Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions

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Abstract

Anatomical studies have established the existence of reciprocal relationships between the main population of monoamine, serotonin (5-HT), norepinephrine (NE) and dopamine (DA) neurons in the brain. The present study was thus conducted to examine the firing activity of 5-HT and NE neurons in DA-depleted rats, as well as the firing activity of DA neurons in 5-HT- or NE-depleted rats. The selective lesion of DA neurons elicited by 6-hydroxydopamine (6-OHDA) decreased the spontaneous firing activity of dorsal raphe (DR) nucleus 5-HT neurons by 60%, thus revealing the excitatory effect of the DA input on these 5-HT neurons. In contrast, the selective lesion of 5-HT neurons produced by 5,7-dihydroxytryptamine (5,7-DHT) enhanced by 36% the firing activity of VTA DA neurons, thereby indicating an inhibitory effect of the 5-HT input on these DA neurons. With regard to the reciprocal interaction between DA and NE neurons, it was observed that the selective loss of DA neurons achieved by the intra-ventral tegmental area (VTA) injection of 6-OHDA increased the firing activity of a subset of locus coeruleus (LC) NE neurons by 47%. The selective loss of NE neurons in response to the intra-LC injection of 6-OHDA enhanced the firing activity of VTA DA neurons by 70%, demonstrating a net inhibitory role of the NE input on VTA DA neurons. These findings have important consequences for antidepressant treatments aimed at enhancing simultaneously 5-HT, NE and DA transmission. Indeed, based on the understanding of such interactions, it may be possible to develop strategies to improve the effectiveness of antidepressant drugs by preventing counter-productive negative feedback actions.

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Introduction

There are reciprocal projections between the major groups of serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine (NE) neurons in the brain (Aston-Jones et al., 1991; Kaehler et al., 1999). The physiological importance of such connections is evidenced by alterations in neuronal activity in lesion experiments. When 5-HT neurons are lesioned, the firing rate of locus coeruleus (LC) NE neurons is enhanced in a sustained fashion by about 70%, as is the case with 5-HT synthesis inhibition (Dremencov et al., 2007; Haddjeri et al., 1997; Reader et al., 1986). When NE neurons are lesioned, dorsal raphe (DR) 5-HT neurons discharge erratically at a low rate, but only for the first few days (Svensson et al., 1975). Although the loss of brain monoamine neurons does not necessarily reflect the pathophysiology of mood disorders, such an experimental approach can be initially used to establish the net excitatory and/or inhibitory nature of a specific neurotransmitter at post-synaptic level. As an example of the clinical relevance of monoaminergic projections, selective 5-HT reuptake inhibition produces a marked inhibition of the spontaneous firing rate of LC NE neurons (Dremencov et al., 2007; Seager et al., 2004, 2005; Szabo et al., 2000). Low doses of atypical antipsychotics, which are now recognized as an effective
augmentation strategy in non-psychotic selective serotonin reuptake inhibitor (SSRI)-resistant depressed patients, reverse this inhibitory action via blockade of 5-HT1A receptors (Berman et al., 2007; Dremencov et al., 2007, Gharabawi et al., 2006a,b; Rapaport et al., 2006; Shelton et al., 2005).

It is well documented that dopamine (DA) neurons of the ventral tegmental area (VTA), giving rise to the mesolimbic/cortical DA system, send projections to the DR (Kalén et al., 1988) and the LC (Beckstead et al., 1979), while in turn, receiving important inputs from the latter nuclei (Hervé et al., 1987). It therefore appears crucial to examine the reciprocal interactions of these three types of neurons to understand the effects of medications acting on monoaminergic systems. In particular, there is growing interest for DA in the field of mood disorders, since drugs that enhance its transmission are clinically effective on their own. For example, the selective D3/D4 agonist pramipexole, customarily used in the treatment of Parkinson’s disease (PD), was shown to be effective in depression as a monotherapy (Barone et al., 2006; Corrigan et al., 2000), as well as an augmentation strategy for SSRI-resistant patients (Goldberg et al., 2004; Lattanzi et al., 2002). Conversely, degeneration of DA neurons in PD patients typically leads to anhedonia and loss of motivation, two symptoms frequently associated with depression (Harro and Oreland, 2001). More importantly, the prevalence of depression can reach 50% in PD patients (McDonald et al., 2003). Taken together, these observations suggest that an attenuation of DA transmission could participate in the pathogenesis of mood disorders, possibly in part through interactions with the 5-HT and/or the NE system(s).

There is consistent evidence regarding the dopaminergic regulation of DR 5-HT neurons. Infusion of the DA agonist apomorphine in the rat DR stimulates the firing rate of 5-HT neurons and the local release of 5-HT, while these effects are partially prevented by the selective D2 receptor antagonist raclopride (Ferre and Artigas, 1993; Martin-Ruiz et al., 2001). The hypothesis that DA interacts with 5-HT neurons, mainly through activation of D2 receptors is also supported by the depolarizing action of quinpirole, and its blockade with the D2 receptor antagonist haloperidol, in rat 5-HT neurons recorded in vitro (Aman et al., 2006; Haj-Dahmane, 2001). The exact nature of the effect of 5-HT on VTA DA neuron activity remains unclear, in that both inhibitory and excitatory roles for 5-HT have been observed. Acute intravenous administration of SSRIs, which probably enhances extracellular 5-HT levels in the VTA, induces a small decrease in the firing rate of VTA DA neurons (Di Mascio et al., 1998; Prisco and Esposito, 1995). However, electrical stimulation of the DR produces two different types of response in the VTA. Some DA neurons exhibit an inhibition-excitation response while others show an initial excitation followed by an inhibition (Gervais and Roulard, 2000).

Descending pathways from the VTA also innervate the LC (Ornstein et al., 1987). In-vivo recordings showed that direct iontophoretic application of DA in the LC of anaesthetized rats, suppresses the firing activity of NE neurons (Elam et al., 1986), while systemic injection of the selective D2 antagonist haloperidol enhances it (Piercey et al., 1994). In turn, functional studies indicate that LC NE neurons modulate DA neurons of the VTA. For instance, the electrical stimulation of the LC as well as the systemic administration of the selective NE reuptake inhibitor reboxetine, both increase NE levels in the VTA, producing excitation of DA neurons (Grenhoff et al., 1993; Linner et al., 2001). In contrast, the local application of NE in the VTA was shown to inhibit the electrical activity of DA neurons (Aghajanian and Bunney, 1977; Grenhoff et al., 1995; White and Wang, 1984).

In order to further elucidate the interactions between DA, 5-HT and NE neurons, the firing activity of 5-HT and NE neurons was examined in DA-depleted rats, as well as the firing activity of DA neurons in 5-HT- or NE-depleted rats.

Material and methods

Animals

Male Sprague–Dawley rats (Charles River, St Constant, QC, Canada) weighing 250–300 g, were used for the experiments. They were housed individually and kept under standard laboratory conditions (12:12 h light/dark cycle with free access to food and water). All animals were handled according to the guidelines of the Canadian Council on Animal Care (CCAC) and protocols in this study were approved by the local Animal Care Committee (Ottawa Health Research Institute, Ottawa, ON, Canada).

Neurotoxic lesions

Rats were anaesthetized with a mixture 1:1 by volume of xylazine (20 mg/ml) and ketamine (100 mg/ml) and placed into a stereotaxic frame with atraumatic ear bars. To study interactions between 5-HT and DA neurons, rats were administered intracerebroventricularly (i.c.v., unilateral) with 5,7-dihydroxytryptamine (5,7-DHT: 200 μg free base in 10 μl of 0.9% NaCl and 0.1% ascorbic acid) or 6-hydroxydopamine...
In-vivo electrophysiological recordings

Ten days after the injection of the neurotoxins, rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed into a stereotaxic frame. The extracellular recordings of the 5-HT, DA and NE neurons in the DR, VTA and LC, were performed using single-barrelled glass micropipettes (R&D Scientific Glass, Spencerville, MD, USA) preloaded with a 2 M NaCl solution. Their impedance typically ranged between 4–7 MΩ.

Recording of DR 5-HT neurons

The single-barrelled glass micropipettes were positioned using the following coordinates (in mm from bregma): AP −0.9, L 1.5, V 3.7 were used to reach the lateral ventricle. The flow rate injection was 1 μl/min and after completion of the i.c.v. infusion of neurotoxins or vehicle, the syringe was left in place for 15 min to allow sufficient diffusion before its withdrawal. One hour before the i.c.v. injection, animals lesioned with 5,7-DHT were pre-treated with the selective NE reuptake inhibitor desipramine (25 mg/kg i.p.) and the selective DA reuptake inhibitor GBR12909 (25 mg/kg i.p.) to prevent loss of NE and DA neurons, respectively. Those lesioned with 6-OHDA were pre-treated with desipramine (25 mg/kg i.p.) and the SSRI fluoxetine (10 mg/kg i.p.) to prevent loss of NE and 5-HT neurons. Control rats (sham-operated) were subjected to the same procedure and received the corresponding pre-treatments 1 h before the unilateral injection of 10 μl vehicle (0.1% ascorbic acid).

To study interactions between central NE and DA neurons, rats received a bilateral injection of 6-OHDA (5 μg free base in 0.5 μl of 0.9% NaCl and 0.1% ascorbic acid) into the LC or VTA to limit the diffusion of the neurotoxin throughout the brain and consequently produce a more selective deafferentation (Reader, 1982). This is of particular interest since intracerebral injection of 6-OHDA may deplete both NE and DA levels (Reader and Gauthier, 1984). The following coordinates were used: AP −1.1, L 1.1, V 5.5 for the LC (in mm from lambda) and AP −5.8, L 0.7, V 8.5 for the VTA (in mm from bregma). Rats that received intra-LC 6-OHDA were pre-treated, 1 h before, with fluoxetine (10 mg/kg i.p.) and GBR12909 (25 mg/kg i.p.) and those that received intra-VTA 6-OHDA, were administered fluoxetine (10 mg/kg i.p.) and desipramine (25 mg/kg i.p.). It is noteworthy that intracerebral administration of 6-OHDA was reported to be more effective in depleting NE than the systemic treatment with DSP4 (Lookingland et al., 1986).

Firing rate and burst analysis

The firing patterns of DA and NE neurons (both displaying a bursting activity) were analysed by spike-interval 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 inter-spike interval shorter than 0.08 s. The termination of bursts was defined as an inter-spike interval (ISI) of ≥0.16 s. The detailed analysis of ISI for

Recording of VTA DA neurons

The single-barrelled glass micropipettes were positioned using the following coordinates (in mm from Bregma): AP −6 to −5.4, L 1 to 0.6, V 7–9. 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). 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).

Recording of LC NE neurons

The single-barrelled glass micropipettes were positioned using the following coordinates (in mm from lambda): 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 excitatory response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982). The compression lasted ~1 s with equal pressure being applied to the paw of rats; once the opposite sides of the forceps made contact with each other, the forceps were then released. Of interest, it has also been reported that the number of elicited bursts is largely independent of paw-compression intensity.

Interactions between monoaminergic neurons
Electrophysiological data were expressed as mean ± S.E.M of the firing rate, number of single spikes, number of bursts and single spikes per burst. Statistical comparisons among DR, VTA and LC of sham-operated and lesioned rats were performed using two-tailed Student’s t tests. The means (number ± S.E.M) of neurons recorded per track in sham-operated and lesioned rats were also compared using a two-tailed Student’s t test. For the lesioning studies, each neurotransmitter peak from the HPLC was converted into values representing ng/mg wet weight tissue based on external neurotransmitter standards of that day. A Student’s t test was used to analyse between-group differences.

Biochemical analysis of brain monoamine levels

The effectiveness and selectivity of the neurochemical lesions was confirmed by measuring 5-HT, NE and DA concentrations at specific brain sites. The frontal cortex and striatum (including the nucleus accumbens) were chosen to determine the extent of 5-HT and DA depletion, respectively, as preferential serotonergic and dopaminergic projections from the DR and the VTA. The hippocampus was selected to examine NE concentration since it has been repeatedly shown to have high levels of this monoamine (Dailly et al., 2006). Immediately after electrophysiological experiments sham-operated rats and lesioned rats were sacrificed, the brain removed and stored at −80 °C. The frontal cortex, hippocampus and striatum were dissected as previously described (Chenu et al., 2006). Each separate brain area was placed in an Eppendorf tube with 500 μl of 0.1 M perchloric acid (containing 1 ng of an internal standard, dihydroxybenzylamine), homogenized using ultrasound and centrifuged at 8000 g for 15 min. The supernatant was analysed for monoamine content using high-performance liquid chromatography analysis (HPLC).

Statistical analysis

Effect of DA neuron lesion on the firing activity of 5-HT neurons in the DR

The mean number of DR 5-HT neurons recorded per track was not significantly different between sham-operated (n=24 tracks) and DA neuron-lesioned rats (n=22 tracks, Table 2). The mean firing frequency of DR 5-HT neurons in DA neuron-lesioned rats was significantly decreased by 60% compared to sham-operated rats (Figure 1).

Results

Neurochemical analyses of the neurotoxic lesions

Rats treated with the i.c.v. injection of 6-OHDA displayed a 70% reduction of DA levels in the striatum (Table 1a). In the DA neuron-lesioned rats, no changes in 5-HT levels were detected in the frontal cortex (Table 1a), the hippocampus and the striatum (data not shown) compared to sham-operated rats. Moreover, no changes in NE levels were reported in these post-synaptic structures (Table 1a, and data not shown) with the exception of the frontal cortex (0.13 ± 0.02 ng/mg vs. 0.22 ± 0.01 ng/mg in lesioned and sham-operated rats, respectively; p < 0.05), suggesting that desipramine does not effectively protect NE terminals within the latter region. The lesion of 5-HT neurons induced by the i.c.v. injection of 5,7-DHT significantly reduced the levels of 5-HT in the frontal cortex by 87%. The selectivity of the 5,7-DHT lesion was confirmed from the observations that the concentrations of NE and DA were not different between lesioned and sham-operated rats in the frontal cortex, hippocampus and striatum (Table 1a, and data not shown).

The lesion of VTA DA neurons elicited by local injection of 6-OHDA produced a significant reduction in DA levels (48%) in the striatum (Table 1b). Interestingly, the depletion of VTA DA neurons produced a similar degree of depletion (50%) in the frontal cortex of lesioned rats compared to sham-operated rats (0.08 ± 0.02 ng/mg vs. 0.19 ± 0.03 ng/mg; p < 0.01). 5-HT and NE levels were unchanged in the frontal cortex, hippocampus and striatum of rats that received the intra-VTA injection of 6-OHDA with respect to the sham-operated animals (Table 1b, and data not shown). The lesion of LC NE neurons achieved by local injection of 6-OHDA significantly decreased the level of NE in the hippocampus by 66% (Table 1b). 5-HT and DA levels were not different between sham-operated and lesioned rats in the frontal cortex, hippocampus and striatum (Table 1b, and data not shown).

Dopaminergic-serotonergic interactions

Effect of DA neuron lesion on the firing activity of 5-HT neurons in the DR

The mean number of DR 5-HT neurons recorded per track was not significantly different between sham-operated (n=24 tracks) and DA neuron-lesioned rats (n=22 tracks, Table 2). The mean firing frequency of DR 5-HT neurons in DA neuron-lesioned rats was significantly decreased by 60% compared to sham-operated rats (Figure 1).
The mean number of VTA DA neurons recorded per track was not significantly different between sham-operated (n = 49 tracks) and 5-HT neuron-lesioned rats (n = 61 tracks, Table 2). In rats with their 5-HT neurons lesioned, the mean firing frequency of VTA DA neurons was significantly increased by 36% compared to sham-operated rats (Figure 2c). In order
to determine whether this increase was due to an alteration of single spike and/or burst activity, a more detailed analysis was performed. The mean number of single spikes/min did not quite reach the pre-determined level of statistical significance in depleted rats compared to sham-operated rats (Figure 2d). However, the number of bursts/min and of single spikes per burst was significantly increased in rats with 5-HT neurons lesioned (Figure 2e, f).

Dopaminergic-noradrenergic interactions

Effect of DA neuron lesion on the firing activity of NE neurons in the LC

The mean number of NE neurons recorded per track was significantly higher in sham-operated rats \( (n = 10) \) than in VTA-lesioned rats \( (n = 36) \) (Table 2). The mean firing frequency of all spontaneously active LC NE neurons was significantly increased by 47% in VTA-lesioned rats compared to sham-operated rats (Table 3).

As previously reported in drug-naive rats (Dremencov et al., 2007), it was found that 77% of NE cells discharged only in single-spike mode while the rest displayed bursting activity. These percentages were not affected by the lesion of VTA DA neurons since 71% and 29% of LC NE neurons exhibited a single spike and bursting activity, respectively. The
mean firing frequency of NE cells discharging with a single spike pattern, was significantly increased by 33% in VTA DA neuron-lesioned rats compared to sham-operated rats (Figure 3c). In addition, among the LC NE cells displaying a bursting activity, the mean firing frequency of LC NE neurons was increased by 59% in VTA DA neuron-lesioned rats compared to sham-operated (Figure 4c). No significant difference was detected between both groups of rats in the mean number of single spikes/min; while the number of bursts/min and single spikes per burst were significantly increased in VTA-lesioned rats (Figure 4e, f).

In addition, it has been observed that the percentage of neurons displaying a sensory-evoked burst firing is significantly increased in VTA DA neuron-lesioned rats compared to sham-operated rats, while no differences were detected in the number of spikes per burst between both groups (Table 3).

<table>
<thead>
<tr>
<th>NE neurons recorded</th>
<th>NE neurons displaying a burst during the pinch</th>
<th>NE neurons displaying a burst during the pinch (%)</th>
<th>Number of spikes/burst</th>
<th>Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 47</td>
<td>15</td>
<td>31 ± 2</td>
<td>4.5</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>VTA DA-lesioned 24</td>
<td>15</td>
<td>63 ± 8*</td>
<td>5.6</td>
<td>3.1 ± 0.4**</td>
</tr>
</tbody>
</table>

VTA, Ventral tegmental area; LC, locus coeruleus. *p < 0.05 and **p < 0.01, relative the sham-operated group of rats.

Discussion
The present electrophysiological study showed that lesioning 5-HT and NE neurons increased the firing activity of VTA DA neurons. Conversely, lesioning DA neurons decreased DR 5-HT neuronal firing but increased that of LC NE neurons.

Reciprocal interactions between DA and 5-HT neurons
The lesion of DA neurons by i.c.v. administration of 6-OHDA produced a profound and selective decrease in brain DA levels (Table 1). In these experimental conditions, the discharge rate of DR 5-HT neurons was reduced by 60% indicating that DA input exerts a tonic excitatory effect on 5-HT neurons in intact brain. It may seem unusual that despite the marked attenuation of DR 5-HT neuronal firing in lesioned rats, the number of DR 5-HT neurons recorded per track was unchanged. However, previous electrophysiological data indicate that conditions that decrease the firing activity of DR 5-HT neurons by about 50% do not necessarily modify the number of neurons found per track (Blier et al., 1986). These findings are consistent with previous in-vivo electrophysiological and neurochemical studies having shown that the systemic administration of the non-selective DA receptor agonist apomorphine, increases the firing rate of 5-HT neurons (Martin-Ruiz et al., 2001), thereby enhancing 5-HT outflow in the rat DR (Ferre et al., 1994; Ferre and
Artigas, 1993; Martin-Ruiz et al., 2001). Likewise, it has been shown by intracellular recordings that both application of DA and the D<sub>2</sub>/D<sub>3</sub> agonist quinpirole in DR slices produces a concentration-dependent membrane depolarization of 5-HT neurons (Haj-Dahmane, 2001). These D<sub>2</sub>-like receptors are probably located on the 5-HT neurons themselves, because the effects of quinpirole were unaffected by tetrodotoxin (which blocks neuronal conduction; Haj-Dahmane, 2001). Furthermore, the pharmacological inactivation of ionotrophic and metabotropic glutamate receptors by the selective antagonists 6,7-dinitroquinoxaline-2,3-dione and 2-amino-5-phosphonopentanoic acid, did not prevent the DA-induced depolarization of DR 5-HT membrane (Haj-Dahmane, 2001) ruling out the possibility that the excitatory effects of DA involved a local release of glutamate. It is well known that the DR is driven by a...
noradrenergic input; therefore a lesion of DA neurons might alter 5-HT neuronal activity indirectly via its interactions with NE neurons. The possibility that the i.c.v. injection of 6-OHDA destroyed NE terminals in the DR should be considered since a reduction of NE levels has been detected in the hippocampus (Table 1, n.s.) and the frontal cortex (data not shown, p < 0.05) in lesioned rats. However, Svensson et al. (1975) demonstrated that the spontaneous firing rate of DR 5-HT neurons after 6-OHDA is unaltered suggesting that a putative loss of NE would not have a sustained impact on 5-HT neuronal activity. In conclusion, these results indicate that the DA input exerts a direct excitatory effect, probably via D2 receptors on DR 5-HT neurons while the influence of other non-dopaminergic afferents is limited.

The i.c.v. injection of 5,7-DHT produced a robust and selective decrease in brain 5-HT levels (Table 1) leading to a 36% increase in the discharge rate of VTA DA neurons. This enhancement of DA neuronal activity resulted from a higher number of bursts and spikes per burst. The putative inhibitory effect of the 5-HT input on DA neurons suggested by the present study is consistent with the finding that SSRIs, which probably raise extracellular 5-HT levels in the VTA, induce a slight decrease in the firing rate of VTA DA neurons (Di Mascio et al., 1998; Prisco and Esposito, 1995). The inhibitory influence of the 5-HT input on DA neurons was further supported by the observation that low doses of the 5-HT1A receptor agonist 8-OHDPAT, known to attenuate the electrical activity of DR 5-HT neurons, increases the firing rate and the number of burst discharge of DA neurons in the VTA (Arborelius et al., 1993; Lejeune and Millan, 1998, 2000; Lejeune et al., 1997), and consequently DA release at the somatodendritic (Chen and Reith, 1995) and terminal levels (Ago et al., 2002; Arborelius et al., 1993; Rasmussen et al., 1994; Tanda et al., 1994). However, high doses of 8-OHDPAT which preferentially activate post-synaptic receptors, also produce increases in the discharge of DA neurons (Lejeune and Millan, 1998). Consequently, the inhibitory effect of the 5-HT input on VTA DA neurons remains debatable. Indeed, initial intracellular recordings showed that 5-HT depolarized 46% of rat VTA DA neurons (Pessia et al., 1994) and stimulated the release of [3H]DA in VTA slices (Beart and McDonald, 1982). In line with these results, microinfusion of 5-HT in the VTA increased DA release in projection areas such as the nucleus accumbens (Guan and McBride, 1989). It was also observed that the electrical stimulation of the DR produces two different types of response in the VTA: some cells exhibit an inhibition-excitation response while other DA neurons show an excitation followed by an inhibition (Gervais and Rouillard, 2000). These results raised the possibility that the regulation of VTA DA neurons involves various post-synaptic 5-HT receptors. The 5-HT1A and 5-HT2C receptor subtypes identified in the VTA (Cornea-Feibert et al., 1999; Nocjar et al., 2002) are of particular interest since their pharmacological activation respectively stimulates and suppresses, VTA DA neuronal activity (Di Giovanni et al., 2000; Di Matteo et al., 2000; Gobert et al., 2000; Millan et al., 2000; Pessia et al., 1994; Prisco et al., 1994) and DA release in the nucleus accumbens.
(De Deurwaerdere et al., 2004; Porras et al., 2002). Overall, it would thus seem that the inhibitory influence of 5-HT input plays a predominant role in the regulation of DA neuronal activity given that the systemic administration of the non-selective 5-HT$_2$ receptor antagonist ritanserin dose-dependently increases the burst firing and the firing rate of VTA DA neurons (Ugedo et al., 1989). Importantly, the lesion of 5-HT neurons might have disrupted more than the serotonergic inputs to the VTA. Growing evidence suggests that feedback loops involving the 5-HT system may control VTA DA neuronal activity. For example, it was proposed that the activation of 5-HT$_1A$ and 5-HT$_2A$ receptor subtypes in the medial prefrontal cortex produces an excitation of VTA DA neurons (Bortolozzi et al., 2005; Diaz-Mataix et al., 2005, 2006). However, in contrast to the present study, a lower tonic stimulation of these pathways in 5-HT-depleted rats should have produced an attenuation of the VTA DA neurons’ firing activity. It may also be claimed that the excitatory effect of the DR 5-HT lesion on VTA DA neurons was indirectly mediated by NE neurons. Indeed, it is well established that NE neurons send projections to the VTA and that a lesion of 5-HT neurons increases the firing activity of the LC NE neurons (Haddjeri et al., 1997). This is, however, unlikely since the present study suggests that NE exerts a robust inhibitory action on VTA DA neurons (Figure 5).

**Reciprocal interactions between DA and NE neurons**

The lesion of VTA DA neurons obtained with local injection of 6-OHDA resulted in a significant and selective reduction of striatal DA levels (Table 1). This selective lesion also results in a 47% increase in the discharge rate of LC NE neurons suggesting that DA exerts an inhibitory action on these NE neurons. Although the majority of LC NE neurons discharge spontaneously in a single-spike mode (Dawe et al., 2001; Dremencov et al., 2007), about 30% of NE neurons exhibit both single and burst patterns of spontaneous firing. In the later population of NE neurons, VTA lesion also elevated the mean discharge rate and the mean discharge rate as the result of a significant increase in the number of bursts and spikes per burst. The putative inhibitory influence of DA input upon LC NE neurons is in line with the observation that direct iontophoretic application of DA suppressing the firing rate of NE neurons (Elam et al., 1986). Similarly, in-vivo extracellular recordings have demonstrated an involvement of D$_2$ receptors in this inhibitory effect, since the systemic administration of antipsychotic drugs, including the D$_2$ receptor antagonist haloperidol, increased LC NE neuronal activity (Nilsson et al., 2005; Piercey et al., 1994). An involvement of 5-HT neurons in the disinhibitory effect of DA neuron lesion on NE neuronal firing should also be considered. Indeed, the decrease in DR 5-HT neuronal activity in response to the VTA lesion could lead to a significant reduction of 5-HT release in the LC, thus contributing to the increase of NE neuronal firing through a lower tonic activation of 5-HT$_{1A}$ receptors (Szabo and Blier, 2002). Such an indirect mechanism, as well as a removal of the direct inhibitory effect of DA in the LC, could explain the present findings that the VTA lesion increased the firing activity of some NE neurons. Despite the elevated discharge of LC NE neurons observed in VTA-lesioned rats, the number of NE neurons recorded was significantly reduced. Since, a partial loss of NE neurons was unlikely on the basis of the neurochemical analysis (Table 1), the possibility that NE neurons were tonically inhibited by a local increase of NE levels was considered. In VTA-lesioned rats, the systemic administration of the $\alpha_2$-adrenoreceptor antagonist idazoxan did not allow the recording of more NE neurons. It thus seems possible that DA could also impinge on LC afferents, such as glutamatergic neurons (Nilsson et al., 2005) that could indirectly participate in activating, at least in part, a subpopulation of LC NE neurons. In line with this hypothesis, it was reported that the stimulation of the VTA can produce an activation of NE neuronal activity (Deutch et al., 1986). It should also be mentioned that the intra-VTA injection of 6-OHDA could have destroyed, at least partly, NE terminals in the VTA. Although, this possibility cannot be completely excluded, it is noteworthy that no reductions of the NE levels were detected either in the hippocampus (Table 1) or in the frontal cortex or striatum of lesioned rats (data not shown). Consequently, the present findings suggest that DA exerts a direct inhibitory action on some LC NE neurons through D$_2$ receptors as well as an indirect activation involving other neuronal system(s).

The lesion of LC induced by the local injection of 6-OHDA resulted in a significant and selective reduction of brain NE levels (Table 1). This lesion increased the discharge rate of VTA DA neurons by 70% owing to a significant higher number of bursts and action potentials per burst. This finding was consistent with those of earlier studies having shown that the systemic administration of low doses of the $\alpha_2$-adrenoreceptor agonist clonidine, which attenuates overall NE transmission (Haddjeri and Blier, 2000; Haddjeri et al., 1998; Szabo and Blier, 2001), also increases the firing activity of VTA DA neurons.
Taken together, these data strongly suggest that NE inputs exert an inhibitory influence on spontaneous VTA DA neuronal activity. In line with this assumption, initial electrophysiological studies have demonstrated that the microiontophoretic application of NE in the VTA reduces the firing of DA neurons while this effect is blocked by the non-selective $\alpha_2$-adrenoceptor antagonist piperoxane (White and Wang, 1984). Thus far, it has been shown that the stimulation of $\alpha_1$-adrenoceptors exerts a direct excitatory influence on VTA DA neurons and an indirect inhibitory effect by activating GABA interneurons (Grenhoff et al., 1995; Steffensen et al., 1998). Thus, an attenuation of GABA release in the VTA, could support the observation reported herein that DA neuronal activity is enhanced in LC-lesioned rats. With regard to $\alpha_2$-adrenoceptors, it has been demonstrated that the local application of clonidine in the VTA does not inhibit DA neurons (Aghajanian and Bunney, 1977) ruling out the possibility that the inhibitory effects of NE involved post-synaptic $\alpha_2$-adrenoceptors. However, divergent results have also been reported. For example, the systemic administration of idazoxan or selective NE reuptake inhibitors, which raise extracellular NE levels in the VTA has been shown to increase the burst firing activity of DA neurons in the VTA (Grenhoff and Svensson, 1989, 1993; Linner et al., 2001; Shi et al., 2000). Clearly, further studies are needed to determine the complex mechanism by which the NE input regulates VTA DA neuronal activity. In particular, it would be relevant to address the issue of the selectivity of NE since the inhibitory effect of NE was found to be prevented by the iontophoretic application of the $D_2$ receptor antagonist sulpiride (White and Wang, 1984). Indirect mechanisms might also be involved in the effect of LC NE neuron depletion on VTA DA neuronal activity. The laterodorsal tegmentum (LDT), an adjacent region to the LC that projects heavily to the VTA (Forster and Blaha, 2000; Oakman et al., 1995; Omelchenko and Sesack, 2005) is mainly concerned. Indeed, inputs to the VTA that arrive from the LDT contain cholinergic, glutamate and GABAergic components which have the ability to regulate not only DA neuron population activity but also their burst firing (Lodge and Grace, 2006, Maskos et al., 2005). Interestingly, evidence indicates that NE excites cholinergic and non-cholinergic neurons in the LDT (Kohlmeier and Reiner, 1999; Koyama and Sakai, 2000) suggesting that an attenuation of NE transmission in the LDT may affect the activity of VTA. As cholinergic and glutamatergic neurons exert excitatory effects on VTA DA neurons, it seems highly unlikely that the decrease in burst firing activity in LC NE-lesioned rats altered those pathways. However, it is more conceivable that the depletion of NE reduces the electrical activity of LDT GABAergic neurons projecting to the VTA. These hypotheses will be of interest to address in future investigations. Nevertheless, so far a direct attenuation of post-synaptic $D_2$ receptor stimulation seems a more plausible hypothesis to explain the increase in DA neuronal activity observed in LC-lesioned rats.

The present lesion experiments emphasize the complex regulation of 5-HT, NE and DA neuronal firing activity. Further electrophysiological studies in combination with local or systemic administration of pharmacological agents will be useful in clarifying the pharmacological bases for these interactions in vivo. Indeed, a better knowledge of such interactions could provide important information for improving the treatment of depression, more specifically for pharmacotherapies aimed at enhancing simultaneously DA, NE and 5-HT transmission without triggering counterproductive negative feedback actions. It is noteworthy that the most of the traditional antidepressant drugs tend to increase the endogenous monoaminergic tone whereas the present study is based on the effect of the removal of this tone. However, those lesioning experiments may initiate novel treatment approaches whose clinical utility remains to be demonstrated.

**Note**

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org).

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**Statement of Interest**

None.

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