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Sustained Administration of Pramipexole Modifies the Spontaneous Firing of Dopamine, Norepinephrine, and Serotonin Neurons in the Rat Brain

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Pramipexole (PPX) is a D_2/D_3 receptor agonist that has been shown to be effective in the treatment of depression. Serotonin (5-HT), norepinephrine (NE) and dopamine (DA) systems are known to be involved in the pathophysiology and treatment of depression. Due to reciprocal interactions between these neuronal systems, drugs selectively targeting one system-specific receptor can indirectly modify the firing activity of neurons that contribute to firing patterns in systems that operate via different neurotransmitters. It was thus hypothesized that PPX would alter the firing rate of DA, NE and 5-HT neurons. To test this hypothesis, electrophysiological experiments were carried out in anesthetized rats. Subcutaneously implanted osmotic minipumps delivered PPX at a dose of 1 mg/kg per day for 2 or 14 days. After a 2-day treatment with PPX the spontaneous neuronal firing of DA neurons was decreased by 40%, NE neuronal firing by 33% and the firing rate of 5-HT neurons remained unaltered. After 14 days of PPX treatment, the firing rate of DA had recovered as well as that of NE, whereas the firing rate of 5-HT neurons was increased by 38%. It was also observed that sustained PPX administration produced desensitization of D_2/D_3 and 5-HT_{1A} cell body autoreceptors, as well as a decrease in sensitivity of α_2-adrenergic cell body autoreceptors. These adaptive changes are implicated in long-term firing rate adaptations of DA, NE and 5-HT neurons after prolonged PPX administration. In conclusion, the therapeutic action of PPX in depression might be attributed to increased DA and 5-HT neurotransmission.

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INTRODUCTION

Dopamine (DA) agonists, such as quinpirole, pergolide, piribedil and bromocriptine have been shown to possess antidepressant-like properties in animal studies and therapeutic action in depressed patients (Anisman *et al*, 1979; Izumi *et al*, 2000; Muscat *et al*, 1992; Waehrens and Gerlach, 1981; Brocco *et al*, 2006). Pramipexole (PPX) is a D_2/D_3 receptor agonist customarily used in treatment of Parkinson's disease and restless legs syndrome (Guttman and Jaskolka, 2001; Piercey, 1998; Reichmann *et al*, 2006). This drug was also shown to be efficacious in treatment of major depressive disorder (MDD) as monotherapy (Corrigan *et al*, 2000; Lattanzi *et al*, 2002), and to be a useful augmentation strategy in treatment-resistant depressed patients (Cassano *et al*, 2004; Goldberg *et al*, 2004; Sporn *et al*, 2000).

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Even though pathophysiological mechanisms of depression have yet to be fully elucidated, a consensus exists for a central involvement of serotonergic (5-HT) and noradrenergic (NE) systems in this disease and in its effective treatment. Furthermore, reciprocal interactions between these two neuronal entities are now well established (Blier, 2001; Szabo and Blier, 2001; Guiard *et al*, 2008a). However, during the last decade substantial data suggesting participation of dopaminergic system in this neuronal network of interactions have emerged (Aman *et al*, 2007; Esposito, 2006; Haj-Dahmane, 2001). Consequently, in the light of an apparent involvement of the DA system in pathophysiology of depression (Dunlop and Nemeroff, 2007), it is important to ascertain the effects of DA on the above-mentioned monoaminergic systems.

Various studies have shown anatomical similarities and functional interactions between the 5-HT neurons of the raphe dorsalis (RD) and the DA neurons of mesencephalic DA systems (Aman *et al*, 2007; Martin-Ruiz *et al*, 2001) that can help to guide research regarding the pharmacological action of antidepressant drugs (Aman *et al*, 2007; Dremencov *et al*, 2004). For instance, D2-like receptors are expressed on

the cell body of 5-HT neurons (Mansour *et al*, 1990; Suzuki *et al*, 1998). This anatomical commonality first suggested that DA might be able to modulate 5-HT neuronal firing. Accordingly, this led to a recent *in vivo* study, which confirmed the existence of the excitatory effect of DA upon RD 5-HT neuronal firing: the mean firing activity of RD 5-HT neurons in DA-lesioned rats was decreased by 60% compared to sham-operated rats (Guiard *et al*, 2008a).

In the locus coeruleus (LC), dopamine is, however, thought to exert an inhibitory effect on NE cells. Several radioligand-binding studies documented presence of D_2 as well as D_3 receptors in the LC (Suzuki *et al*, 1998; Yokoyama *et al*, 1994). As predicted, pharmacological blockade of these receptors or selective lesioning of ventral tegmental area (VTA) DA neurons enhances LC NE neuronal activity (Guiard *et al*, 2008a; Piercey *et al*, 1994). This suggests a negative influence of VTA DA neurons on LC NE neurons.

Clinical attenuation of depressive symptoms correlates in time with desensitization of autoreceptors achieved after long-term treatment with pharmacological agents acting on the respective neuronal systems. Waning of the responsiveness of somatodendritic 5-HT_{1A} autoreceptor following chronic administration of selective serotonin reuptake inhibitor (SSRI) was previously described (Blier and De Montigny, 1983; Pineyro and Blier, 1999). It was observed that attenuated autoreceptor regulation leads to an overall increase in 5-HT transmission in the presence of 5-HT reuptake inhibitor (Chaput *et al*, 1986; Haddjeri *et al*, 1998a). Analogously, desensitization of terminal α_2-adrenergic autoreceptor as a result of sustained NE reuptake inhibition has been described using electrophysiology and microdialysis (Lacroix *et al*, 1991; Parini *et al*, 2005). Similarly biochemical and electrophysiological aspects of dopaminergic autoreceptor desensitization have also been described in a large body of literature (Jeziorski and White, 1989; Pitts *et al*, 1995; Subramaniam *et al*, 1992).

The *in vivo* electrophysiological studies that we present here were designed to test the hypothesis that acute and sustained administration of the D_2/D_3 receptor agonist PPX will alter not just DA neuronal activity, but that of 5-HT and NE neurons as well. This endeavor was prompted by reports of the clinical effectiveness of PPX in the MDD treatment (Cassano *et al*, 2004, Goldberg *et al*, 2004; Lattanzi *et al*, 2002; Maj *et al*, 1997; Sporn *et al*, 2000), the presence of D_2 as well as D_3 receptors in VTA, LC, and RD (Levant *et al*, 1993; Suzuki *et al*, 1998; Yokoyama *et al*, 1994), as well as the existence of reciprocal interactions between DA, NE, and 5-HT systems involved in the pathophysiology of depression (Millan *et al*, 2000a; Tremblay and Blier, 2006).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, St Constant, QC), weighing 270–320 g at the time of recording, were used for the experiments. They were kept under standard laboratory conditions (12:12 h light/dark cycle with access to food and water *ad libitum*). All animal handling and procedures were carried out according to the guidelines of the Canadian Council on Animal Care and protocols of this study were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, ON, Canada).

Treatments

Rats were anesthetized with isoflurane for the subcutaneous implantation of osmotic minipumps, delivering PPX at a daily dose of 1 mg/kg for 2 or 14 days. Control rats were implanted with minipumps delivering physiologic saline.

In Vivo Electrophysiological Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. To maintain a full anesthetic state, chloral hydrate supplements of 100 mg/kg, i.p. were given as needed to prevent any nociceptive reaction to paw pinching. Extracellular recordings of the 5-HT, DA, and NE neurons in the RD, the VTA and the LC, respectively, were obtained using single-barreled glass micropipettes. Their tips were of 1–3 μm in diameter and impedance ranged between 4 and 7 MΩ. All glass micropipettes were filled with a 2 M NaCl solution. Using this approach, during all recordings signal-to-noise ratio was between 2 and 10, therefore making spike amplitude discrimination extremely reliable. In cases when more than one neuron was recorded simultaneously, neurons were discriminated automatically by the Spike 2 software based on the spike shape and amplitude. Prior to electrophysiological experiments, a catheter was inserted in the lateral tail vein for systemic i.v. injection of appropriate pharmacological agents when applicable.

Recording of the VTA DA neurons. Single-barreled glass micropipettes were positioned using the following coordinates (in mm from λ): AP, +3.0 to +3.8; L, 1–0.6; V, 6.5–9. The presumed DA neurons were identified according to the well-established electrophysiological properties *in vivo*: a typical triphasic action potential with a marked negative deflection; a characteristic long duration (>2.5 ms) often with an inflection or 'notch' on the rising phase; a slow spontaneous firing rate (0.5–5 Hz) with an irregular single spiking pattern with slow bursting activity (characterized by spike amplitude decrement; Grace and Bunney, 1983). Additionally, as previously described, a criterion of duration (>1.1 ms from the start of the action potential to the negative trough) was used (Ungless *et al*, 2004).

Recording of the LC NE neurons. Single-barreled glass micropipettes were positioned using the following coordinates (in mm from λ): AP, −1.0 to −1.2; L, 1.0–1.3; V, 5–7. Spontaneously active NE neurons were identified using the following criteria: regular firing rate (0.5–5.0 Hz) and positive action potential of long duration (0.8–1.2 ms) exhibiting a brisk excitation followed by period of silence in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982b).

Recording of the RD 5-HT neurons. Single-barreled glass micropipettes were positioned using the following

coordinates (in mm from λ): AP, +1.0 to 1.2; L, 0 ± 0.1; V, 5–7. The presumed 5-HT neurons were then identified by applying the following criteria: a slow (0.5–2.5 Hz) and regular firing rate and long-duration (2–5 ms) bi- or triphasic extracellular waveform (Aghajanian and Vandermaelen, 1982a).

Dose–Response Curves

Dose–response curves were generated to determine response of DA, NA, and 5-HT neurons to acute i.v. administration of PPX. Dose–response curves were also constructed for systemic i.v. administration of the DA agonist apomorphine, the 5-HT autoreceptor agonist lysergic acid diethylamide (LSD), and the α_2-adrenergic agonist clonidine to assess the effect of sustained administration of PPX on the sensitivity of D_2/D_3, 5-HT_{1A}, and α_2-adrenergic autoreceptors. Dose–response curves were obtained using only the initial response to the first dose injected to a single neuron of each rat. Dose–response curves were plotted using GraphPad software.

Firing Rate and Burst Analysis

The firing patterns of DA and NE neurons were analyzed by interspike interval burst analysis, following the criteria set by Grace and Bunney (1984). The onset of a burst was defined as the occurrence of two spikes with an interspike interval shorter than 0.08 s. The termination of burst was defined as an interspike interval of 0.16 s or longer. Burst activity of 5-HT neurons mostly occurs in doublets. Furthermore, 5-HT burst firing was analyzed using the following parameter: the onset of a burst, defined as the occurrence of two spikes with an interspike interval of 0.01 s or shorter (Hajós and Sharp, 1996).

Statistical Analysis

All results are expressed as mean ± SEM, unless otherwise specified. Statistical comparisons between differences in spontaneous firing rate and burst activity DR, VTA, and LC of control and PPX-treated rats were carried out by using one-way analysis of variance and multiple comparison procedures using Fisher's PLSD *post hoc* test. Data were obtained from three to five rats per experimental group. Statistical significance was taken as $p < 0.05$.

Drugs

Pramipexole was generously provided by Boehringer Ingelheim Pharmaceuticals (Ingelheim, Germany); S 33084 was generously provided by Servier Research Institute (Paris, France); L-741626 was purchased from Tocris Biopharmaceuticals (Bristol, UK); apomorphine, haloperidol, clonidine, idazoxan, and WAY 100635 were purchased from Sigma (St Louis, MO, USA); LSD was obtained through Health Canada. All drugs except haloperidol and S 33084 were dissolved in distilled water. Haloperidol and S 33084 were dissolved in distilled water acidified with lactic acid (followed by pH control and normalization, as needed).

RESULTS

Effects of Acute Systemic Administration of PPX on the Mean Firing Rate of VTA DA, LC NE, and RD 5-HT Neurons

Intravenous injection of PPX led to a dose-dependent inhibition of DA spontaneous firing, inducing a complete suppression at a dose of 100 μg/kg (Figure 1). Dose–response values were obtained using only the initial response to the first dose injected to a single neuron of each rat ($n = 14$ in 14 rats). In contrast, administration of PPX in doses of up to 3–6 mg/kg did not produce any significant effect on 5-HT and NE discharge rate (data not shown).

Effects of PPX Administration for 2 and 14 Days on the Mean Firing Rate and Burst Activity of VTA DA Neurons

The mean firing rate of recorded DA neurons in vehicle-treated rats was 4.2 ± 0.33 Hz ($n = 41$ in eight rats). A 2-day treatment with PPX at a dose of 1 mg/kg per day resulted in a 40% attenuation of the spontaneous firing of DA neurons ($n = 41$ in eight rats) when compared to the vehicle-treated rats. However, following 14 days of treatment with the same dose of PPX, the firing activity of DA neurons had fully recovered ($n = 41$ in seven rats; Figure 2a).

Burst firing activity, characteristic of most DA neurons, was significantly altered by PPX. In controls, 24% of all spikes were occurring in bursts, 81% of neurons exhibited burst firing, with an average of 29 bursts per minute (assessed only in neurons exhibiting burst firing; Figure 2b). After 2 days of PPX administration at dose of 1 mg/kg, there was no alteration of the percentage of neurons displaying burst-mode activity. However, the number of bursts per minute was decreased by 50% and the percentage of spikes occurring in bursts was not changed when compared to the control level. Interestingly, after 14 days of PPX administration, the number of bursts per minute returned to the baseline level (Figure 2b). Despite the recovery of this parameter, the percentage of spikes occurring in bursts significantly decreased to 70% of control level (Figure 2b), possibly due to significantly decreased number of neurons exhibiting burst activity (Figure 2b).

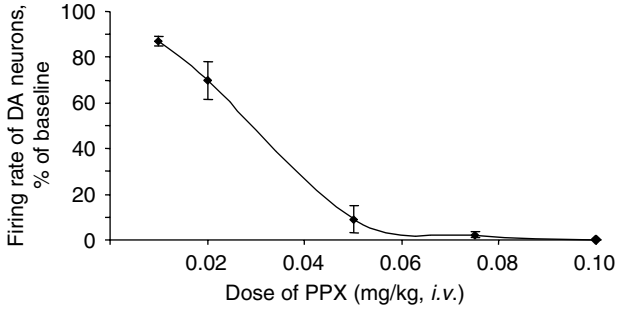

Figure 1 Effect of acute systemic administration of pramipexole (PPX) on firing rate of ventral tegmental area (VTA) dopamine (DA) neurons when compared to the prior injection of the PPX.

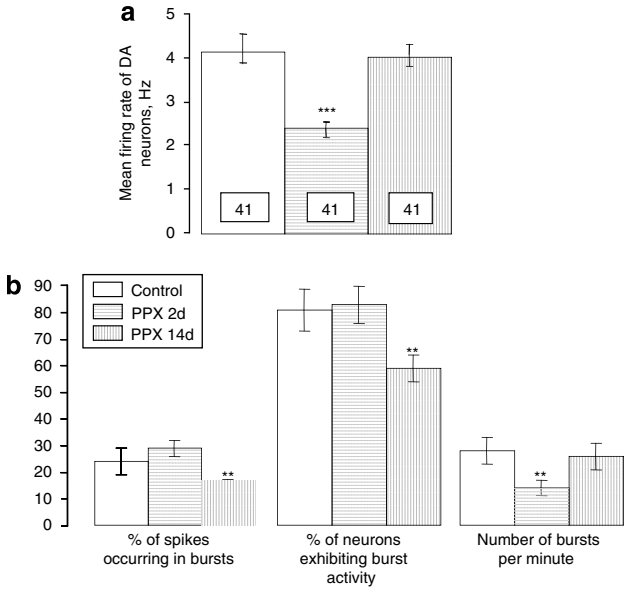

Figure 2 Effect of acute and sustained administration of pramipexole (PPX) on ventral tegmental area dopamine (DA) spontaneous firing rate (a) and burst activity (b). (a) The number of neurons recorded in each group is displayed in respective histogram. (b). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded ($n=41$ in control, $n=41$ in PPX 2-day-treated rats and $n=41$ in PPX 14-day-treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity ($n=33$ in control, $n=34$ in PPX 2-day-treated rats and $n=24$ in PPX 14-day-treated rats). The data expressed as mean firing rate ± SEM. $^{**}p<0.01$, $^{***}p<0.001$; *n*, number of neurons; SEM, standard error mean.

Assessment of Long-Term Administration of PPX on the Function of the D2-like Autoreceptors

To explain the recovery of firing of DA neurons following the 14-day administration of PPX, the responsiveness of D_2/D_3 autoreceptors was assessed using the i.v. administration of DA agonist apomorphine. Injection of apomorphine in doses of 10–40 μg/kg led to a dose-dependent inhibition of DA firing activity in control rats. Injection of 30 μg/kg of apomorphine resulted in a complete and lasting inhibition of spontaneous firing in the control group ($ED_{50}=13\pm1.1$ μg/kg; $n=8$ in eight rats). In contrast, rats subjected to 14 days of PPX administration responded to apomorphine only to a minor extent. Apomorphine administered in doses of up to 1000 μg/kg induced an inhibition of only up to 33% (Figure 5; $n=7$ in seven rats). To determine if the attenuated response to apomorphine was due to a true desensitization of DA autoreceptors, or to a competition of apomorphine with PPX at the autoreceptor sites, the effect of apomorphine was examined after a washout period (minipumps delivering PPX were taken out under isoflurane anesthesia and electrophysiological recordings were carried out 24 h later). After PPX was washed out, a complete inhibition of DA neuronal firing was achievable with 175 μg/kg of apomorphine ($ED_{50}=32.7\pm1.5$ μg/kg; $n=7$ in seven rats), thus indicating that despite apparent competition between the two agonists, a desensitization of the D2 receptors had occurred (Figure 3). Besides the sensitivity of the autoreceptor, other parameters such as spontaneous and burst-mode firing of the DA neurons were not affected after the 24 h PPX washout, when

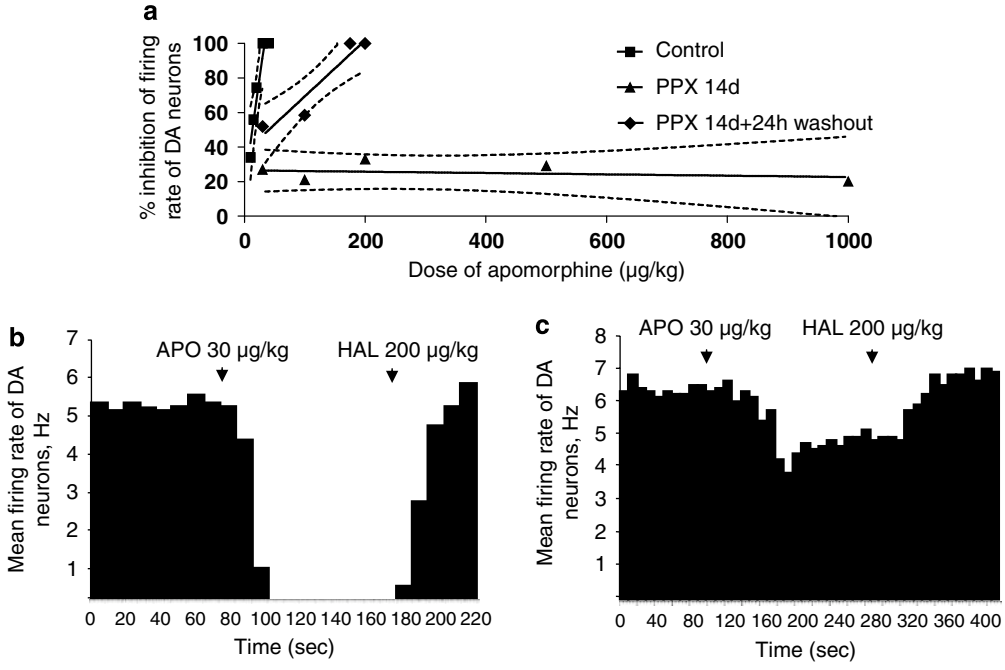

Figure 3 Assessment of the sensitivity of the ventral tegmental area (VTA) dopamine (DA) D2 autoreceptor: (a) Relationship between the degree of suppression of VTA DA firing activity and doses of D2 receptor agonist apomorphine administered intravenously in control, 14-day pramipexole (PPX)-treated rats and 14-day PPX-treated rats after 24-h PPX washout. Outer lines represent the standard error of the regression line. Representative integrated firing rate histogram illustrating the effect of apomorphine administration in control (b) and 14-day PPX-treated rats (c). Effect of the D2 agonist apomorphine is reversed by administration of the D2 antagonist haloperidol in both control, PPX 14-day-treated rats and 14-day PPX-treated rats after 24-h PPX washout.

compared to the PPX 14-day-treated group tested with the minipump delivering the drug present in the rats (data not shown).

Assessment of the Role of D_2 and D_3 Receptors in the Suppressant Effect of PPX Administration on VTA DA Firing

As PPX is an agonist on both D_2 and D_3 receptors, pharmacological dissection of observed inhibitory action of PPX on DA neuronal firing was attempted using highly selective antagonists of D_2 and D_3 receptors (data not shown). Dopamine neuronal firing in control rats was suppressed by an i.v. bolus injection of PPX (100 μg/kg). To determine whether PPX was acting on the firing activity of the DA neuron via D_2 and/or D_3 receptors, the selective D_2 receptor antagonist L-741626 or the selective D_3 receptor antagonist S 33084 were injected thereafter in doses of 250–500 μg/kg. These doses were based on previous studies (Millan *et al*, 2000b). Several experiments yielded inconsistent results possibly due to heterologous distribution of D_2 and D_3 receptors on VTA DA neurons and their similar effects on DA spontaneous firing.

Effects of PPX Administration for 2 and 14 Days on the Mean Firing Rate and Burst Activity of LC NE Neurons

The mean firing rate of NE neurons in vehicle-treated rats was 1.7 ± 0.11 Hz ($n = 61$ in five rats). Similarly to DA neurons, a 2-day regimen of PPX led to a significant 33% decrease in firing activity of NE neurons ($n = 53$ in four rats) compared to controls. Norepinephrine neuronal firing returned to the baseline levels after 14 days of PPX administration (Figure 4a; $n = 41$ in four rats).

Overall burst activity, represented by the percentage of spikes occurring in bursts, was drastically decreased by both short- and long-term treatment with PPX (Figure 4b). This change is potentially attributed to the significant decrease in the number of bursts per minute (assessed only in neurons exhibiting burst firing) in response to both 2- and 14 days of PPX treatment compared to baseline control level (Figure 4b). The percentage of NE neurons exhibiting burst activity, however, was not changed by PPX treatment (Figure 4b).

Assessment of Short-Term Treatment with PPX on the Function of The α_2-Adrenergic Autoreceptor

Dopamine-induced decrease of the NE firing rate has recently been attributed to the stimulation of α_2-adrenergic autoreceptors (Guiard *et al*, 2008b). If this receptor is solely responsible for the inhibition of spontaneous firing of NE neurons by PPX, then its blockade would lead to the same increase of firing rate in vehicle- and PPX-treated rats (See Szabo and Blier, 2002). To address this possibility, the selective α_2-adrenoreceptor antagonist idazoxan was administered at a dose 1 mg/kg in the control group (six rats) and in rats given PPX for 2 days (six rats). Firing rates of the NE neurons were recorded prior to and following administration of the antagonist in both groups. Despite significantly lower initial rates of discharge in PPX-treated group, they were equalized in both groups after idazoxan

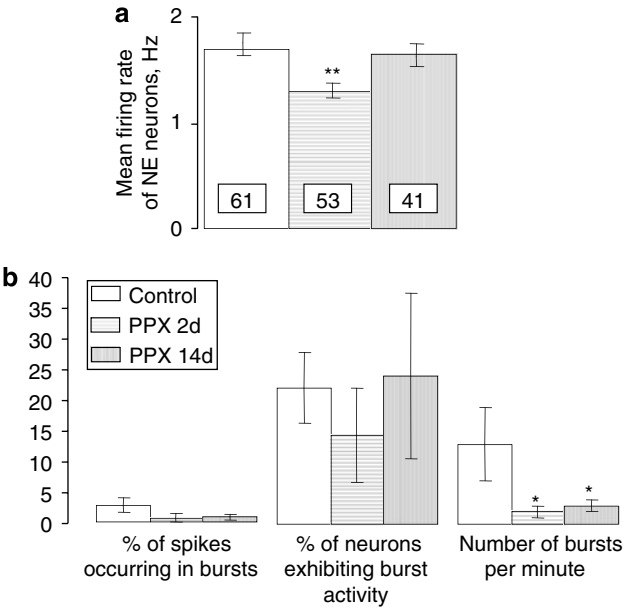

Figure 4 Effect of acute and sustained administration of pramipexole (PPX) on locus coeruleus norepinephrine (NE) spontaneous firing rate (a) and burst activity (b). (a) The number of neurons recorded in each group is displayed in respective histogram. (b). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded ($n = 61$ in control, $n = 53$ in PPX 2-day-treated rats, and $n = 41$ in PPX 14-day-treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity ($n = 14$ in control, $n = 8$ in PPX 2-day-treated rats, and $n = 6$ in PPX 14-day-treated rats). The data expressed as mean firing rate ± SEM. *$p < 0.05$, **$p < 0.01$; *n*, number of neurons, SEM, standard error mean.

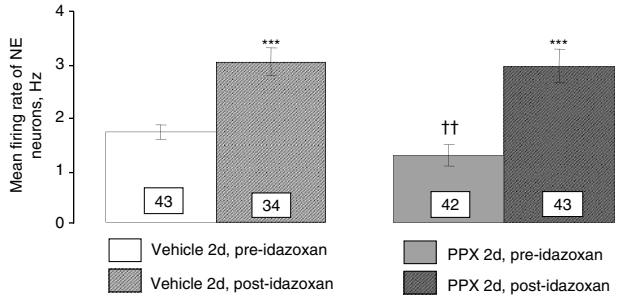

Figure 5 Effect of the α_2-adrenoreceptor antagonist idazoxan on locus coeruleus norepinephrine (NE) neuronal firing in control and 2-day pramipexole (PPX)-treated rats. †† indicates decreased NE neuronal firing in 2-day PPX-treated rats when compared to control. The number of neurons recorded in two electrode descents before and after i.v. idazoxan administration recorded displayed in each column. The data expressed as mean firing rate ± SEM. ***$p < 0.001$.

administration (Figure 5), thus indicating that no other receptor than α_2-adrenoreceptors contributed to the inhibition of firing of NE neurons by PPX.

Effect of Long-Term PPX Administration on the Function of the α_2-Adrenergic Autoreceptor

In an attempt to explain the recovery of the mean firing of NE neurons following the 14-day administration of PPX, the sensitivity of the cell body α_2-adrenergic autoreceptor was

assessed using the α_2-adrenoreceptor agonist clonidine. Although the ED_{50} values for clonidine in the PPX-treated rats ($ED_{50} = 3.4 \pm 1.1$ μg/kg; $n = 6$ in 6 rats) did not significantly differ from the controls ($ED_{50} = 2.7 \pm 1.2$ μg/kg; $n = 10$ in 10 rats), there was some evidence for an attenuated responsiveness of the α_2-adrenergic autoreceptor based on the differential doses required to completely inhibit firing between PPX-treated and control rats. The dose of clonidine required for silencing of NE neurons in control rats was determined to be 5 μg/kg; however, chronic treatment with PPX 1 mg/kg per day resulted in a marked attenuation of the inhibitory effect of clonidine, with a required does of 15 μg/kg for complete inhibition of firing (Figure 6).

Effects of PPX Administration for 2 and 14 days on the Mean Firing Rate and Burst Activity of RD 5-HT Neurons

The baseline firing rate of 5-HT neurons (controls: 1.0 ± 0.1 Hz; $n = 51$ in five rats) remained unchanged after 2-day treatment with PPX (1 mg/kg per day; $n = 65$ in six rats). However, after 14 days of the same regimen, the spontaneous firing of 5-HT neurons was increased by 38% ($n = 66$ in six rats) (Figure 7a). This increase was observed in both single-spike and burst-firing neurons (data not shown).

As for the mean firing rate of 5-HT neurons, the percentage of spikes occurring in bursts was not changed by the 2-day PPX administration. It was, however, significantly elevated after the drug was administered for 14 days (Figure 7b). This change was not due to the increase in the number of neurons exhibiting burst activity, as this parameter was not altered by either 2 or 14-day PPX administration, when compared to saline-treated rats (Figure 7b). Thus, the observed increase in percentage of spikes occurring in bursts is likely due to the substantial difference in the number of bursts per minute at different stages of the treatment (assessed only in neurons exhibiting burst firing). On average, 5-HT neurons in vehicle-treated rats exhibited four bursts per minute. After 2 days of PPX administration, this parameter was amplified by 50%, and after 14 days of PPX administration it increased even more, reaching a level of 150% of control (Figure 7b).

Effect of Long-Term PPX Administration on the Function of the 5-HT_{1A} Autoreceptor

Given the potent inhibitory role of the 5-HT_{1A} autoreceptor on 5-HT neuronal firing, its sensitivity had to be determined to explain the elevated firing activity of 5-HT neurons in 14-day PPX-treated rats. The degree of 5-HT neuron firing rate suppression due to LSD, a 5-HT autoreceptor agonist, was assessed. LSD is considered to be a more reliable tool for testing 5-HT_{1A} autoreceptor sensitivity and was chosen over the other widely used 5-HT_{1A} agonist 8-OH-DPAT because, unlike the latter, it does not have an effect on postsynaptic cortical 5-HT_{1A} receptors and therefore does not activate a feedback loop (Blier *et al*, 1987).

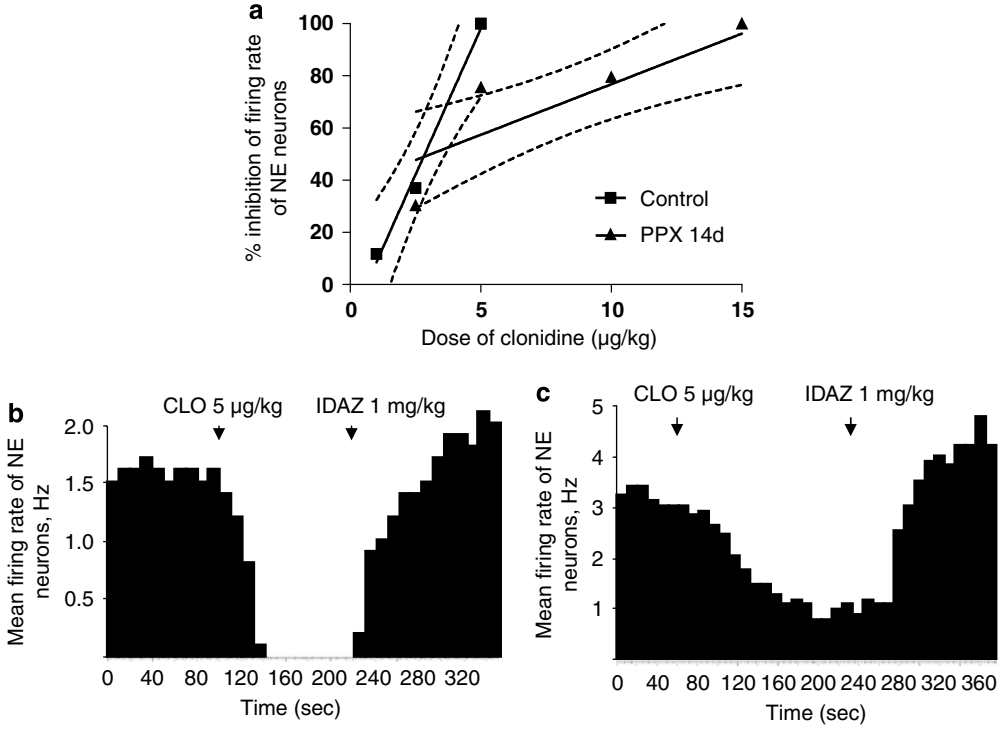

Figure 6 Assessment of the sensitivity of the locus coeruleus (LC) norepinephrine (NE) α_2-adrenergic autoreceptor. (a) Relationship between the degree of suppression of LC NE firing activity and doses of α_2 agonist clonidine administered intravenously in control and 14-day pramipexole (PPX)-treated rats. Outer lines represent the standard error of the regression line. Representative integrated firing rate histogram illustrating the effect of clonidine administration in control (b) and 14-day PPX-treated rats (c). Effect of the α_2 receptor agonist clonidine is reversed by administration of the α_2 receptor antagonist idazoxan in both control and PPX 14-day-treated rats.

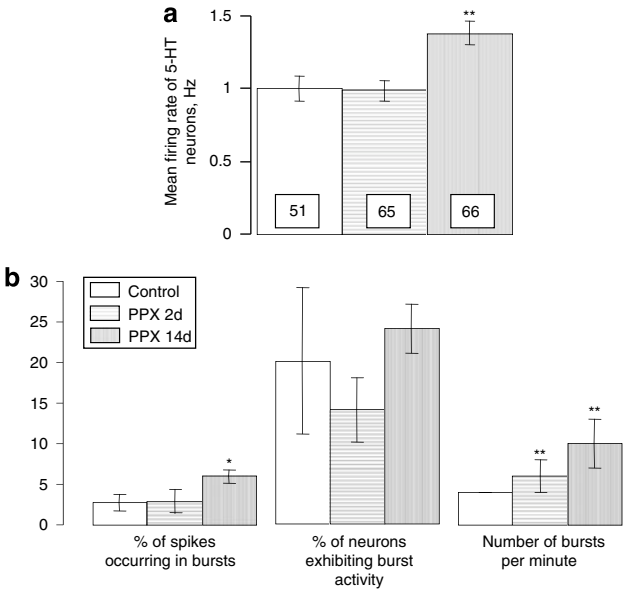

Figure 7 Effect of acute and sustained administration of pramipexole (PPX) on raphe dorsalis serotonin (5-HT) spontaneous firing rate (a) and burst activity (b). (a) The number of neurons recorded in each group is displayed in respective histogram. (b). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded ($n = 51$ in control, $n = 65$ in PPX 2-day-treated rats, and $n = 66$ in PPX 14-day-treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity ($n = 17$ in control, $n = 9$ in PPX 2-day-treated rats, and $n = 16$ in PPX 14-day-treated rats). The data expressed as mean firing rate ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$; *n*, number of neurons, SEM, standard error mean.

A dose-dependent suppression of the 5-HT firing was observed with the administration of LSD in the range of 1–10 μg/kg. In control rats, LSD completely suppressed the firing activity of 5-HT neurons with a dose of 10 μg/kg ($ED_{50} = 5.6 \pm 1.1$ μg/kg; $n = 7$ in seven rats), whereas in rats treated with PPX 1 mg/kg per day for 14 days, the dose required for complete inhibition was 40 μg/kg ($ED_{50} = 15.1 \pm 1.2$ μg/kg; $n = 6$ in six rats; Figure 8).

DISCUSSION

The present electrophysiological study documented the effects of acute and prolonged administration of the D2-like receptor agonist PPX on VTA DA, LC NE, and RD 5-HT neuronal firing. The decrease in the mean spontaneous firing of DA and NE neurons observed after 2-day PPX treatment was no longer present after prolonged administration, although their burst activity remained attenuated. Serotonergic neurons, which did not show any response to acute or subacute administration of PPX, significantly increased their firing rate and burst activity after prolonged treatment.

As expected from the negative feedback action of DA D2-like autoreceptors (localized on the VTA DA neurons) on DA firing (Piercey *et al*, 1996), their acute as well as short-term activation by PPX resulted in a reduction of DA spontaneous discharge rate. As is the case with other DA agonists (Pitts *et al*, 1995), sustained treatment with PPX (which overstimulates somatodendritic D_2/D_3 receptors), led to a decrease in their responsiveness and a subsequent restoration of the mean firing rate of DA neurons. This adaptive change was found to be due to the desensitization of the D2-like autoreceptors. PPX and apomorphine have a similar affinity for the D2-like receptors (Piercey, 1998). To rule out the possibility of competition between these two pharmacological agents at the autoreceptor sites, a 24-h washout period was carried out. This procedure allowed to determine that, even in rats subjected to sustained PPX administration, a complete inhibition of the DA firing activity with DA autoreceptor agonist apomorphine was still possible. Therefore, there was some competition between PPX and apomorphine for the autoreceptor site when the minipumps delivering the drug were present in the rats. Indeed, the dose of apomorphine needed to fully suppress DA firing was nevertheless six times greater than that in control rats, thus clearly indicating desensitization of the autoreceptor. This finding is consistent with previously reported decreased sensitivity and density of D2-like receptors, following chronic administration of the D2-like agonist quinpirole (Pitts *et al*, 1995; Subramaniam *et al*, 1992)

Interestingly, the burst activity of DA neurons was also modulated by PPX administration. The physiological role of the latter mode of firing needs to be emphasized. It has been shown to lead to increased transmitter release for the same number of impulses delivered at regular intervals during the same time period (Gonon, 1988). Chronic PPX treatment resulted in a decrease in the percentage of neurons discharging in bursts. On the other hand, the number of bursts per minute returned to baseline levels, and the overall DA firing rate was accordingly markedly attenuated. The recovery of the DA tonic activity in the presence of the decreased burst-type activity after chronic stimulation of D_2/D_3 receptors by PPX implies a compensation from the single spiking activity of DA neurons. This difference on the two types of DA firing mode might be explained by PPX agonism on both D_2 and D_3 cell body receptors because it was proposed they contribute to tonic and phasic suppression of DA tone, respectively (Millan *et al*, 2000a). PPX, being slightly more potent at D_3 than D_2 receptors, might affect them in different ways, when administered chronically. However, PPX acting on both subtypes of DA autoreceptors makes it difficult to fully differentiate their effects on DA firing.

Acute PPX administration is known to inhibit neuronal firing in the nucleus accumbens, a postsynaptic target of VTA DA neurons (Piercey, 1998). However, the long-term outcome of chronic PPX treatment on postsynaptic neurons has not been examined. It is anticipated that the recovery of firing of DA neurons, despite a 29% reduction of spikes occurring in bursts, may lead to a net enhancement of the DA transmission in these structures because of the presence of PPX, which directly activates postsynaptic neurons. A definite answer will have to come from the assessment of the tonic activation of D_2/D_3 receptors in postsynaptic areas.

The presence of D2-like receptors in the LC was previously put into evidence in a number of studies (Suzuki *et al*, 1998; Yokoyama *et al*, 1994). It has been presumed that DA exerts an inhibitory influence on NE neurons through these receptors. For example, systemic administration

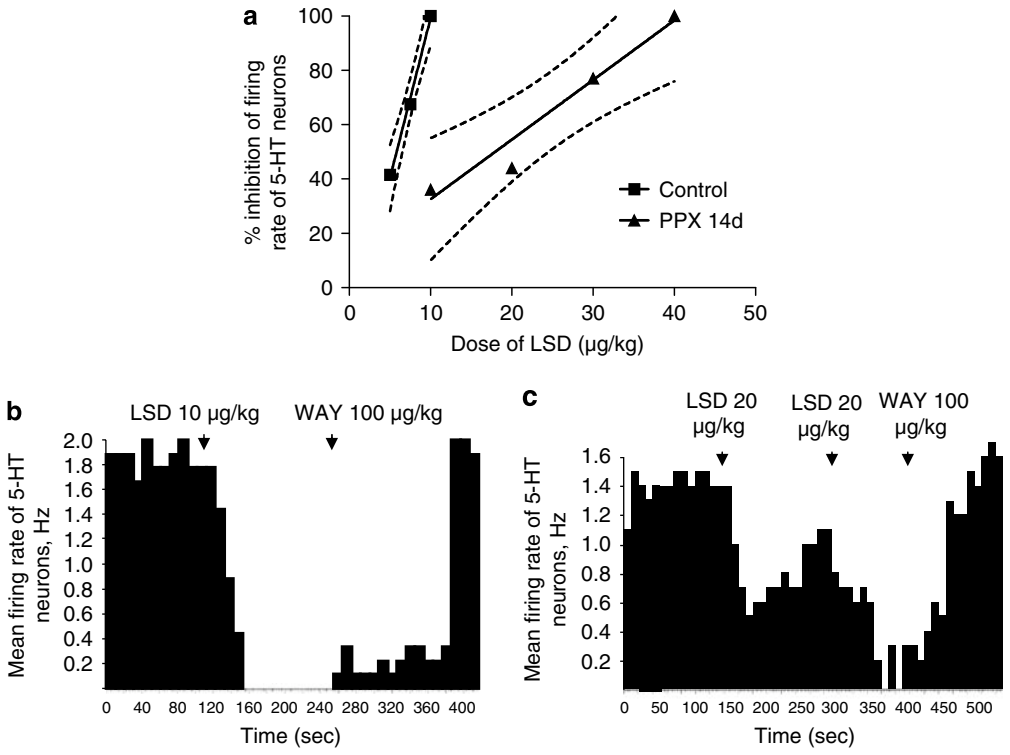

Figure 8 Assessment of the sensitivity of the raphe dorsalis (RD) serotonin (5-HT) 5-HT_{1A} autoreceptor. (a) Relationship between the degree of suppression of RD 5-HT firing activity and doses of 5-HT_{1A} autoreceptor agonist lysergic acid diethylamide (LSD) administered intravenously in control and 14-day PPX-treated rats. Outer lines represent the standard error of the regression line. Representative integrated firing rate histogram illustrating the effect of LSD administration in control (b) and 14-day PPX-treated rats (c). Effect of the 5-HT_{1A} autoreceptor agonist LSD is reversed by administration of the 5-HT_{1A} antagonist WAY in both control and PPX 14-day-treated rats.

of the selective D2-like receptor antagonist haloperidol enhances the spontaneous firing activity of NE neurons in the LC (Piercey, 1994). Moreover, a recent study showed a 47% increase in firing of NE neurons in VTA-lesioned rats (Guiard *et al*, 2008a). Accordingly, in the current study it was found that despite the absence of any effect due to an i.v. bolus, a 2-day PPX regimen did reduce NE firing activity by 30% (Figure 4a).

It is noteworthy that PPX has some affinity for α_2-adrenoreceptors ($K_i = 188$ nM; Piercey *et al*, 1996). It is thus possible, though unlikely, that accumulation of PPX after 2 days of sustained infusion can directly activate α_2-adrenergic autoreceptors. On the other hand, availability of NE is known to be inversely proportional to the levels of endogenous DA (Misu *et al*, 1985). Thus, a short-term PPX treatment resulting in a decreased DA tone causes disinhibition of NE release in the LC. Increased synaptic availability of NE would, therefore, stimulate inhibitory α_2-adrenergic autoreceptors. Consequently it was hypothesized that the initial decrease in firing activity of NE neurons in 2-day PPX-treated rats is due to α_2-adrenoreceptor stimulation. This possibility was supported by the ability of the α_2-adrenergic antagonist idazoxan to increase spontaneous firing to equal levels in controls and rats subacutely given PPX (Figure 5), thus indicating that observed NE inhibition in 2-day-treated rats is mediated solely by α_2-adrenergic receptors.

Interestingly, after chronic PPX treatment, NE neurons regained their normal firing rate. This adaptive change likely occurred due to a decreased responsiveness of the cell body α_2-adrenergic autoreceptor (Figure 6). This modification can be attributed either to the direct effect of PPX on the α_2-adrenoreceptors or, more likely, to activation of the α_2-adrenoreceptors by endogenous NE, levels of which are likely to be increased due to dampened inhibitory influence of the DA neuronal system.

Unlike for the spontaneous firing of NE neurons, their burst-mode discharge did not recover after chronic treatment with PPX, thus suggesting an involvement of modulating factors different from those affecting single-spike firing activity. Even though mechanisms affecting NE burst firing are not fully understood, it may be speculated that an increase in 5-HT neurotransmission, exerting a suppressant action on NE neurons, may prevent burst-mode activity from recovery. For instance, burst firing of NE neurons completely disappears during sustained administration of the potent SSRI escitalopram (Dremencov *et al*, 2007). Another possibility would be that PPX dampens the glutamatergic activation of LC neurons coming from the nucleus paragigantocellularis, which has been shown to drive the activity of NE neurons (Ennis and Aston-Jones, 1988).

Previous reports documented that the firing activity of 5-HT neurons is positively influenced by administration of dopaminergic agonists without direct 5-HT effects (Haj-Dahmane, 2001). On the basis of these data, an increase in the discharge rate of 5-HT neurons in rats acutely treated with PPX was expected. Surprisingly, both

an i.v. bolus injection and a 2-day regimen of PPX failed to alter 5-HT neuronal firing. Nonetheless, after prolonged stimulation of the D2-like receptors by systemic PPX administration, the frequency of 5-HT neuron firing was significantly increased. Considering that other types of drugs that directly or indirectly lead to an enhancement of serotonergic neurotransmission cause a desensitization of somatodendritic 5-HT_{1A} autoreceptor (Haddjeri *et al*, 1998b; Haddjeri and Blier, 2000; Kreiss and Lucki, 1995), it was assumed that similar adaptation could occur in response to chronic PPX administration. Indeed, the observed shift of the LSD dose–response curve (Figure 8a) implies a desensitization of somatodendritic 5-HT_{1A} autoreceptors following prolonged administration of PPX. Taking into consideration that PPX has no affinity for 5-HT_{1A} receptors, such a desensitization probably resulted from an indirect action evoked by PPX. The latter observation is in line with previous *in vivo* and *in vitro* studies suggesting enhancement of the 5-HT tone in response to the stimulation of RD D2-like receptors by various pro-dopaminergic agents (Ferre and Artigas, 1993; Haj-Dahmane, 2001).

Even though mechanisms involved in burst-mode firing of 5-HT neurons are not well established, it is hypothesized that observed elevation of this parameter in response to sustained PPX administration likely results from the activation of D2-like receptors located on 5-HT neurons. This assumption is supported by the fact that both application of DA as well as DA agonists produces a train of action potentials in 5-HT neurons *in vitro* (Aman *et al*, 2007). On the other hand, pharmacological blockade of the 5-HT_{1A} autoreceptors was reported to produce an increase in the number of bursts in the RD 5-HT neurons exhibiting burst-mode activity (Hajós *et al*, 1995). As similar changes in burst firing were observed in rats chronically treated with PPX, desensitization of the 5-HT_{1A} autoreceptors might serve as another possible mechanism responsible for increased 5-HT bursting.

Indeed, the latter mode of discharge activity functionally correlates with increased release of 5-HT (Gartside *et al*, 2000). These observations, in addition to the 38% increase in the mean firing rate of 5-HT neurons, suggest that the long-term administration of PPX should enhance tonic activation of the postsynaptic 5-HT receptors. These alterations of 5-HT neuronal function may be an important contributor to the antidepressant effect of PPX.

Considering the facilitatory effect of chronic PPX administration on DA neurotransmission resulting from a normalized mean firing rate of DA neurons in the presence of sustained activation of postsynaptic D2-like receptors by PPX, it can be hypothesized that drugs possessing pro-dopaminergic properties act through both the DA and the 5-HT systems. In conclusion, the present study provided possible mechanism(s) of action of PPX, which likely underlies its clinical effectiveness in the treatment of depression. These results also serve as yet another line of evidence for the central involvement of reciprocal interactions between the monoaminergic systems involved in the pathophysiology and/or therapeutics of depression.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that the present study was fully funded by the CIHR grant to PB. Beside this PB has financial involvements with these companies. OC and ME have no financial involvements to disclose.

1	Biovail	C
2	Cyberonics	C, G
3	Eli Lilly & Company	C, SB
4	Forest Laboratories	CE
5	Janssen Pharmaceuticals	C, SB, CE, G
6	Lundbeck	G, C, SB
7	Organon Pharmaceuticals	C, SB, G
8	Sepracor	C, G
9	Wyeth Ayerst	C, SB, G, CE
10	Sanofi-Aventis	C
11	AstraZeneca	G, SB
12	Takeda	C
13	Novartis	C
14	Shire	C

C = Consultant.
SB = Speaker's Bureau.
G = Grant Funding.
CE = Contract employee.
The above companies have no relevance to the work covered in the submission.

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