Long-term administration of monoamine oxidase inhibitors alters the firing rate and pattern of dopamine neurons in the ventral tegmental area

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Abstract

Monoamine oxidase inhibitors (MAOIs) exert their antidepressant action by increasing the function of the serotonin (5-HT), norepinephrine and dopamine (DA) systems. There is, however, limited electrophysiological data on the effects of MAOIs on DA neurons. The effects of 2-d and 21-d administration of three MAOIs were investigated (clorgyline, selective MAOI-A; deprenyl, selective MAOI-B; phenelzine, non-selective MAOI) on the firing activity of DA neurons in the ventral tegmental area using in-vivo electrophysiology in rats. Short-term clorgyline (1 mg/kg) and phenelzine (2.5 mg/kg) was devoid of effect on DA neurons, whereas prolonged administration significantly decreased their firing rate (by 30% and 20%, respectively), number of bursts (by 80% and 45%, respectively), and percentage of spikes occurring in bursts only in clorgyline-treated rats (70%). Deprenyl (0.25 mg/kg) was without effects. DA firing was restored in clorgyline-treated rats by inhibiting 5-HT synthesis using para-chlorophenylalanine (p-CPA; 300 mg/kg . d for three consecutive days). The 5-HT₃ antagonist ondansetron (0.5 mg/kg) was devoid of effect in control rats, but completely reversed the alterations of DA neuronal activity in clorgyline-treated rats. An attenuation of DA neuronal activity was thus produced by prolonged blockade of MAOA activity. The absence of effect of MAOA inhibition after subacute administration suggested an indirect mechanism. This was confirmed by the observation that p-CPA antagonized the effects of clorgyline. Since ondansetron completely reversed the effects of clorgyline on DA neuronal activity, the effects of MAOA inhibition appeared to be mediated by 5-HT₃ receptors.

Key words: Burst activity, dopamine, firing rate, MAO inhibitors, 5-HT₃ receptors.

Introduction

Monoamine oxidase inhibitors (MAOIs) were discovered in the 1950s and became the first class of medication active in the treatment of unipolar depression. Monoamine oxidases (MAO) are enzymes that catalyze the oxidative deamination of monoamines. Two isoforms of this enzyme have been identified in the mammalian brain on the basis of their substrate affinities: MAOA and MAOB (Johnston, 1968). MAOA is responsible for the metabolism of both serotonin (5-HT) and norepinephrine (NE), whereas the MAOB metabolize phenylethylamine and histamine. Dopamine (DA) and tyramine are common substrates for both subtypes (Krishnan, 2007). MAOIs used as antidepressants (e.g. clorgyline; Lipper et al., 1979) potency inhibit at least the A isoforms. In contrast, the MAOB inhibitor deprenyl is devoid of effect in the treatment of depression at low doses, which selectively inhibit MAOB, but it exerts an antidepressant response at higher doses that also inhibit MAOA (Knoll and Magyar, 1972; Mann et al., 1984; Robinson, 2002). Only drugs that inhibit MAOA modify the neuronal activity of both 5-HT and NE systems. Indeed, it was previously demonstrated that subacute...
administration of clorgyline for 2 d decreased the firing rate of both 5-HT and NE neurons, but only the firing rate of 5-HT neurons recovered after prolonged administration (21 d). This recovery was attributed to a desensitization of cell body 5-HT receptors (Blier and de Montigny, 1985). These results are in line with others showing that the pharmacological inhibition of MAOA, or its constitutive deletion, induce an increase in tissue and extracellular levels of both 5-HT and NE in various brain areas (Blier et al., 1986b; Evrard et al., 2002; Finberg et al., 1993). On the other hand, neither 5-HT nor NE neuronal firing was modified by short-term or long-term treatment with deprenyl used at a dose that only inhibits MAOB subtype (Blier and de Montigny, 1985; Blier et al., 1986b).

Several lines of evidence suggest that low levels of DA may play an important role in depression, and that an enhancement of dopaminergic transmission may underlie, at least in part, the antidepressant response (Dailly et al., 2004; Willner, 1997). The effect of sustained administration of MAOIs on the firing activity of ventral tegmental area (VTA) DA neurons have, however, never been studied. Such results may lead to a better understanding of the monoaminergic pathways involved in the mechanism of action of MAOIs. Indeed, a recent study has demonstrated that a lesion of 5-HT or NE neurons induces an increase in the firing rate of VTA DA neurons (Guirard et al., 2008). Interactions between these three systems are then functionally important. It is thus conceivable that MAOI-A could exert direct (via inhibition of DA degradation) and/or indirect (via an enhancement in 5-HT and/or NE function) effect(s) on VTA neurons.

Material and methods

Animals

Male Sprague–Dawley rats (Charles River, St Constant, QC, Canada) weighing 250–300 g, at the time of recording were used for the experiments. The rats were maintained under standard laboratory conditions [12 h light–dark cycle (lights on 07:00 hours) with free access to food and water]. Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA), a burr hole was drilled for recordings. Supplemental doses (100 mg/kg i.p.) were given to prevent any nociceptive reaction to pinching of the hind paws. Body temperature was maintained at 37 °C throughout the experiment using a thermistor-controlled heating pad. The extracellular recordings of DA neurons were carried out using single-barreled glass micropipettes. The tips of electrodes were broken back to 1–3 μm and filled with 2 M NaCl solution. The impedance of the electrodes ranged between 4 and 7 MΩ. Prior to the electrophysiological experiments, a catheter was inserted in a lateral tail vein for systemic i.v. injection of pharmacological agents. All the experiments were carried out in accordance with the Canadian Council on Animal Care, for the care and use of laboratory animals and protocols were approved by the institutional Animal Care Committee (IMHR Animal Care Committee).

Treatments

Rats were anaesthetized with isoflurane in order to subcutaneously implant the osmotic Alzet mini-pumps (Alza, Palo Alto, CA, USA), which delivered clorgyline (1 mg/kg . d, Sigma-Aldrich, St Louis, MO, USA), phenelzine (2.5 mg/kg . d; Sigma-Aldrich) or deprenyl (0.25 mg/kg . d, Tocris, Hornby, ON, Canada) for 2 d or 21 d. These regimens were chosen because prior results in our laboratory showed adequate and/or selective effect on MAOs (Blier et al., 1986b). Control rats were implanted with mini-pumps delivering physiological saline. The selective 5-HT1C receptor antagonist SB242084 (0.5 mg/kg . d; Sigma-Aldrich) and the 5-HT3 receptor antagonist ondansetron (0.1 mg/kg twice a day; Sigma-Aldrich) were injected subcutaneously for the last 3 d. The last injection was given 1 h prior to electrophysiological recordings. To study the effect of clorgyline on 5-HT-depleted rats, para-chlorophenylalanine methyl-ester hydrochloride (p-CPA; Sigma-Aldrich) was dissolved in distilled water and injected i.p. for three consecutive days (300 mg/kg . d, with the last injection 24 h before recording).

Recording of VTA DA neurons

VTA DA neurons were recorded with single-barreled glass micropipettes positioned at the following coordinates (in mm from bregma): AP –6 to –5.4, L 0.6 to 1, V –7 to –9. The DA neurons were identified by the established electrophysiological criteria in vivo: a triphasic action potential (duration >2.5 ms) with an inflection (‘notch’) in the rising phase and a marked negative deflection, a regular firing rate (2–10 Hz) with an irregular single-spiking pattern and bursting activity (Grace and Bunney, 1983, 1984; Grace et al., 2007). As previously described, a criterion of duration (>1.1 ms from the start of the action potential to the negative trough) was also used (Ungless et al., 2004). The classical filters parameters (50 Hz to 0.8 kHz) were
used (Marinelli et al., 2006). The equipment used for recording included the data acquisition interface CED Micro 1401 mk and Spike2 software (Cambridge Electronic Design, Cambridge, UK). The parameters studied for DA neurons were: the firing rate (frequency), the number of single spikes/min, the inter-spike interval (ISI) between single spikes, the number of spontaneously active cells (average number of cells found per electrode descent through the VTA) and the bursting pattern.

**Burst analysis**

The firing pattern of recorded DA neurons was analysed by ISI burst analysis following the criteria established by Grace and Bunney (1984). The onset of a burst was defined as the occurrence of two spikes with an ISI < 0.08 s. The termination of bursts was defined as an ISI ≥ 0.16 s. Burst parameters studied were: the number of bursts/min, the percentage of spikes in bursts, the number of spikes/burst and the ISI within the bursts.

**Statistical analysis**

The results were expressed as means ± S.E.M. of the firing rates, the number of single spikes, the number of bursts and the percentage of spikes occurring in bursts. Statistical comparisons were carried out using a one-way ANOVA (treatment as the main factor) for the effect of MAOIs followed by Fisher’s PLSD post-hoc test as appropriate (Sigmastat software, Chicago, IL, USA).

The results of the combination studies were analysed by a two-way ANOVA with treatment (clorgyline or NaCl) and co-treatment (NaCl, p-CPA or 5-HT receptor antagonist) as main factors. In case of significance, the initial test was followed by post-hoc tests. Statistical significance was set at p < 0.05.

**Results**

**Effect of MAOIs on DA VTA neurons**

**Clorgyline**

Administration of clorgyline (1 mg/kg, d) for 2 d did not modify the firing pattern of VTA DA neurons. Administration of clorgyline for 21 d markedly decreased the spontaneous firing rate of VTA DA neurons by 30% in comparison to the saline-treated rats (p < 0.001) and the 2-d clorgyline-treated animals (p < 0.01, Figures 1, 2). Burst analysis showed a decrease in the number of bursts/min and the percentage of spikes occurring in bursts in comparison to the saline-treated rats (bursts/min: -80%, p < 0.001; percentage of spikes/burst: -70%, p < 0.01) and the 2-d clorgyline-treated animals (bursts/min: -80%, p < 0.01; percentage of spikes in bursts: -75%, p < 0.01). When bursts occurred, the number of spikes/burst was decreased by 20% in the 21-d treatment group in comparison to the control and the short-term treatment group (p < 0.05 and p < 0.01, respectively). Unlike those effects, neither the number of single spikes/min, nor the ISI within the bursts were changed (Table 1). The number of spontaneously active cells was increased by 65% after prolonged clorgyline treatment in comparison to saline- and 2-d-treated animals (p > 0.05).

The same clorgyline-treated group (21 d) was used throughout the study.

**Phenelzine**

Administration of phenelzine (2.5 mg/kg) for 2 d did not modify the firing pattern of VTA DA neurons.
Administration of phenelzine for 21 d slightly decreased the spontaneous firing rate of VTA DA neurons by 20% in comparison to the saline-treated rats ($p<0.05$, Table 2) and by 25% in comparison to the 2-d-treated animals ($p<0.05$). Similarly to clorgyline-treated animals, the ISI between single spikes was increased after 3 wk of phenelzine exposure in comparison to saline- and 2-d-treated animals ($p<0.01$). A trend for an attenuation of the bursting pattern (number of bursts and percentage of spikes in bursts) was observed, but was not statistically significant. No other trends were obtained in the other parameters studied. The number of single spikes/min, number of spikes/burst, ISI in burst and number of cells/track were not significantly changed (see Table 2).

**Deprenyl**

Administration of deprenyl (0.25 mg/kg . d) for two consecutive days did not modify the firing pattern of VTA DA neurons (Table 2). Administration of deprenyl for 21 d induced an increase in the ISI between single spikes in comparison to the saline treatment group ($p<0.05$). This increase in ISI could explain the slight trend in the decrease in the mean firing rate ($p=0.19$).

**Effect of 5-HT-depletion on VTA DA neurons in control and clorgyline-treated rats**

Since all three MAOIs were devoid of effect after short-term administration, it appeared that changes in the firing pattern of DA neurons obtained with clorgyline and phenelzine were related to an indirect effect. This is because at that regimen of clorgyline, MAOA is maximally inhibited after 2 d and the increase in tissular 5-HT and NE concentration is already maximal (Blier et al., 1986b). This alteration of firing could thus be due to an enhancement in 5-HT or NE function. Since the firing rate of 5-HT neurons, but not that of NE neurons, recovers over a 3-wk period, the 5-HT system was first investigated. The most effective MAOI clorgyline was thus tested in 5-HT-depleted rats using $p$-CPA, in comparison to 5-HT-depleted control animals.

$p$-CPA treatment (300 mg/kg . d for three consecutive days) increased the firing rate of DA neurons (saline +22%, clorgyline +90%), the number of bursts/min (saline +115%, clorgyline +1000%), the percentage of spikes in bursts (saline +150%, clorgyline +600%), and the number of spikes/burst (+25% and +40% in the saline- and clorgyline-treated groups, respectively; Table 1). The two-way ANOVA performed on the firing rate showed a significant effect

![Graphs showing the effect of clorgyline treatment on VTA DA neurons.](http://ijnp.oxfordjournals.org/)

**Figure 2.** Effect of clorgyline treatment (2 d and 21 d) on the firing activity of ventral tegmental area dopamine neurons. Data are expressed as means ± S.E.M. of (a) the firing rate, (b) the number of bursts/min, (c) the percentage of spikes in bursts and (d) the number of spontaneously active cells found per track. The number of neurons recorded in each group is shown at the foot of the histograms. Data were analysed by a one-way ANOVA followed by Fisher’s PLSD test. **$p<0.01$, ***$p<0.001$ indicates a significant effect of clorgyline in comparison to the control group.
Table 1. Effect of p-CPA, SB242084 and ondansetron on the electrical activity of ventral tegmental area dopamine neurons in rats treated with clorgyline (21 d)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firing rate (Hz)</th>
<th>No. of bursts/min</th>
<th>% Spikes occurring in burst</th>
<th>No. of single spikes/min</th>
<th>Spikes/burst</th>
<th>ISI burst (ms)</th>
<th>ISI single spike (ms)</th>
<th>Cells/track</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>NaCl × NaCl</td>
<td>3.6 ± 0.2</td>
<td>14.3 ± 2.2</td>
<td>16 ± 2</td>
<td>172 ± 7</td>
<td>2.6 ± 0.1</td>
<td>64 ± 2</td>
<td>398 ± 27</td>
<td>1.2 ± 0.1</td>
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<tr>
<td>(n = 62)</td>
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<td>(n = 53)</td>
<td>(n = 53)</td>
<td>(n = 62)</td>
</tr>
<tr>
<td>Clorg × NaCl</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.8</td>
<td>5 ± 1</td>
<td>140 ± 13</td>
<td>2.1 ± 0.1</td>
<td>63 ± 3</td>
<td>633 ± 84</td>
<td>2.0 ± 0.3</td>
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<td>(n = 31)</td>
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<td><strong>p</strong>-CPA</td>
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<tr>
<td>NaCl × p-CPA</td>
<td>4.4 ± 0.3</td>
<td>30.7 ± 4.4</td>
<td>40 ± 4</td>
<td>148 ± 14</td>
<td>3.3 ± 0.2</td>
<td>64 ± 3</td>
<td>563 ± 72</td>
<td>1.6 ± 0.3</td>
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<td>(n = 23)</td>
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<td>(n = 22)</td>
<td>(n = 23)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>Clorg × p-CPA</td>
<td>4.2 ± 0.3</td>
<td>29.9 ± 5.8</td>
<td>33 ± 5</td>
<td>154 ± 15</td>
<td>2.9 ± 0.2</td>
<td>65 ± 3</td>
<td>408 ± 81</td>
<td>1.4 ± 0.3</td>
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<td>(n = 18)</td>
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<td>SB242084</td>
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<tr>
<td>NaCl × SB</td>
<td>4.6 ± 0.3</td>
<td>17.9 ± 3.3</td>
<td>20 ± 4</td>
<td>215 ± 15</td>
<td>3.0 ± 0.2</td>
<td>70 ± 3</td>
<td>300 ± 23</td>
<td>1.2 ± 0.3</td>
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<td>(n = 23)</td>
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<td>(n = 22)</td>
<td>(n = 23)</td>
<td>(n = 18)</td>
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<tr>
<td>Clorg × SB</td>
<td>2.9 ± 0.2</td>
<td>7.4 ± 1.8</td>
<td>11 ± 3</td>
<td>153 ± 12</td>
<td>2.3 ± 0.1</td>
<td>66 ± 2</td>
<td>511 ± 55</td>
<td>2.2 ± 0.5</td>
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<td>Ondansetron</td>
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<tr>
<td>NaCl × Ond</td>
<td>3.6 ± 0.2</td>
<td>11.5 ± 3.5</td>
<td>16 ± 5</td>
<td>183 ± 14</td>
<td>2.6 ± 0.2</td>
<td>62 ± 4</td>
<td>376 ± 49</td>
<td>1.3 ± 0.3</td>
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<td>(n = 16)</td>
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<td>(n = 13)</td>
<td>(n = 13)</td>
<td>(n = 16)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>Clorg × Ond</td>
<td>3.5 ± 0.2</td>
<td>14.1 ± 3.7</td>
<td>17 ± 4</td>
<td>170 ± 10</td>
<td>2.7 ± 0.1</td>
<td>69 ± 3</td>
<td>373 ± 25</td>
<td>1.7 ± 0.3</td>
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<td>(n = 22)</td>
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<td>(n = 17)</td>
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<td>(n = 13)</td>
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*p < 0.05, **p < 0.01, ***p < 0.001 in comparison to the NaCl corresponding group.

Table 2. Effect of short-term and long-term treatment with monoamine oxidase inhibitors on the firing activity of ventral tegmental area dopamine neurons

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firing rate (Hz)</th>
<th>No. of bursts/min</th>
<th>% Spikes occurring in burst</th>
<th>No. of single spikes/min</th>
<th>Spikes/burst</th>
<th>ISI burst (ms)</th>
<th>ISI single spike (ms)</th>
<th>Cells/track</th>
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<tr>
<td>Control</td>
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<tr>
<td>2 d</td>
<td>3.6 ± 0.2</td>
<td>14.3 ± 2.2</td>
<td>16 ± 2</td>
<td>172 ± 7</td>
<td>2.6 ± 0.1</td>
<td>64 ± 2</td>
<td>398 ± 27</td>
<td>1.2 ± 0.1</td>
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<td>(n = 62)</td>
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<td>(n = 53)</td>
<td>(n = 53)</td>
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<tr>
<td>21 d</td>
<td>3.5 ± 0.3</td>
<td>12.3 ± 4.2</td>
<td>14 ± 5</td>
<td>177 ± 15</td>
<td>2.5 ± 0.1</td>
<td>63 ± 2</td>
<td>397 ± 42</td>
<td>1.0 ± 0.1</td>
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<td>(n = 21)</td>
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<td>21 d</td>
<td>3.0 ± 0.3</td>
<td>9.8 ± 2.7</td>
<td>14 ± 4</td>
<td>149 ± 16</td>
<td>3.0 ± 0.3</td>
<td>70 ± 4</td>
<td>530 ± 51</td>
<td>1.3 ± 0.1</td>
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<td>(n = 30)</td>
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<td>(n = 20)</td>
<td>(n = 20)</td>
<td>(n = 30)*</td>
<td>(n = 23)</td>
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</table>

*p < 0.05, **p < 0.01, in comparison to the control group.
The administration of SB242084 (0.5 mg/d) for 3 d before recording increased the firing rate in both saline-treated (+25%) and clorgyline-treated (+20%) rats in comparison to their respective controls (Table 2). SB242084, however, did not antagonize the inhibitory effect of clorgyline on the firing rate of DA neurons located in the VTA. SB242084 did not reverse the inhibitory effect of clorgyline on the bursting pattern (% of spikes in bursts: \(-45\%\), \(p<0.05\); bursts/min: \(-60\%\), \(p<0.01\); spikes/burst: \(-25\%\), \(p<0.01\)), as well as the decrease in the number of single spikes/min (\(p<0.01\), Table 1). The two-way ANOVA performed on firing rates showed a significant effect of treatment (NaCl × clorgyline: \(F_{1,130} = 5.68\), \(p<0.05\)) and 5-HT depletion (NaCl × p-CPA: \(F_{1,130} = 24.27\), \(p<0.001\)). Sustained clorgyline administration significantly decreased the firing rate of DA neurons in control (\(p<0.001\)), but not in depleted rats. Moreover, 5-HT depletion significantly increased the firing rate of DA neurons in both control (\(p<0.05\)) and clorgyline-treated groups (\(p<0.001\), Figure 3).

**Combined administration of clorgyline and the 5-HT\textsubscript{2C} receptor antagonist SB242084 or the 5-HT\textsubscript{2} receptor antagonist ondansetron on the firing activity of DA neurons**

The mean number of DA neurons recorded per track remained higher in clorgyline-treated rats than in their controls, even with the co-administration of SB242084. The administration of SB242084 (0.5 mg/d) for 3 d before recording increased the firing rate in both saline-treated (+25%) and clorgyline-treated (+20%) rats in comparison to their respective controls (Table 2). SB242084, however, did not antagonize the inhibitory effect of clorgyline on the firing rate of DA neurons located in the VTA. SB242084 did not reverse the inhibitory effect of clorgyline on the bursting pattern (% of spikes in bursts: \(-45\%\), \(p<0.05\); bursts/min: \(-60\%\), \(p<0.01\); spikes/burst: \(-25\%\), \(p<0.01\)), as well as the decrease in the number of single spikes/min (\(p<0.01\), Table 1). The two-way ANOVA performed on firing rates showed a significant effect of treatment (NaCl × clorgyline: \(F_{1,130} = 5.68\), \(p<0.05\)) and 5-HT depletion (NaCl × p-CPA: \(F_{1,130} = 24.27\), \(p<0.001\)). Sustained clorgyline administration significantly decreased the firing rate of DA neurons in control (\(p<0.001\)), but not in depleted rats. Moreover, 5-HT depletion significantly increased the firing rate of DA neurons in both control (\(p<0.05\)) and clorgyline-treated groups (\(p<0.001\), Figure 3).

**Effect of serotonergic depletion with the serotonin synthesis inhibitor para-chlorophenylalanine (p-CPA) on the firing activity of dopamine neurons in the ventral tegmental area.** The number of neurons recorded in each group is shown at the foot of the histograms. Data were analysed by a two-way ANOVA followed by a Newman–Keuls test. *** \(p<0.001\) indicates a significant effect of clorgyline in comparison with the corresponding control group. # \(p<0.05\), ### \(p<0.001\) indicates a significant effect of 5-HT depletion between groups receiving the same treatment.
One hypothesis explaining the absence of prolonged treatment with clorgyline (Lamensdorf et al., 1996) is that firing returned to normal after prolonged administration (21 d; Blier and de Montigny, 1985; Blier et al., 1986a; Haddjeri et al., 1998). In the present experiments, MAOA inhibition decreased the firing rate of VTA DA neurons after 2 d, but not 2 d, thus suggesting an indirect effect of MAOA inhibition on the firing of VTA DA neurons (Figure 2). This is in line with the absence of effect of the MAOB inhibitor deprenyl (Table 2). Indeed, DA is also a substrate for MAOB and an increase in DA extracellular levels in the rat striatum occurs after prolonged treatment with deprenyl, whereas a decrease is obtained after prolonged treatment with clorgyline (Lamensdorf et al., 1996). One hypothesis explaining the absence of an inhibitory effect of deprenyl on the firing rate of DA VTA neurons could be that deprenyl increased the extracellular levels of endogenous DA in the terminal areas, but not in the somatodendritic region, thereby not causing any increased stimulation of these D₂ autoreceptors. Indeed, it appears to be a characteristic of DA neurons that somatodendritic release of DA is consistently lower than terminal release (Kalivas et al., 1989). These results are in line with those showing that bupropion, which releases DA in post-synaptic structures, leaves unaltered the firing rate of VTA DA neurons after 2 d and 14 d of administration (El Mansari et al., in press; Li et al., 2002; Nomikos et al., 1989).

The inhibitory effect of MAOA inhibitors on DA firing could have been related to an increase in 5-HT and/or NE. Indeed both monoaminergic systems are known to have an inhibitory effect on the firing activity of DA neurons of the VTA. For example, the local application of NE (or NE agonists) in the VTA inhibits the firing activity of DA neurons (Aghajanian and Bunney, 1977; Grenhoff et al., 1995; White and Wang, 1984). A similar inhibitory effect of 5-HT has been widely described (Adell and Artigas, 2004; Esposito, 2006). This is in line with recent results showing that a lesion of 5-HT or NE neurons increases the firing rate of VTA DA neurons (Guiard et al., 2008). However, this inhibitory effect of 5-HT remains debatable because some authors have reported that local infusion of 5-HT in the VTA increased the amount of DA released in the nucleus accumbens (Guan and McBride, 1989). Nonetheless, the effect of clorgyline on VTA DA neuron firing is more likely to be related to 5-HT than to NE. Indeed, Mateo et al. (2001) have shown that 1 mg/kg clorgyline induces an increase in extracellular levels of NE in the locus coeruleus and its projection areas after acute administration. In contrast, MAOA inhibition increase the extracellular levels of 5-HT in projection areas after sustained, but not acute, administration with a time-course similar to that of the desensitization of somatodendritic 5-HT₁A autoreceptors (Celada and Artigas, 1993; Ferrer and Artigas, 1994; Pineyro and Blier, 1999). If the inhibitory effect of clorgyline on VTA DA neurons had been related to the NE system, it should have thus occurred after the 2-d exposure. In order to completely rule out an effect of NE on the inhibitory activity of clorgyline on VTA DA neurons, experiments using a neurotoxin specific to the NE system (i.e. DSP-4) could have been performed. However, the current results showed that an inhibition of the synthesis of 5-HT using p-CPA totally reversed the inhibitory effect of clorgyline in VTA DA neurons. It is therefore likely that only 5-HT was involved in...
the inhibitory effect of clorgyline. Aulakh et al. (1988) have demonstrated that, in control rats, p-CPA induces a decrease in 5-HT tissue levels of 95%, but that this decrease is only 60% in clorgyline-treated animals, in comparison to their respective controls. Considering the inhibitory effect of 5-HT on VTA DA neurons, this suggests that for an equivalent level of depletion, the firing rate of DA neurons could be higher in clorgyline-depleted rats than in control-depleted rats.

Numerous studies have investigated the effect of 5-HT$_{3C}$ receptor ligands on the firing activity of VTA DA neurons. It was demonstrated that 5-HT$_{3C}$ receptor agonists decrease the firing rate of DA neurons, whereas 5-HT$_{3C}$ receptor antagonists increase both the firing rate and bursting pattern of those neurons (Alex and Pehek, 2007). It was thus presumed that the inhibitory effect of clorgyline on VTA DA neurons might be related to an enhanced activation of 5-HT$_{3C}$ receptors by endogenous 5-HT. In the present study, however, the selective 5-HT$_{3C}$ receptor antagonist SB242084 did not antagonize the inhibitory effect of clorgyline, even if blockade of the 5-HT$_{3C}$ receptor increased the firing rate of VTA DA neurons by 20% in both saline- and clorgyline-pretreated rats (Figure 4). In contrast, Dremencov et al. (2008) have observed that SB242084 antagonizes the inhibitory effect of the SSRI escitalopram on the VTA DA neuron. This suggests that post-synaptic effects of MAOIs and SSRIs did not involve the same receptors. A difference in post-synaptic activity following chronic antidepressant treatment (SSRIs vs. MAOIs) has already been demonstrated. For example, a subpopulation of 5-HT$_{3}$ receptors in limbic areas are desensitized after chronic treatment with the SSRI paroxetine, but not after chronic treatment with the selective MAOA inhibitors befoxatone and moclobemide (Blier and Bouchard, 1994; Mongeau et al., 1994).

The 5-HT$_{3}$ receptor antagonist ondansetron alone did not modify the firing pattern of VTA DA neurons, but antagonized the inhibitory effect of clorgyline on VTA DA firing activity (Figure 5). The inhibitory effect of MAOA inhibition on VTA DA firing activity could therefore be attributable to an activation of 5-HT$_{3}$ receptors. Interestingly, a long-term treatment with clorgyline did not modify the number of single spikes/min, suggesting that the difference in the firing rate was only due to a difference in bursting activity. It is now well established that VTA DA neurons receive GABA-mediated inhibitory post-synaptic potentials (IPSPs) that regulate the number of spontaneously active neurons in the VTA (Grace et al., 2007). It is therefore probable that the increase in the number of spontaneously active neurons (following clorgyline treatment) was due to a disinhibition of VTA DA neurons through a decrease in IPSPs. Burst activity is regulated by excitatory glutamatergic input from the tegmental nuclei (latero-dorsal tegmentum – LDTg; and pedunculopontine tegmentum – PPTg; Grace et al., 2007). It is therefore reasonable to assume that clorgyline exerts its inhibitory effect by activating 5-HT$_{3}$ receptors that, in turn, inhibit the activity of glutamatergic input from the tegmental nuclei. The PPTg receives afferents from the amygdala, and it has been demonstrated that activation of 5-HT$_{3}$ receptors in the amygdala facilitates an inhibitory GABAergic current (Koyama et al., 2002), whereas the 5-HT-induced facilitation of GABAergic current is blocked by 5-HT$_{3}$ antagonists (Ropert and Guy, 1991). These observations suggest that the inhibitory effect of clorgyline on VTA DA firing might have been dependent on the activation of 5-HT$_{3}$ receptors in the amygdala that are responsible for the inhibition of the LDTg, the PPTg, and subsequently the VTA.

A role for 5-HT$_{3}$ receptors located in the VTA still cannot be excluded. The presence of 5-HT$_{3}$ receptors in the VTA remains, however, controversial. Indeed, 5-HT$_{3}$ binding was detected in the rat VTA using $[^{3}H]$quipazine (Perry, 1990), whereas studies using more selective ligands with a higher affinity than quipazine did not identify the presence of 5-HT$_{3}$ binding sites in the rat VTA (Barnes et al., 1990; Hamon, 1992; Laporte et al., 1992). Nonetheless, local perfusion of the 5-HT$_{3}$ receptor agonist m-chlorophenyl-biguanide in the VTA, via a microdialysis probe, induces a dose-dependent increase in extracellular level of DA (Campbell et al., 1996; Liu et al., 2006). Therefore very scarce 5-HT$_{3}$ receptors possibly present in the VTA might still be functionally important. It is also of interest that local or intracerebroventricular infusion of 5-HT$_{3}$ receptor agonists increased DA extracellular levels (Chen et al., 1992; Jiang et al., 1990) in the medial prefrontal cortex and nucleus accumbens, and that chronic 5-HT$_{3}$ antagonism decreases extracellular DA levels in the rat nucleus accumbens (Invernizzi et al., 1995). It is therefore possible that an indirect activation of the 5-HT$_{3}$ receptor by clorgyline could induce an increase in extracellular levels of DA in the VTA which in turn would activate the somatodendritic D$_{3}$ receptors, thereby decreasing the firing rate of VTA DA neurons.

Taken together the present results imply that ondansetron could be used as an augmentation strategy when combined with MAOA inhibitors for the treatment of depression resulting in intensification of DA transmission due to restoration of DA neuronal firing.
This is in line with behavioural results showing that 5-HT3 receptor blockade can induce an antidepressant-like effect by itself (Ramamoorthy et al., 2008), and can be used to potentiate the activity of various antidepressants (citalopram, fluoxetine, fluvoxamine and agomelatine; Bourin et al., 2004; Redrobe and Bourin, 1997). Further investigations are, nevertheless, necessary to determine whether such a recovery in DA neuronal firing, following the combination of ondansetron with clorgyline, would lead to an additional increase in extracellular DA in forebrain areas.

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Statement of Interest

None.

References


