immediately before the injection. For the antagonism experiment using the pretreatment protocol, firing rates were integrated over a 5-min period starting immediately after the 8-OH-DPAT challenge injection, and were compared with 1-min preinjection baseline firing rates, to provide an overall measure of treatment effects.

2.9. Drugs

p-MPPI hydrochloride was a gift from Dr. Alan Frazer at the University of Texas, San Antonio, USA. (±)-8-OH-DPAT hydrobromide was obtained from Research Biochemicals International, Natick, USA. *p*-MPPI was dissolved in sterile water, whereas 8-OH-DPAT was dissolved in sterile bacteriostatic saline. Both drugs required sonication to dissolve. Drugs were prepared immediately before each trial. Dosages of both drugs refer to the salt.

2.10. Histology

Animals were anesthetized with ketamine hydrochloride (20 mg/kg, i.m.), and direct anodal current was passed through the recording electrode (20 μA for 40 s) at sites from which acceptable units were recorded. Cats were overdosed with pentobarbital sodium (100 mg/kg, i.v.) and then perfused intracardially with physiological saline, followed by 10% formalin, and 5% potassium ferrocyanide in formalin to produce a Prussian blue reaction. Frozen sections (50 μm) were cut through the midbrain region, mounted on slides and stained with neutral red.

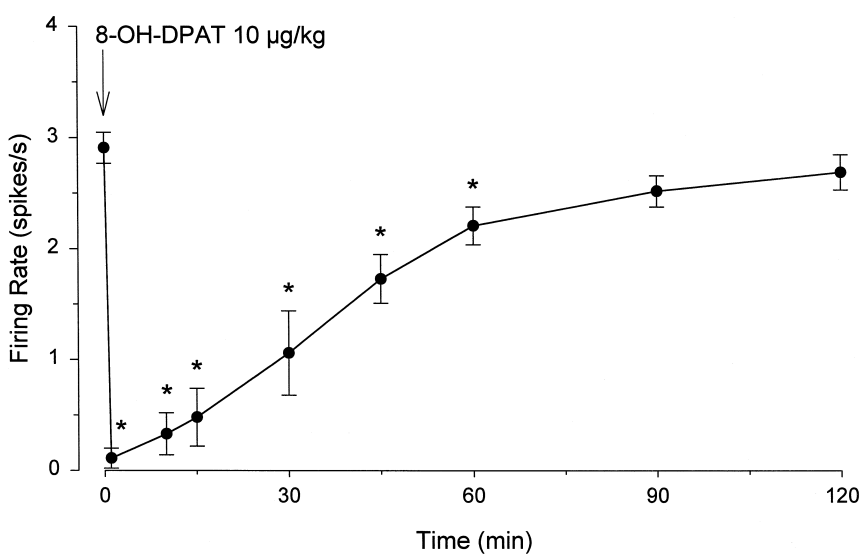

Fig. 2. Duration of the inhibitory action of 8-OH-DPAT (10 μg/kg, i.v.) on the spontaneous activity of serotonergic dorsal raphe nucleus neurons in awake cats. Values are means ± S.E.M.; $n = 7$ cells. The first two time points after 8-OH-DPAT injection are 1 and 10 min. * $P < 0.05$ vs. baseline by Student–Newman–Keuls test.

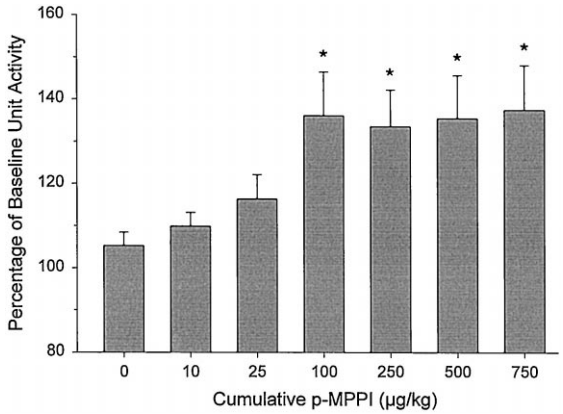

Fig. 3. Dose-response relationship for the activation of serotonergic dorsal raphe nucleus neurons produced by i.v. administration of *p*-MPPI. Values are means ± S.E.M.; $n = 7$ cells. *p*-MPPI was administered in increasing doses at 5-min intervals and the maximum effect of each injection was determined. The baseline firing rate of these neurons was 2.99 ± 0.17 spikes/s. $* P < 0.05$ vs. 0 µg/kg (water vehicle control) by Student–Newman–Keuls test.

2.11. Statistical analysis

Data are expressed as means ± S.E.M. Unit activity was analyzed using a one-way repeated-measures analysis of variance (ANOVA) and post-hoc Student–Newman–Keuls test, or where appropriate, paired or unpaired *t*-test. In all cases, $P \leq 0.05$ was taken as statistically significant.

3. Results

3.1. Characteristics of serotonergic neuronal activity

Data were obtained from 20 serotonergic dorsal raphe nucleus neurons recorded in 10 cats. All neurons exhibited the characteristic changes in firing rate across the sleep/wake cycle, as described previously (Fornal et al., 1996). Thus, the mean discharge rate of these neurons was

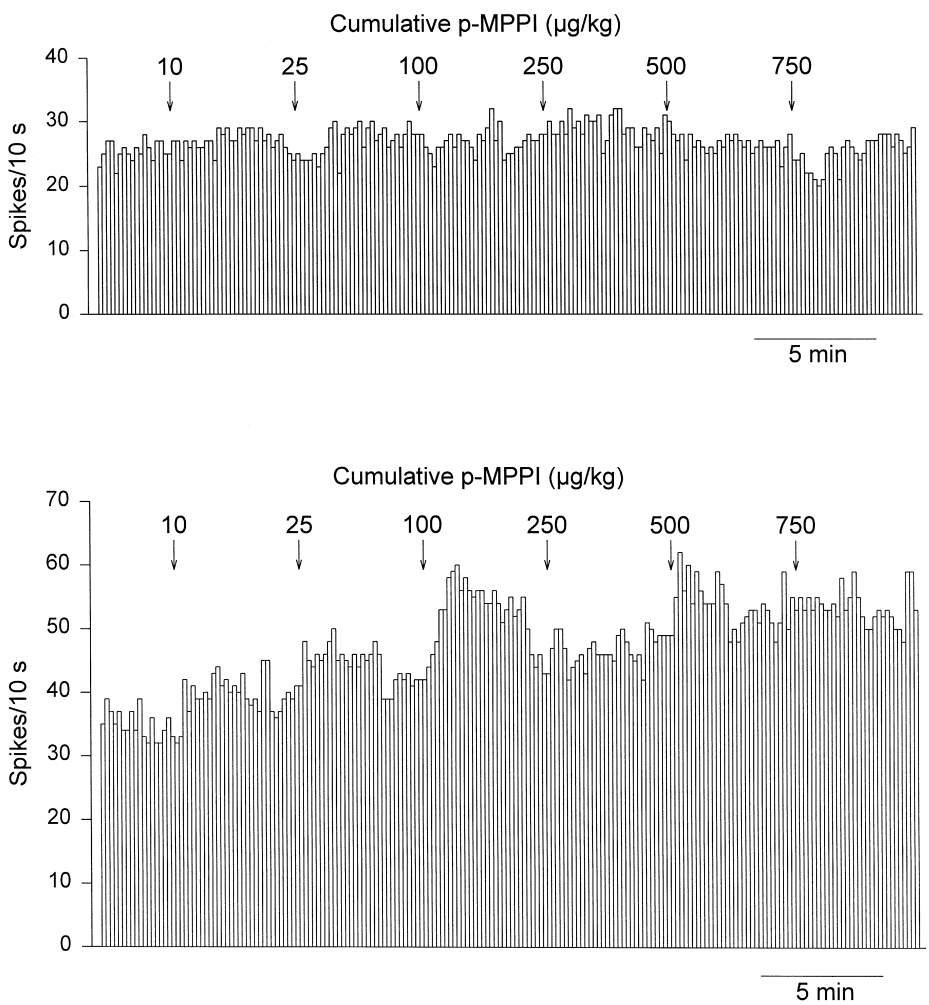

Fig. 4. Integrated firing rate histograms showing the response of two individual serotonergic dorsal raphe nucleus neurons to i.v. administration of cumulative doses of *p*-MPPI (10–750 µg/kg). Both cells were recorded in the same cat, on different days. Injections were given at 5-min intervals, as indicated by the arrows. The cell in the top panel was unaffected by *p*-MPPI, whereas the cell in the bottom panel responded with an increase in firing rate after *p*-MPPI.

highest during active waking, slightly lower during quiet waking, approximately half the waking level during SWS (middle of episode), and lowest during REM sleep (Fig. 1). Most of the cells studied (16/20) completely ceased firing during REM sleep. Nineteen neurons were tested with the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (10 μg/kg, i.v.). All 19 cells displayed the characteristic inhibitory response to the drug. The mean percentage decrease in neuronal activity produced by 10 μg/kg of 8-OH-DPAT was 99 ± 1%. The activity of seven of these cells was monitored for 2 h after 8-OH-DPAT. As shown in Fig. 2, neuronal discharge rates gradually recovered to preinjection baseline rates over the course of 90 min.

3.2. Effects of p-MPPI on serotonergic neuronal activity

Systemic administration of *p*-MPPI (10–750 μg/kg) increased the spontaneous firing rate of serotonergic dorsal raphe nucleus neurons in a dose-dependent manner (Fig. 3). The stimulatory effect of *p*-MPPI appeared to be maximal at a cumulative dose of 100 μg/kg, since no additional increase in neuronal activity was observed with higher doses. Not all cells were responsive to *p*-MPPI. Fig. 4 shows the response of two individual cells recorded in the same animal to *p*-MPPI; one neuron exhibited an increase in firing rate (up to 170% of baseline), whereas the other neuron was unresponsive to the drug. The degree to which cells responded to *p*-MPPI appeared to be related to their responsiveness to 8-OH-DPAT, with cells demonstrating lower sensitivity to 8-OH-DPAT, typically displaying smaller increases in unit activity after *p*-MPPI.

The time course of the neuronal activation produced by the maximally effective dose of *p*-MPPI (100 μg/kg) is shown in Fig. 5. This dose produced a significant increase in firing rate within 1 min. The stimulatory effect of

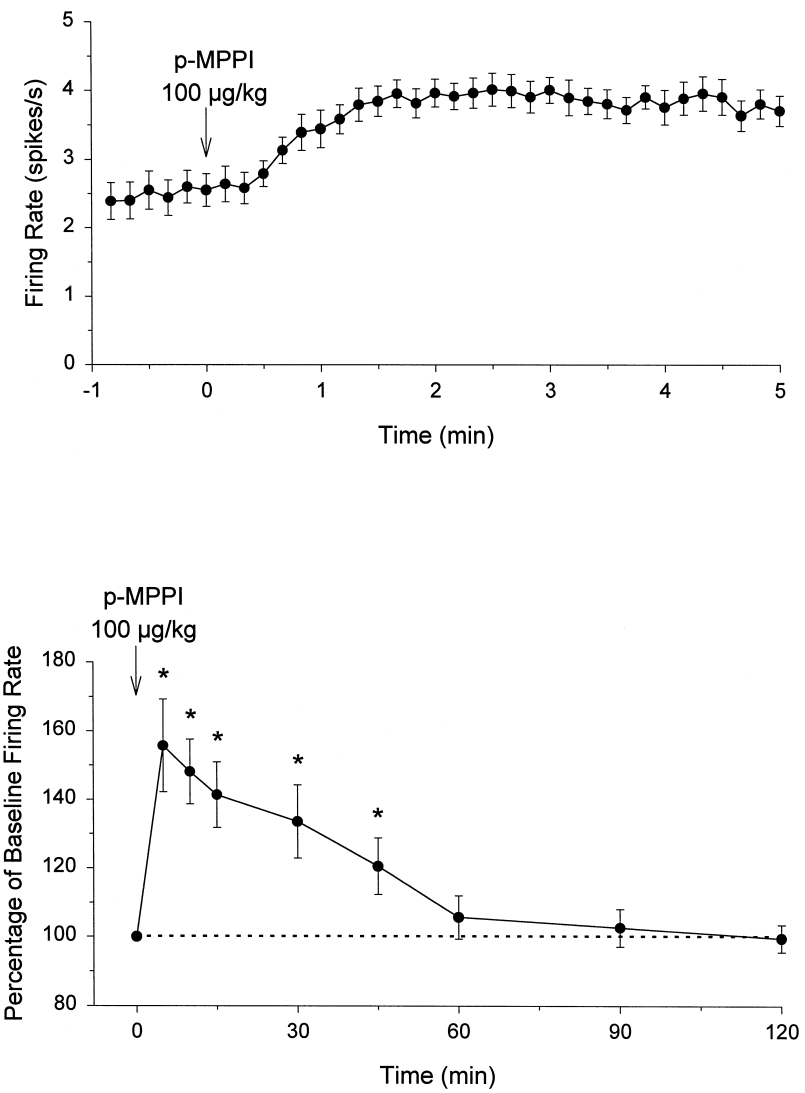

Fig. 5. Time course of the increase in serotonergic dorsal raphe nucleus neuronal activity produced by i.v. administration of *p*-MPPI (100 μg/kg) in awake cats. Values are means ± S.E.M.; $n = 8$ cells. Arrows indicate time of drug injection. *p*-MPPI produced a significant increase in neuronal activity within 1 min of the injection (top panel). The stimulatory effect of *p*-MPPI persisted for at least 45 min (bottom panel). The horizontal dashed line in the bottom panel represents baseline unit activity. $* P < 0.05$ vs. baseline by Student–Newman–Keuls test.

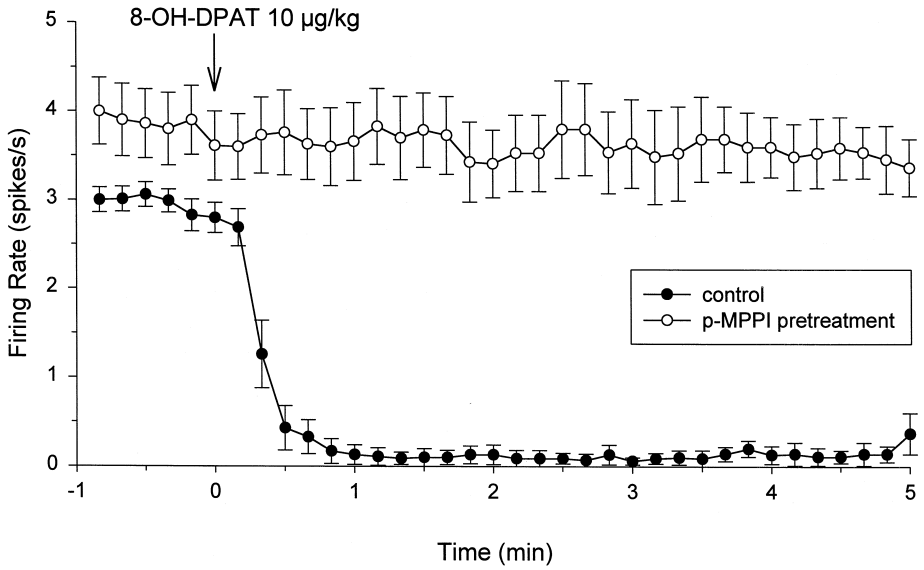

Fig. 6. Response of serotonergic dorsal raphe nucleus neurons to an i.v. challenge dose of 8-OH-DPAT (10 μg/kg) before (control) and after pretreatment with *p*-MPPI (750 μg/kg; cumulative dose). Values are means ± S.E.M.; $n = 7$ cells. Arrow indicates time of injection. *p*-MPPI pretreatment completely blocked the neuronal suppression produced by 8-OH-DPAT.

p-MPPI lasted for at least 45 min. By 60 min postinjection, neuronal activity returned to baseline levels.

p-MPPI administration by itself did not produce any appreciable changes in behavior or in polygraphic indices. During these trials, several cats fell asleep at a time when *p*-MPPI was still capable of exerting a substantial stimulatory effect on waking neuronal activity. Serotonergic dorsal raphe nucleus neurons showed a decrease in activity with sleep onset, and ceased firing during REM sleep in a manner similar to that observed during the nondrug baseline condition, despite the presence of *p*-MPPI. The mean discharge rate of these neurons during SWS after *p*-MPPI injection was not significantly different from the pretreatment SWS level (1.27 ± 0.16 spike/s vs. 1.51 ± 0.25 spike/s, respectively; $n = 7$ cells; paired *t*-test, $t = 1.56$). Likewise, the mean firing rate during REM sleep after *p*-MPPI was not significantly different from the baseline REM sleep level (0 spike/s vs. 0 spike/s, respectively; $n = 4$ cells). The stimulatory effect of *p*-MPPI, however, was once again evident when cats awakened from sleep, as indicated by a significant increase in waking discharge rates compared with pretreatment baseline levels (3.30 ± 0.14 spikes/s vs. 2.37 ± 0.25 spikes/s.; $n = 7$ cells; paired *t*-test, $t = 5.77$, $P < 0.01$). Thus, the ability of *p*-MPPI to increase serotonergic neuronal activity was found to vary in association with the behavioral state.

3.3. Effects of p-MPPI on the neuronal suppression produced by 8-OH-DPAT

Under control conditions (no pretreatment), 8-OH-DPAT at 10 μg/kg produced a rapid and nearly complete suppression of neuronal activity (Fig. 6). The prior administration of *p*-MPPI (750 μg/kg) prevented the inhibitory response to 8-OH-DPAT. Neuronal discharge rates immediately before 8-OH-DPAT injection were significantly elevated by *p*-MPPI in these experiments (paired *t*-test, $t = 2.61$, $P < 0.05$). To further evaluate the effectiveness of *p*-MPPI as a 5-HT_{1A} receptor antagonist, 8-OH-DPAT was given in increasing amounts (up to a cumulative dose of 80 μg/kg) in an attempt to overcome the receptor blockade produced by *p*-MPPI. As shown in Fig. 7, the

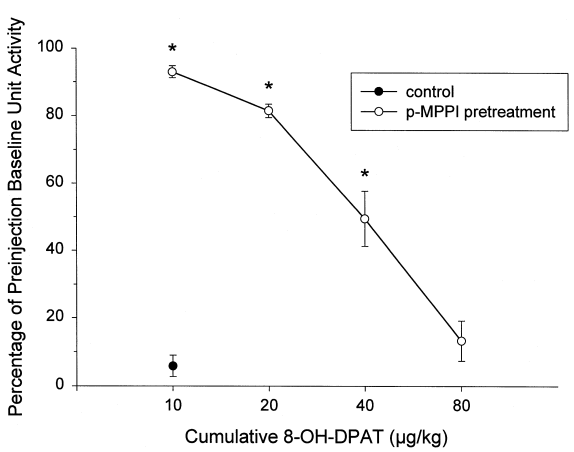

Fig. 7. Effect of *p*-MPPI pretreatment (750 μg/kg; cumulative dose) on the ability of 8-OH-DPAT to inhibit the activity of serotonergic dorsal raphe nucleus neurons in awake cats. Values are means ± S.E.M.; $n = 7$ cells. 8-OH-DPAT was administered in increasing doses at 5-min intervals. The effects of 8-OH-DPAT on neuronal activity were assessed over the entire 5-min period following each injection. The preinjection 8-OH-DPAT baseline firing rates of these neurons were 2.95 ± 0.14 spikes/s in the control (no pretreatment) condition and 3.85 ± 0.39 spikes/s after *p*-MPPI pretreatment. $* P < 0.05$ vs. control by Student–Newman–Keuls test.

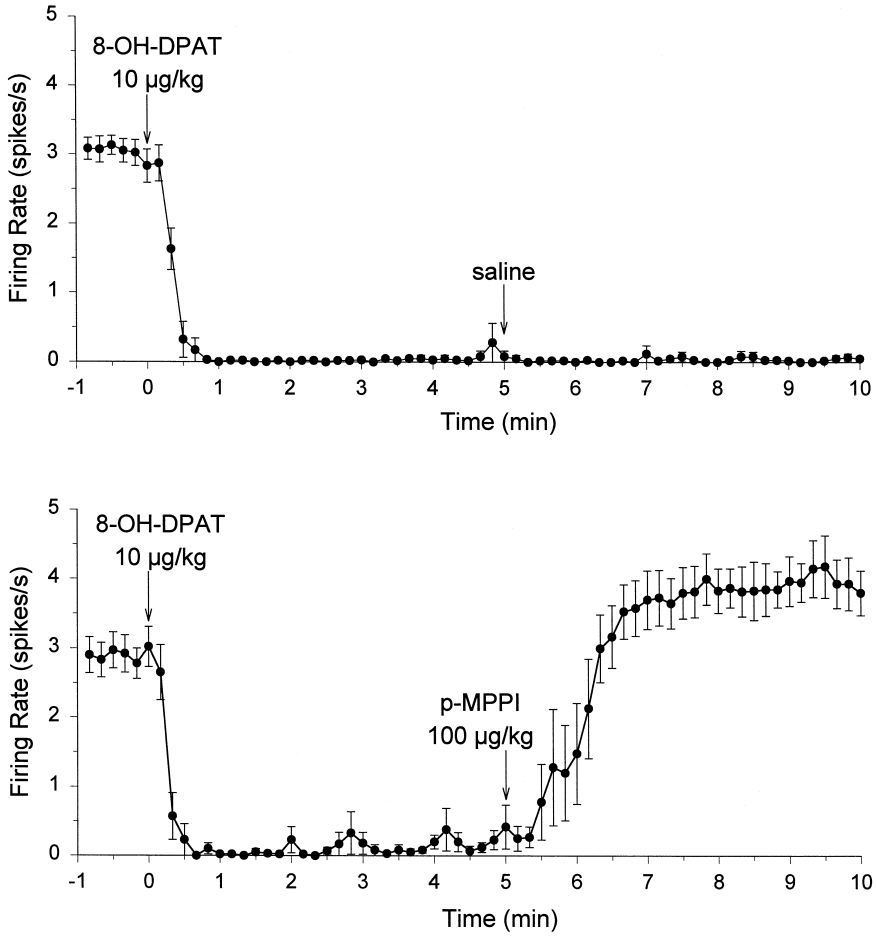

Fig. 8. Response of serotonergic dorsal raphe nucleus neurons to i.v. administration of 8-OH-DPAT (10 μg/kg) and subsequent administration of saline or *p*-MPPI (100 μg/kg, i.v.). Values are means ± S.E.M; $n = 6$ cells for both the saline and *p*-MPPI treatment groups. Arrows indicate time of drug injections. *p*-MPPI rapidly reversed the inhibitory action of 8-OH-DPAT on neuronal activity, whereas saline control injections had no effect.

antagonism produced by *p*-MPPI was surmountable. Complete or nearly complete inhibition of cell firing could still be achieved in the presence of *p*-MPPI if the challenge dose of 8-OH-DPAT was increased by at least eight-fold. This suggests a competitive interaction between these two drugs.

We also examined the ability of *p*-MPPI to restore the discharge of serotonergic dorsal raphe nucleus neurons after unit activity was completely suppressed by prior injection of 8-OH-DPAT. As shown in Fig. 8, administration of *p*-MPPI (100 μg/kg) rapidly reestablished cell firing in animals pretreated with 10 μg/kg of 8-OH-DPAT and significantly elevated the activity of these neurons to 142 ± 11% of pre-drug baseline levels. In contrast, saline control injections had no effect on the neuronal inhibition produced by 8-OH-DPAT.

4. Discussion

The present study demonstrates that the putative selective 5-HT_{1A} receptor antagonist *p*-MPPI increases the spontaneous activity of serotonergic dorsal raphe nucleus neurons in behaving cats, and also blocks the inhibition of serotonergic neuronal activity produced by the selective 5-HT_{1A} receptor agonist 8-OH-DPAT. This antagonism could be demonstrated both as a shift in the inhibitory potency of 8-OH-DPAT following *p*-MPPI pretreatment and as a reversal of the already suppressed neuronal activity produced by prior injection of 8-OH-DPAT. Overall, these results indicate that *p*-MPPI is a highly effective 5-HT_{1A} autoreceptor antagonist in the awake cat.

The phenylpiperazine derivative *p*-MPPI was recently described as a selective 5-HT_{1A} receptor antagonist in vitro (Kung et al., 1994). In vivo, *p*-MPPI antagonizes the 5-HT behavioral syndrome and the decrease in body temperature induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT (Thielen and Frazer, 1995; Thielen et al., 1996; Allen et al., 1997). Since both of these responses are thought to reflect the activation of postsynaptic 5-HT_{1A} receptors (Tricklebank, 1985; Millan et al., 1993; Allen et al., 1997), these results indicate that *p*-MPPI has antagonist properties at postsynaptic 5-HT_{1A} receptors. In addition, *p*-MPPI was found to antagonize 8-OH-DPAT-induced reduction in

brain 5-HT turnover (Thielen et al., 1996) and pretreatment with *p*-MPPI prevented the ability of 8-OH-DPAT to reduce extracellular 5-HT levels in the striatum (Allen et al., 1997), responses mediated by presynaptic 5-HT_{1A} receptors. These latter results are consistent with the present electrophysiological data.

A number of compounds, such as NAN-190, BMY 7378, and methyl-4{4-[4-(1,1,3-trioxo-2*H*-1,2-benziosothiazol-2-yl)butyl]-1-piperazinyl}1*H*-indole-2-carboxylate (SDZ 216-525), have been described as 5-HT_{1A} receptor antagonists. In vivo studies have demonstrated that these compounds do in fact block the functional effects of 5-HT_{1A} receptor agonists at postsynaptic 5-HT_{1A} receptors (Hjorth and Sharp, 1990; Sharp et al., 1990; Lanfumey et al., 1993). However, these compounds show 'agonist-like' activity when examined at presynaptic 5-HT_{1A} receptors. Thus, systemic administration of these drugs inhibits 5-HT synthesis and reduces extracellular 5-HT levels in the brain (Hjorth and Sharp, 1990; Sharp et al., 1990; Sharp et al., 1993; Millan et al., 1994). The inhibition of firing of serotonergic dorsal raphe nucleus neurons is a very sensitive measure of 5-HT_{1A} autoreceptor activation. Both NAN-190 and BMY 7378 produce a dose-dependent inhibition of serotonergic neuronal activity in our cat preparation (Fornal et al., 1994b). Other putative nonselective 5-HT_{1A} receptor antagonists, such as propranolol and pindolol, also suppress the activity of serotonergic dorsal raphe neurons and fail to block the inhibitory response of these neurons to 8-OH-DPAT (Fornal et al., 1994b; Fornal et al., 1997). Thus, these drugs can not be considered as effective 5-HT_{1A} autoreceptor antagonists following systemic administration.

Recently, the novel phenylpiperazine derivatives (*S*)-WAY 100135 and WAY 100635 were proposed as selective 5-HT_{1A} receptor antagonists (Fletcher et al., 1993; Forster et al., 1995). In our studies, (*S*)-WAY 100135 acts as a partial agonist at 5-HT_{1A} autoreceptors (Fornal et al., 1996). In contrast, WAY 100635 appears to act as a potent and selective 5-HT_{1A} autoreceptor antagonist (Fornal et al., 1996). Thus, WAY 100635 increases the firing rate of serotonergic dorsal raphe neurons and strongly blocks the action of 8-OH-DPAT. The present study shows that the pharmacological profile of *p*-MPPI on serotonergic neuronal activity is similar to that of WAY 100635. One difference between these two drugs was that *p*-MPPI seemed to have a much shorter duration of action than WAY 100635. At equal doses (100 μg/kg), the stimulatory effect of *p*-MPPI on neuronal activity lasted for approximately 45 to 60 min, whereas the effect of WAY 100635 persisted for up to 4 h. Furthermore, the maximal increase in neuronal activity produced by *p*-MPPI was less than that observed after WAY 100635, suggesting that WAY 100635 is a more efficacious antagonist. The nonselective 5-HT_{1A} receptor antagonist spiperone also increases the firing rate of serotonergic dorsal raphe nucleus neurons in the awake cat (Fornal et al., 1994a). However, whereas WAY 100635 and *p*-MPPI were devoid of behavioral effects, spiperone produced behavioral sedation in animals, probably due to its potent dopamine antagonist properties.

A common finding with *p*-MPPI, WAY 100635 and spiperone was that their stimulatory effect on neuronal activity was behavioral state-dependent. Thus, the increase in serotonergic cell firing produced by these antagonists was only evident when the animal was awake and alert; tonic discharge rates during SWS and REM sleep were unchanged from baseline levels (Fornal et al., 1994a; Fornal et al., 1996). This implies that 5-HT_{1A} autoreceptors play an important regulatory role in 5-HT neurotransmission under physiological conditions, but only during the waking state. This is what can be expected of a negative feedback system, i.e., it should be engaged when neurons are firing at a relatively high rate and disengaged at lower firing rates. Thus, under conditions in which serotonergic neurons are known to be relatively inactive (as during sleep), autoreceptor blockade has little, if any, effect on neuronal activity. However, during periods of increased behavioral arousal when serotonergic neurons are firing at a much higher rate, autoreceptor blockade is manifested as an increase in the activity of serotonergic neurons. This provides further evidence for the potential physiological significance of the negative feedback inhibition mediated by 5-HT_{1A} autoreceptors.

The increase in firing rate observed with *p*-MPPI contrasts with the lack of effect seen in the anesthetized rat preparation using comparable doses of the drug (Waszcak et al., 1996). This may be due to the relatively low firing rate (typically 1 spike/s) of serotonergic neurons under anesthesia and/or to the elimination of the influence of behavioral state. Thus, there may be too little 5-HT released onto 5-HT_{1A} autoreceptors to exert significant feedback inhibition.

The spontaneous activity of serotonergic neurons throughout the brainstem is strongly behavioral state-dependent, with activity being highest during waking, intermediate during SWS, and lowest during REM sleep (see review by Jacobs and Fornal, 1991). Recent neurochemical studies confirm this behavioral state-dependency in 5-HT neurotransmission. Extracellular 5-HT levels in both the dorsal raphe nucleus (Portas and McCarley, 1994; Portas et al., 1998) and the frontal cortex (Portas et al., 1998) of experimental animals are highest during waking, significantly reduced during SWS, and at their lowest point during REM sleep. A preliminary study in human epileptic patients also demonstrates the same pattern of extracellular 5-HT levels across the sleep/wake cycle in both the amygdala and the orbital frontal cortex (Wilson et al., 1997). Furthermore, in rats, extracellular 5-HT levels in other brain regions, such as the hypothalamus, hippocampus, striatum, and cerebellum are higher during the active phase, compared with the inactive phase, of the animal's light/dark cycle (Mendlin et al., 1996; Rueter and Jacobs, 1996), providing further evidence that 5-HT neurotrans-

mission is strongly related to behavioral state. These data clearly indicate a close correspondence between the spontaneous activity of serotonergic neurons and 5-HT release in the dorsal raphe nucleus and other brain regions. Furthermore, since 5-HT_{1A} receptor antagonists do not appear to modify the tonic firing rate of serotonergic neurons during SWS or REM sleep, the hypothesis that 5-HT_{1A} autoreceptors mediate the suppression of serotonergic neuronal activity during sleep (Cespuglio et al., 1990) appears unlikely. Instead, afferent input from other neurotransmitter systems, such as γ-aminobutyric acid (GABA), seem more likely to mediate the sleep-related decreases in serotonergic dorsal raphe neuronal activity (Levine and Jacobs, 1992; Nitz and Siegel, 1997).

The activation of serotonergic neuronal activity induced by 5-HT_{1A} autoreceptor antagonists during wakefulness has important implications, since it suggests that 5-HT neurotransmission is under a tonic inhibitory influence mediated by 5-HT_{1A} autoreceptors. The function of these receptors may be to prevent or dampen neuronal activation in response to increases in excitatory inputs. We have previously shown that the discharge rate of serotonergic neurons is unresponsive to a wide variety of stressors, such as pain, loud noise, physical restraint, pyrogen administration, and insulin-induced hypoglycemia (Jacobs et al., 1990; Jacobs and Fornal, 1991). This stability of serotonergic neuronal activity may be related to the engagement of the autoreceptor mechanism, which serves to regulate 5-HT neurotransmission within a relatively narrow range. Thus, the pharmacological blockade of 5-HT_{1A} autoreceptors may have pronounced effects on 5-HT neurotransmission. Surprisingly, data from in vivo microdialysis studies show that selective 5-HT_{1A} receptor antagonists, such as WAY 100635 and *p*-MPPI, have little or no effect on extracellular 5-HT levels in frontal cortex (Gartside et al., 1995; Invernizzi et al., 1996), striatum (Allen et al., 1997), and hippocampus (Hjorth et al., 1997). This argues against the notion that 5-HT_{1A} autoreceptors play an important role in modulating the activity of the serotonergic system under physiological conditions. However, we want to underscore the importance of controlling for the influence of behavioral state when studying drug effects on 5-HT neurotransmission. As discussed above, 5-HT_{1A} receptor antagonists only increase neuronal activity during wakefulness. This may explain the lack of effect of 5-HT_{1A} receptor antagonists on extracellular 5-HT levels in studies conducted under general anesthesia (Sharp and Hjorth, 1990; Gartside et al., 1995; Hjorth et al., 1997), or in unanesthetized animals, but during their inactive phase of the light/dark cycle (Routledge et al., 1993; Invernizzi et al., 1996; Allen et al., 1997).

As shown in the present study, not all neurons were responsive to systemic *p*-MPPI (Fig. 4). In general, the neurons which were less responsive to *p*-MPPI were also less responsive to the 5-HT_{1A} receptor agonist 8-OH-DPAT. One may speculate that this is related to the density or the sensitivity of the autoreceptors on individual neurons (Jacobs and Fornal, 1991; Fornal et al., 1994a). A neuron with few or less sensitive autoreceptors will presumably be less responsive to pharmacological manipulations. One may also speculate that faster firing neurons have fewer or less sensitive autoreceptors than slower firing neurons. Thus, the density and/or sensitivity of the 5-HT_{1A} autoreceptors may be an important factor regulating the firing rate of the individual neurons. This is consistent with the data obtained for serotonergic neurons in the medullary raphe nuclei, which are faster-firing, but less responsive to 5-HT_{1A} receptor agonists (Jacobs and Fornal, 1991; Veasey et al., 1995).

In conclusion, *p*-MPPI acts as an effective 5-HT_{1A} autoreceptor antagonist, similar to WAY 100635, and in contrast to many other putative 5-HT_{1A} receptor antagonists. Our data also underscore the importance of controlling for the influence of behavioral state when studying the effects of drugs on central 5-HT neurotransmission.

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