# Impact of substance P receptor antagonism on the serotonin and norepinephrine systems: relevance to the antidepressant/anxiolytic response

**Pierre Blier, MD, PhD; Gabriella Gobbi, MD, PhD; Nasser Haddjeri, PhD; Luca Santarelli, MD; Gina Mathew, BSc; René Hen, PhD**

Blier — University of Ottawa Institute of Mental Health Research, Ottawa, Ont.; Gobbi — Department of Psychiatry, McGill University, Montréal, Que.; Haddjeri — Université Claude Bernard, Lyons, France; Santarelli, Hen — Department of Psychiatry and Pharmacology, Columbia University, NY; Mathew — Department of Psychiatry and Neuroscience, University of Florida, Gainesville, Fla.

Substance P (neurokinin-1 [NK1]) receptor antagonists appear to be effective antidepressant and anxiolytic agents, as indicated in 3 double-blind clinical trials. In laboratory animals, they promptly attenuate the responsiveness of serotonin (5-hydroxytryptamine [5-HT]) and norepinephrine (NE) neurons to agonists of their cell-body autoreceptors, as is the case for some antidepressant drugs that are currently in clinical use. Long-term, but not subacute, antagonism of NK1 receptors in rats increases 5-HT transmission in the hippocampus, a property common to all antidepressant treatments tested thus far. This enhancement seems to be mediated by a time-dependent increase in the firing rate of 5-HT neurons. Mice with the NK1 receptor deleted from their genetic code also have an increased firing rate of 5-HT neurons. Taken together, these observations strongly suggest that NK1 antagonists could become a new class of antidepressant and anxiolytic agents.

Les antagonistes des récepteurs de la substance P (neurokinine-1 [NK1]) semblent efficaces comme antidépresseurs et anxiolytiques, ainsi que l'indiquent trois études cliniques à double insu. Chez des animaux de laboratoire, ces agents atténuent rapidement la réponse des neurones à sérotonine (5-hydroxytryptamine [5-HT]) et à norépinéphrine (NE) aux agonistes de leurs autorécepteurs du corps cellulaire, comme dans le cas de certains antidépresseurs actuellement utilisés en clinique. L'antagonisme à long terme mais non subaigu des récepteurs de la NK1 chez le rat accroît la transmission de la 5-HT dans l'hippocampe, caractéristique commune à tous les traitements aux antidépresseurs étudiés à ce jour. Une augmentation de la fréquence de décharge des neurones de la 5-HT liée au temps semble intervenir dans cette élévation. Les souris dont on a éliminé le récepteur de la NK1 du code génétique ont aussi une fréquence de décharge plus élevée des neurones de la 5-HT. Globalement, ces observations indiquent fortement que les antagonistes de la NK1 pourraient devenir une nouvelle catégorie d'antidépresseurs et d'anxiolytiques.

## Introduction

There are several families of antidepressant drugs available to treat major depression. When used as monotherapy, they produce remission rates below 50%.[1,2] In treatment-resistant cases, the combined use of 2 agents with different mechanisms of action can improve this success rate significantly, especially

**Correspondence to: Dr. Pierre Blier, University of Ottawa Institute of Mental Health Research, 1145 Carling Ave., Lady Grey Bldg., Rm. 2043, Ottawa ON K1Z 7K4; fax 613 792-3935; pblier@rohcg.on.ca**

Medical subject headings: antidepressive agents; depression; norepinephrine; receptors, adrenergic; receptors, neurokinin-1; serotonin; substance P.

*J Psychiatry Neurosci* 2004;29(3):208-18.

Submitted July 4, 2003; Revised Oct. 29, 2003; Accepted Nov. 11, 2003

when several of these dual strategies are used in a sequential manner. Nevertheless, even though the newer antidepressant agents are not toxic in overdosage, they may still produce undesirable side effects throughout the prolonged treatment periods indicated for recurrent depression. For instance, selective serotonin reuptake inhibitors (SSRIs) may produce, in a significant proportion of patients, sexual dysfunctions that will not abate over time. Consequently, there is an unmet need for medications that will restore euthymia, while being relatively free of cumbersome side effects that decrease patient comfort and could compromise compliance.

The use of a substance P (neurokinin-1 [NK1]) receptor antagonist as a novel approach to treat depression was first reported in 1998.[3] In this first trial, the effects of MK-869 were superior to placebo, as was the case for the SSRI paroxetine, in a double-blind study carried out in 4 reputable centres. Because the trial was carried out in depressed patients with moderate-to-severe anxiety, the results suggested that this new agent could be an antidepressant as well as an anxiolytic. It was striking that the side-effect profile of this drug was relatively innocuous, which added to the credibility of the results in such a double-blind study design. The results of a second controlled trial were recently published using another antagonist simply denoted "compound A." The effects of this NK1 antagonist were superior to placebo in a standard study design. Initially, it was claimed that the NK1 antagonist acted independently of the serotonin (5-HT) and the norepinephrine (NE) systems. However, it was demonstrated in the late 1970s that substance P applied directly on locus coeruleus NE neurons in anesthetized rats increases their spontaneous firing rate.[4] It was later shown, using a selective nonpeptide antagonist, that the excitatory effect of substance P on NE neurons is indeed mediated by NK1 receptors.[5] Furthermore, in humans, substance P is co-localized in dorsal raphe 5-HT neurons.[6] Finally, Shirayama et al[7] reported in 1996 that long-term administration of antidepressant drugs decreased substance P levels in the rat forebrain. Taken together, these observations made the purported absence of monoaminergic actions in the psychotropic action of NK1 antagonists highly improbable (Fig. 1).[8] We present a brief summary of the first endeavours that have linked NK1 receptor antagonism to alterations in the 5-HT and the NE systems with regard to physiologic and clinical actions.

## Localization of NK1 receptors in the dorsal raphe and the locus coeruleus

Immunocytochemistry studies have revealed that NK1 receptors are present on NE neurons in the locus coeruleus (Fig. 2).[9,10] Therefore, the excitatory effect of substance P applied by microiontophoresis onto NE neurons in extracellular recording experiments could well be mediated by a direct action on these neurons. In contrast, NK1 receptors in the dorsal raphe are not localized on 5-HT neurons.[9,10] Consistent with the latter observation, in-vitro intracellular recordings showed that substance P does not exert direct postsynaptic actions on 5-HT neurons. In this type of preparation, substance P does activate 5-HT neuronal firing, but via interneurons, because this action is blocked by tetrodotoxin, which blocks neuronal conduction from afferent inputs within the slice.[11]

## Effects of a single administration of NK1 antagonists on the firing activity of NE and 5-HT neurons

Single intravenous injections of most NK1 antagonists do not modify the spontaneous firing rate of NE neurons in rats (Fig. 3).[12,13] Similarly in guinea pigs, using an in-vitro slice preparation, the firing activity of NE neurons is not altered by exposure to the NK1 antagonist L760,735.[14] In mice, however, systemic injection of the NK1 antagonist RP67580 does increase spiking activity as well as the burst-firing activity of NE neurons.[15] One cannot invoke a methodological difference to explain these divergent results. Rather, differential properties of the drugs themselves may help explain this phenomenon. For instance, a partial agonistic action of RP67580 at NK1 receptors could possibly account for its enhancing action on NE neuronal firing. Another possibility would be actions, or lack of actions, of such drugs at NK3 receptors, the activation of which leads to an increased excitability of NE neurons.[16] The NK1 antagonists, despite generally leaving unchanged the spontaneous firing rate of NE neurons, promptly attenuate the responsiveness of the cell-body autoreceptor to the systemic injection of the $\alpha_2$-adrenergic agonist clonidine (Fig. 3).[12] The latter observation suggests that these neurons could be a predominant target of these drugs. Indeed, dampening the negative feedback role exerted by the $\alpha_2$-adrenergic autoreceptor would tend to increase

synaptic NE, because an enhancement of NE transmission underlies the antidepressant action of NE reuptake inhibitors.[1]

A single injection of the NK1 antagonist GR 205,171 was reported to increase the concentration of NE collected from a microdialysis probe implanted in rat frontal cortex. This enhancement could possibly result from the capacity of this drug to increase the firing rate of locus coeruleus neurons by 50%, as observed after its systemic injection.[17] It is nevertheless difficult to grasp the effect of this drug on NE neuronal firing, because the basal discharge rates of the neurons tested were not provided.

Shortly after the intravenous injection of NK1 antagonists, the firing rate of 5-HT neurons remained unaffected, as was the responsiveness of their cell-body 5-$HT_{1A}$ autoreceptors.[12] However, after a delay of a few minutes, there was a clear decreased responsiveness of 5-HT neurons to a 5-$HT_{1A}$ autoreceptor agonist.[13] The time course of the latter effect was interpreted as a possible primary action of the antagonists on NE neurons. Indeed, not only was the action of clonidine on NE neurons almost immediately attenuated after the injection of the NK1 antagonists, but the suppressant action of this $\alpha_2$-adrenergic agonist on 5-HT neuronal firing was also rapidly attenuated (Fig. 4).[13] The inhibitory action of clonidine on 5-HT neuronal firing was attributable to a decreased NE output acting normally on excitatory $\alpha_1$-adrenergic receptors located on the cell body of 5-HT neurons (Fig. 1).[18,19]

As is the case for NE neurons, several minutes after the systemic injection of RP68750 in mice, the firing rate of 5-HT neurons is markedly enhanced.[9,15] This property of RP68750 was put to use in order to docu-

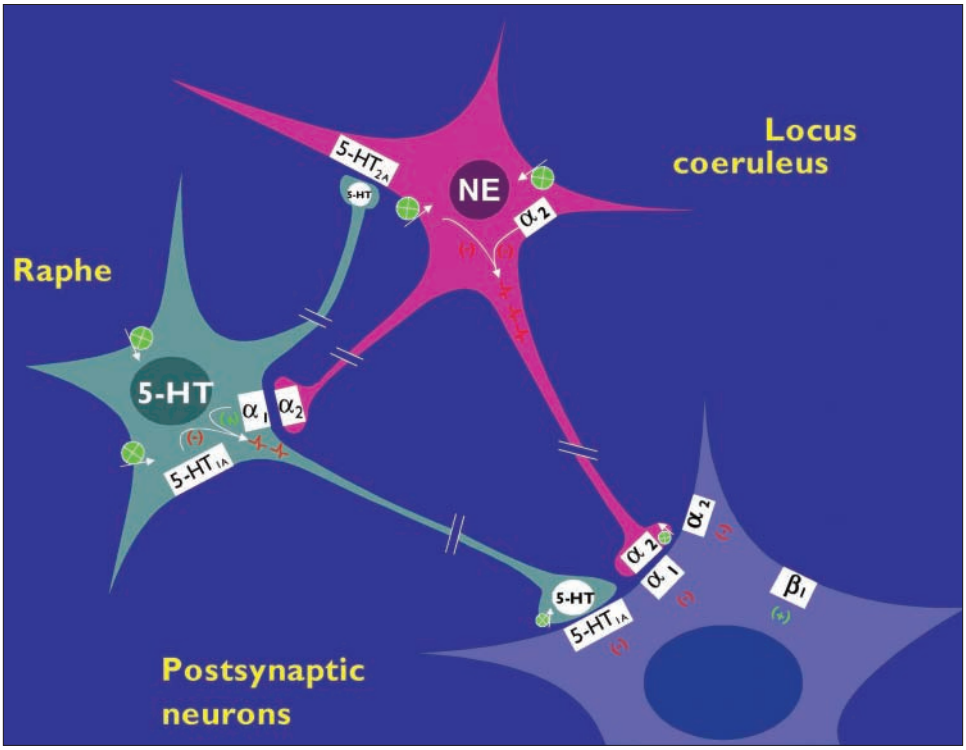


**Fig. 1: Interconnections between serotonin (5-HT) and norepinephrine (NE) neurons in the brain stem and their common projection sites in the forebrain. The pathway from the locus coeruleus to the dorsal raphe is a monosynaptic projection terminating on $\alpha_1$-adrenergic receptors on 5-HT neurons, as depicted. The pathway from the dorsal raphe to the locus coeruleus is composed of an apposition of 3 synapses, with the excitatory 5-$HT_{2A}$ receptor being located on a γ-aminobutyric acid (GABA) interneuron projecting to the NE neurons of the locus coeruleus.[8] At the level of the projections of 5-HT neurons, there is an $\alpha_2$-adrenergic receptor, not depicted on the diagram, which exerts a negative feedback action on 5-HT release. The (+) and (–) signs represent excitatory and inhibitory actions, respectively, on the function of the neurons. The firing activity of the neurons is represented by the action potential waves in red on the axons of the neurons.**

ment the possible role of NE neurons in mediating the enhancing action of NK1 receptor antagonism on the firing of 5-HT neurons. The activating action of RP68750 on 5-HT neuronal firing was thus studied in NE neuron-lesioned rats. The drug was no longer effective in enhancing the firing rate of 5-HT neurons in rats pretreated with the selective NE neurotoxin DSP-4. These results indicate that intact NE neurons are necessary for the action of NK1 receptor antagonism on 5-HT neuronal firing.

The enhancing effect of NK1 antagonists on the firing of 5-HT neurons may also involve other neuronal systems known to modulate the activity of these mesencephalic neurons. For instance, the lateral habenula is a structure that sends projections to dorsal raphe 5-HT neurons that use the neurotransmitter γ-aminobutyric acid (GABA) and possibly substance P as well.[20,21] Interfering with the habenuloraphe projection has been shown to alter the capacity of NK1 antagonists to enhance the firing rate of 5-HT neurons.[22]

## Electrophysiologic studies in mutant mice that lack the NK1 receptor gene

Although the NK1 antagonists described in the previous section are fairly selective, and some of these

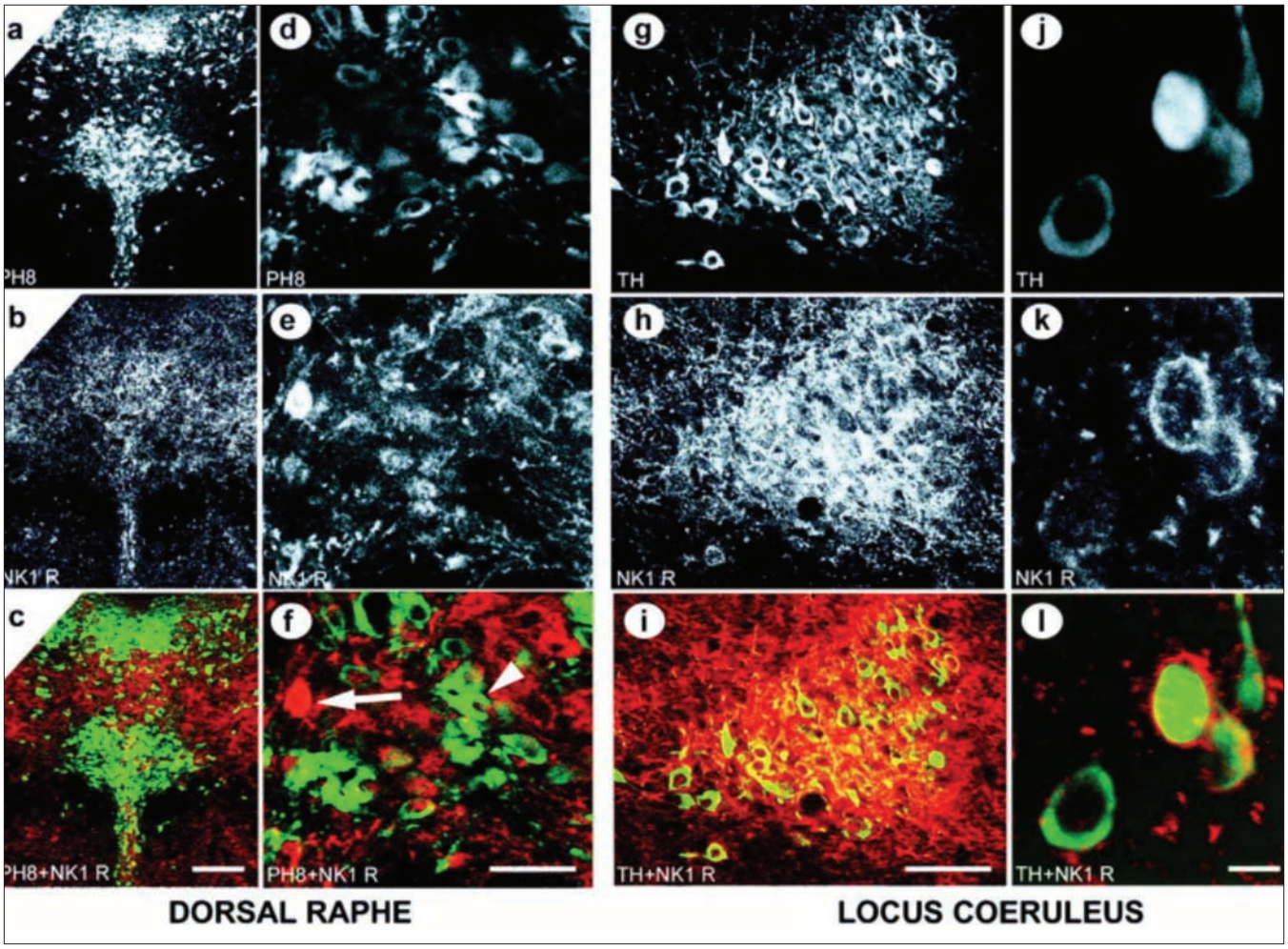


**Fig. 2: Photomicrographs of 5-HT neurons in the dorsal raphe labelled with antibodies for tryptophan hydroxylase (a and d) and for the neurokinin-1 receptors (NK1 R) (b and e). In the lowest 2 left-side images (c and f), the upper images are superimposed, showing that the NK1 immunoreactivity is not located on the 5-HT neurons themselves. The arrowhead indicates a neuron stained with the tryptophan hydroxylase antibody (PH8) and the arrow shows an NK1 receptor-positive neuron in the same area. Photomicrographs of NE neurons in the locus coeruleus labelled with antibodies for tyrosine hydroxylase (g and j) and the NK1 R (h and k). In the lowest 2 right-side images (i and l), the upper images are superimposed, showing that the immunoreactivity is located on the NE neurons themselves. Reproduced with permission from the National Academy of Sciences (*Proc Natl Acad Sci U S A* 2001;98:1912-7).[10]**

results were validated using enantiomers that lacked NK1 properties, the mediation of the effects on 5-HT and NE neurons by these drugs had to be ascertained. To this end, mice lacking 1 (NK1 +/–) or 2 (NK1 –/–) alleles of the NK1 receptor gene were studied.[9]

The mean firing activity of 5-HT neurons was significantly higher in NK1 +/–, or heterozygous, mice than in the wild-type mice (NK1 +/+). In the NK1 –/–, or homozygous, mice, the mean firing rate of 5-HT neurons was even higher than in the NK1 +/– mice (Fig. 5A). Similar electrophysiologic experiments carried out in raphe slices prepared from such mutant mice did not reveal an increased firing activity of 5-HT neurons.[23] It is important to mention, however, that 5-HT neurons do not fire spontaneously in such an in-vitro preparation.[21] This is because they become isolated from their noradrenergic activating input from the locus coeruleus. Consequently, 5-HT neurons have to be activated using the $\alpha_1$-adrenergic agonist phenylephrine to discharge within their normal firing range. It is, therefore, not possible to draw firm conclusions about the impact of drugs such as the NK1 antagonists on the spontaneous firing rate of 5-HT neurons from these experiments because their firing has to be artificially driven.

At this point, one may wonder how the firing rate of 5-HT neurons could be enhanced with a normally functioning 5-$HT_{1A}$ autoreceptor. In fact, the sensitivity

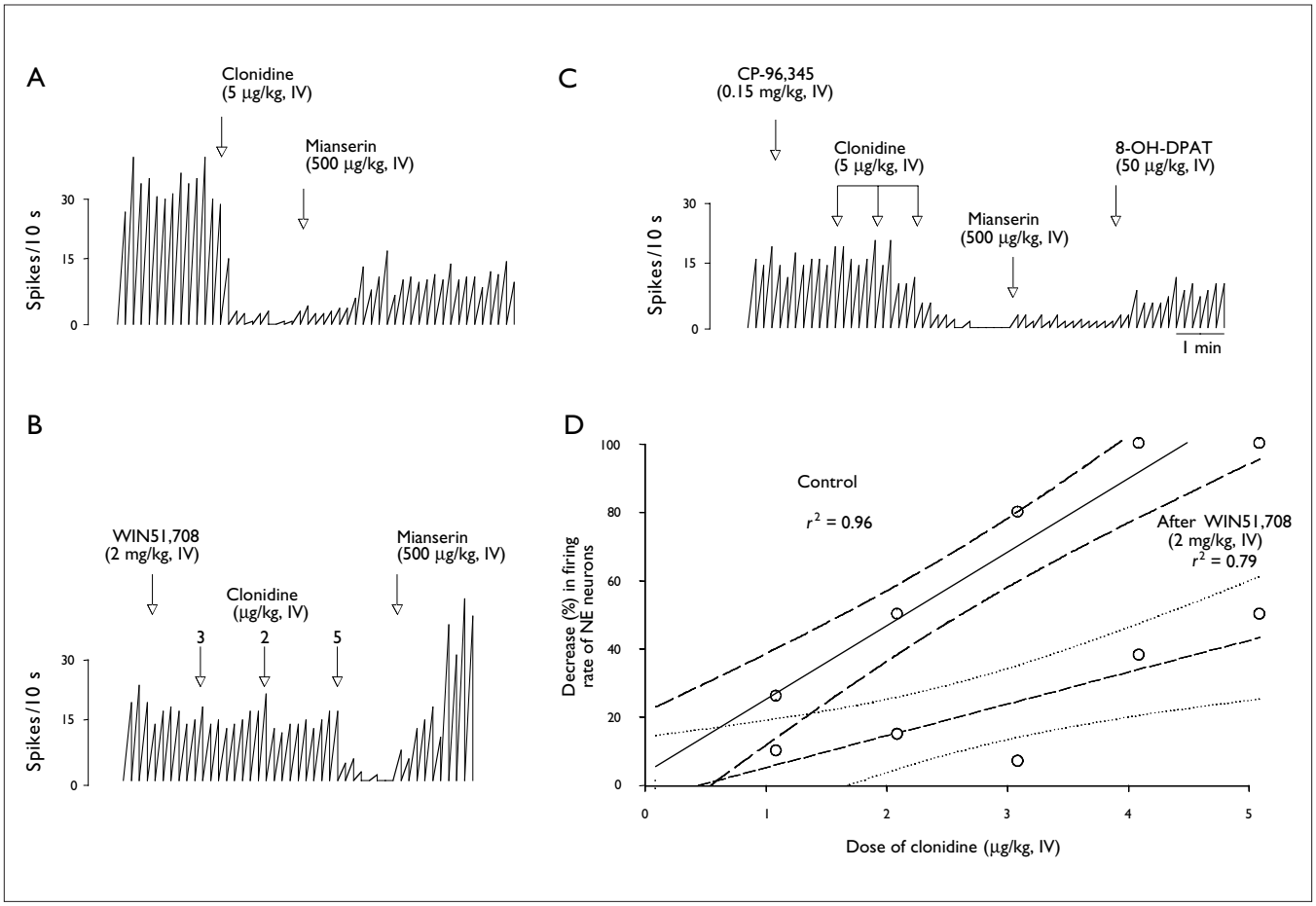


**Fig. 3: Integrated firing-rate histograms of NE neurons (A–C) recorded in the locus coeruleus of anesthetized rats, showing their attenuated responsiveness to the $\alpha_2$-adrenergic receptor agonist clonidine after the systemic injection of the NK1 receptor antagonists. Mianserin was used as an $\alpha_2$-adrenergic autoreceptor antagonist to ascertain the noradrenergic nature of the neurons recorded and to show that the suppression of firing was not merely the result of losing the recording signal. In D are represented the dose-response curves for intravenous injections of 5 µg/kg of clonidine in drug-naïve rats and in rats that had received an NK1 antagonist beforehand. Each symbol represents the response of 1 neuron to the first dose of clonidine in 1 rat. The curved lines depict the standard errors of the regression lines. The correlation coefficients ($r^2$) are given within the graph. Reproduced with permission from Lippincott Williams & Wilkins (*Neuroreport* 2000;11:1323-7).**[12]

of this autoreceptor, assessed with direct microiontophoretic application of the 5-$HT_{1A}$ autoreceptor agonist 8-OH-DPAT, was found to be attenuated in relation to the NK1 receptor genetic load of the mice (Fig. 5B).[8] It is noteworthy that bath application of another 5-$HT_{1A}$ autoreceptor agonist in raphe slices also provided support for this decreased responsiveness of 5-HT neurons in NK1-mutant mice.[23] Therefore, it can be concluded that interference with the NK1 receptor, whether it is achieved with an antagonist or by its constitutional deletion, leads to a desensitization of the 5-$HT_{1A}$ autoreceptor, thereby allowing increased firing activity of 5-HT neurons. This desensitization of the 5-$HT_{1A}$ autoreceptor is explainable, at least in part, by a downregulation of 5-$HT_{1A}$ binding sites in the dorsal raphe nucleus.[9]

It is important to mention that the sensitivity of postsynaptic 5-$HT_{1A}$ receptors in the hippocampus is not altered in NK1 –/– mutant mice when compared with that of their wild-type congeners (Fig. 5C). This observation adds to the long list of data clearly indicating that, despite being encoded by the same gene, presynaptic and postsynaptic 5-$HT_{1A}$ receptors have distinct physiologic properties.[24] The preserved function of postsynaptic 5-$HT_{1A}$ receptors in the hippocampus in the presence of desensitized presynaptic 5-$HT_{1A}$ autoreceptors would indicate that overall 5-HT transmission is enhanced in some parts of the forebrain. This possibility is consistent with the less anxious phenotype exhibited by the NK1 –/– mice.[9]

With regard to NE neurons, it was observed that their firing rate was unchanged in NK1 –/– mice. It would, therefore, appear that the constitutional absence of the NK1 gene does not lead to increased electrical activity of NE neurons in the locus coeruleus. These results are thus consistent with the unchanged

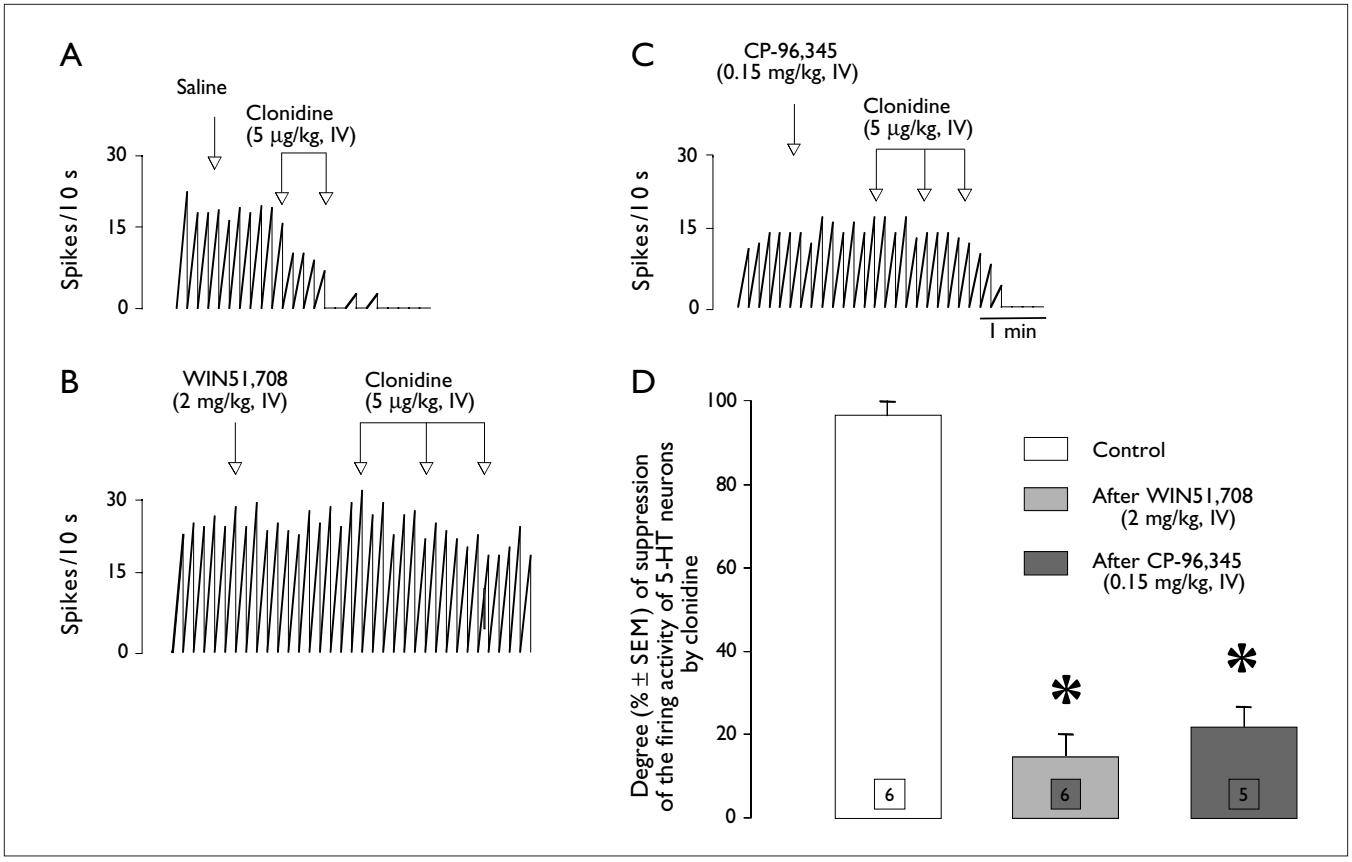


**Fig. 4: Integrated firing-rate histograms of 5-HT neurons (A–C) recorded in the dorsal raphe of anesthetized rats, showing their attenuated responsiveness to the $\alpha_2$-adrenergic autoreceptor agonist clonidine after the systemic injection of the NK1 receptor antagonists. In D are represented the mean responses to the first dose of clonidine (5 µg/kg) in drug-naïve rats and in rats that had received an NK1 antagonist beforehand. *$p < 0.05$ when compared with the control value using the Student's *t* test. Reproduced with permission from Lippincott Williams & Wilkins (*Neuroreport* 2000;11:1323-7).[12]**

firing rate of NE neurons after sustained administration of NK1 antagonists.[10]

## Effect of NK1 receptor manipulations on the extracellular level of 5-HT as assessed with microdialysis

Two studies have thus far examined the concentration of 5-HT in the extracellular compartment of the frontal cortex in the brain of laboratory animals using NK1 antagonists and gene deletion.[23,25] Although some of these results are consistent with those obtained with the electrophysiologic approach, others are not. In NK1 –/– mutant mice, as well as in mice that have had long-term treatment with an NK1 antagonist, the baseline extracellular level of 5-HT is not altered when compared with that of NK1 +/+ mice. The enhancing action of the SSRI paroxetine on the latter parameter is, however, potentiated in the NK1 –/– animals and in wild-type mice that have had long-term treatment with an NK1 antagonist. This accrued effect of the SSRI is consistent with a desensitization of the 5-$HT_{1A}$ autoreceptor, allowing a greater increase in 5-HT levels in the presence of 5-HT reuptake inhibition. These results stand in contrast with the increased tonic activation of 5-$HT_{1A}$ receptors in the rat hippocampus by long-term administration of an NK1 antagonist in electrophysiologic experiments.[26] It is conceivable that an increase in synaptic 5-HT was not detected by the microdialysis approach, which assesses 5-HT in the extracellular compartment, as a result of active 5-HT transporters that effectively clear diffusing 5-HT.

## Behavioural experiments that address the anxiolytic and antidepressant potential of NK1 antagonists in laboratory animals

In the first experiments using the NK1 antagonist MK-869, the drug, upon a single injection, suppressed ultrasonic vocalizations in guinea-pig pups separated from their mother.[3] The model is thought to represent a valid model of anxiety, and several antidepressant and anxiolytic drugs are effective in this paradigm. Similarly, administration of benzodiazepines and the NK1 antagonist RP68750 decreased stress responses in mice.[9] For instance, wild-type mice that received these drugs spent more time in the fear-producing part of an elevated plus maze. The same phenomenon was observed in NK1-mutant mice.

## Effects of long-term administration of NK1 antagonists on 5-HT transmission and on the NE system

Because NK1 antagonists are effective in treating depression, but with a delayed onset of action as is the case for other types of antidepressant drug, it was deemed essential to study their impact on 5-HT transmission after long-term treatment. The first series of experiments examined the firing rate of rat dorsal raphe 5-HT neurons after 2-day treatment with the NK1 antagonist CP-96,345.[26] The firing rate of 5-HT neurons increased by 50% over that of rats treated with saline (Fig. 6). This was accompanied by a significant attenuation of the sensitivity of the 5-$HT_{1A}$ autoreceptor. When rats were treated for 14 days, the firing rate of 5-HT neurons was increased by 90% and the degree of desensitization of the 5-$HT_{1A}$ autoreceptor was greater than in the 2-day treated group (Fig. 6). This degree of increase in firing was about the

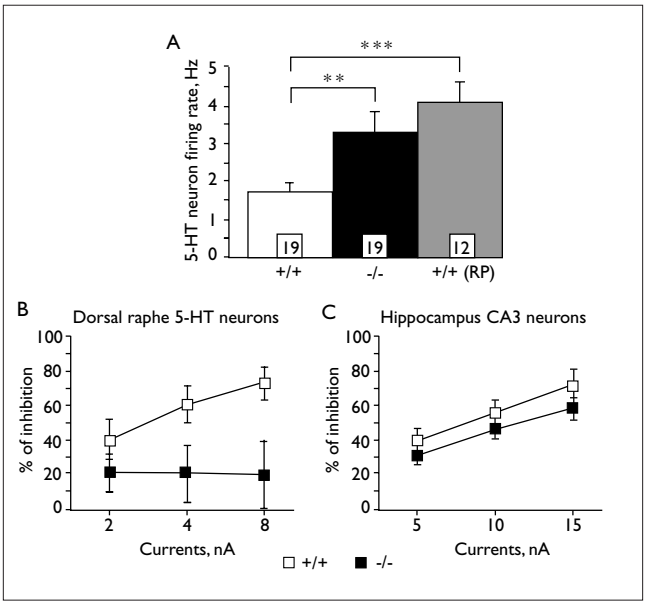


**Fig. 5A: Mean (and standard error of the mean [SEM]) firing rates of 5-HT neurons recorded in the dorsal raphe of anesthetized wild-type mice (NK1 +/+), in null mutant mice (NK1 –/–) and in wild-type mice after the systemic injection of the NK1 antagonist RP 67580.**
**B: Decreased responsiveness of 5-HT neurons and of (C) hippocampus CA3 pyramidal neurons to the direct iontophoretic application of the 5-$HT_{1A}$ agonist 8-OH-DPAT, using 3 currents (nA) to eject the drug from the recording electrode, in NK1 –/– mice when compared with that assessed in wild-type mice. Reproduced with permission from the National Academy of Sciences (*Proc Natl Acad Sci U S A* 2001;98:1912-7).[10]**

same as that observed in mutant mice lacking 5-$HT_{1A}$ receptors.[27]

In order to determine whether these alterations lead to a significant increase in 5-HT neurotransmission in the forebrain, the degree of activation of 5-$HT_{1A}$ receptors on CA3 hippocampus pyramidal neurons was assessed in rats treated with the same regimen of the NK1 antagonist CP-96,345.[21] In this model, it was previously shown that various types of antidepressant treatment, including electroconvulsive shock treatment, increase the degree of activation of postsynaptic 5-$HT_{1A}$ receptors.[28] A 2-day treatment with CP-96,345 did not alter the degree of activation of 5-$HT_{1A}$ receptors in the hippocampus (Fig. 7). In contrast, a 14-day treatment produced a marked enhancement of this parameter (Fig. 7). As is the case with the NK1 –/– mice, the responsiveness of the postsynaptic 5-$HT_{1A}$ receptors to 5-HT itself, tested by direct application of 5-HT through the recording electrode, was unchanged by long-term treatment with the NK1 antagonist. This delayed increase in overall 5-HT transmission is therefore consistent with the time lag these drugs require to exert their antidepressant or anxiolytic action in depressed patients.

We have recently investigated whether NK1 receptor antagonists can interfere with the function of 5-HT terminals (G.M. and P.B., unpublished observations, 2003). In-vitro exposure of rat hippocampus slices to an NK1 antagonist did not alter evoked [$^3$H]5-HT release from preloaded slices. The sensitivity of the terminal 5-$HT_{1B}$ autoreceptor was unaltered by the perfusion of an NK1 antagonist. The sensitivity of the $\alpha_2$-adrenergic receptor located on 5-HT terminals was also unaffected by exposure to an NK1 antagonist. The latter presynaptic receptor exerts an inhibitory action on 5-HT release that is similar to that of the 5-$HT_{1B}$ autoreceptor.[29] Consequently, it would appear that the increase in 5-HT transmission observed in the hippocampus in vivo after long-term NK1 receptor antagonism results mainly from the marked increase in firing rate of 5-HT neurons permitted by the desensitization of the 5-$HT_{1A}$ autoreceptor.[26]

A 2-day treatment with the same regimen of CP-96,345 that produced a 50% increase in 5-HT neuronal firing left unaltered the firing rate of locus coeruleus NE neurons.[13] Experiments using another NK1 antagonist, CP-94,999, produced identical results

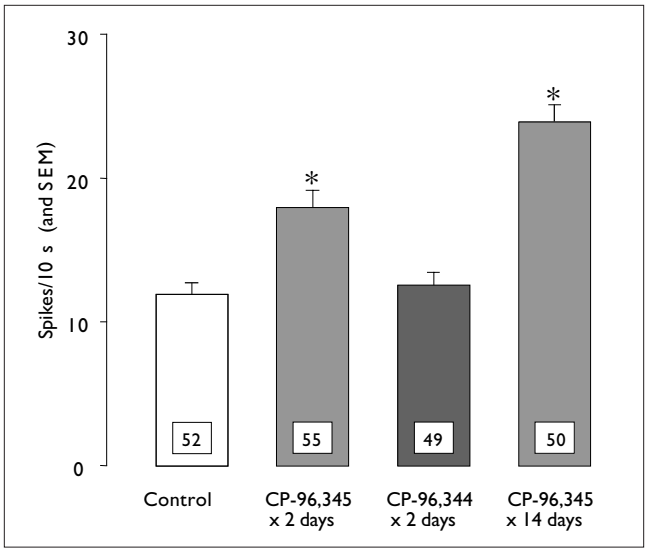


**Fig. 6: Histograms illustrate the mean (and SEM) firing rate of 5-HT neurons recorded in the dorsal raphe of anesthetized saline-treated rats and in anesthetized rats that received the active NK1 receptor antagonist CP-96,345 or the NK1-inactive enantiomer CP-96,344. The number of neurons recorded in each group is indicated at the bottom of each histogram. *$p < 0.05$ when compared with the control group. Reproduced with permission from Elsevier (*Biol Psychiatry* 2001;50:191-9).[26]**

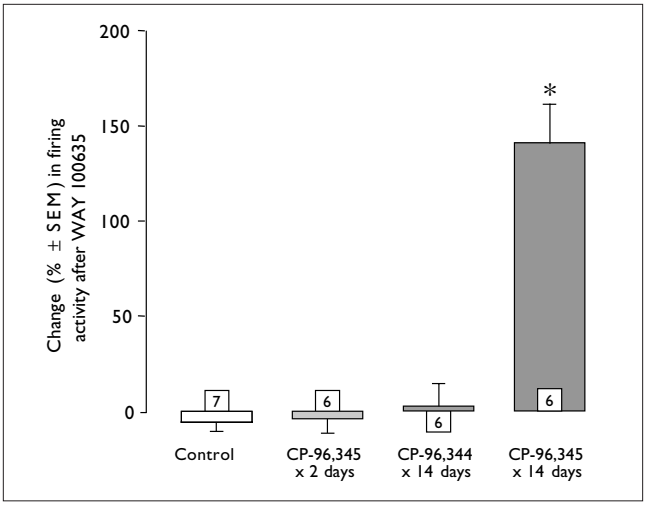


**Fig. 7: Changes in the spontaneous firing activity of hippocampus CA3 pyramidal neurons, recorded in anesthetized rats, after the intravenous injection of the 5-$HT_{1A}$ antagonist WAY 100,635 to determine the degree of activation of postsynaptic 5-$HT_{1A}$ receptors in that forebrain structure. An increase in firing rate represents a disinhibitory effect exerted by the antagonist, thereby preventing an increased inhibitory tone being mediated by increased 5-HT transmission in the hippocampus. The number of rats tested in each group is given within each histogram. The variance is expressed as the SEM. Note that only the long-term treatment with the active NK1 antagonist (CP-96,345) increased 5-HT transmission. *$p < 0.05$ when compared with the control group. Reproduced with permission from Elsevier (*Biol Psychiatry* 2001;50:191-9).[26]**

after 2-day treatment. Prolongation of the treatment to 7 and 14 days did not change the firing rate of NE neurons. These results using NK1 antagonists endowed with moderate-to-low affinity for the rat NK1 binding site are nevertheless consistent with the unaltered firing rate of NE neurons in NK1-mutant mice. It is noteworthy that long-term treatment of guinea pigs with the NK1 antagonist L760,735 increased the burst-firing pattern of NE neurons when recorded ex vivo in slices.[14] This mode of firing leads to greater release of monoamine neurotransmitters for the same number of action potentials that occur in a regular fashion.[30–32] It is nevertheless difficult to ascertain the net output of the locus coeruleus after this treatment, because the mean firing rate of NE neurons was decreased from 1.6 Hz to 1.0 Hz, whereas the degree of burst firing was increased from 27% of the neurons discharging in this mode in the controls to 90% in the treated guinea pigs.

As for the 5-HT system, the evoked release of tritium from slices preloaded with [$^3$H]NE and the responsiveness of the terminal $\alpha_2$-adrenergic autoreceptor were studied in the rat hippocampus during exposure to an NK1 antagonist and after long-term treatment (G.M. and P.B., unpublished observations, 2003). The function of NE terminals was unaffected by NK1 antagonist exposure. In contrast, NE release was significantly decreased after 2-week treatment with an NK1 antagonist. The terminal $\alpha_2$-adrenergic autoreceptor was normosensitive after 14-day treatment with an NK1 antagonist. Consequently, it would appear that long-term treatment with an NK1 antagonist does not increase NE neurotransmission, as is the case with 5-HT transmission at 5-HT$_{1A}$ receptors in the hippocampus.[26] On the contrary, overall NE transmission could well be attenuated after such a long-term treatment. Further work needs to be carried out to clarify the effect of long-term administration of NK1 antagonists on overall NE transmission.

## Common actions of various antidepressants and NK1 antagonists on 5-HT neurons

Long-term treatment with SSRIs, monoamine oxidase inhibitors or 5-HT$_{1A}$ agonists desensitizes the 5-HT$_{1A}$ autoreceptor, presumably by their capacity to tonically activate it.[33] They do not produce, however, an increased firing rate of 5-HT neurons as the NK1 antagonists do. This is likely because, although these drugs decrease the function of the autoreceptor, they still significantly activate it. In contrast, 2 established antidepressant drugs have been reported to drive up the firing rate of 5-HT neurons; these are mirtazapine and bupropion.[31,34–35] Both drugs increase the synaptic availability of NE. Mirtazapine acts by blocking the $\alpha_2$-adrenergic autoreceptors at the cell body of NE neurons, thereby increasing firing, and at the level of their terminals to enhance NE release. Bupropion acts by increasing NE release on excitatory $\alpha_1$-adrenergic receptors on the cell body of 5-HT neurons, thereby driving up their firing rate. As is the case with NK1 antagonists, the enhancing action of mirtazapine and bupropion on 5-HT neurons is lost in NE neuron-lesioned rats. These results therefore indicate that NK1 antagonists could be categorized with antidepressants that primarily exert their action on 5-HT neurons via the noradrenergic system (Fig. 7).

## Substance P and non-monoaminergic transmission

Substance P can exert profound actions on various types of neurons other than 5-HT and NE neurons in the central nervous system. Little is known about its actions in forebrain structures implicated in depression. Plasma levels of substance P have been reported to be elevated in depression, and although the relevance of this pool of neurotransmitter is not as important as that in the cerebrospinal fluid, a statistically significant decrease proportional to clinical improvement with antidepressant treatment has been recorded.[36] A postmortem study has also documented a decrease in NK1-binding density in the brain of depressed patients.[37] Taken together with the observations that antidepressant drugs decrease substance P levels in the brains of laboratory animals, the latter change in binding density may represent an attempt of the brain to compensate for an excess of this excitatory neurotransmitter. Much work remains to be done to clarify the role of substance P in depression.

## Conclusion

Electrophysiologic, neurochemical and behavioural studies have provided evidence for effects of NK1 antagonists on the 5-HT and NE systems similar to those produced by various types of antidepressant drug. Clinical studies carried out with such drugs thus far are compatible with their antidepressant or anxiolytic

potential. The monoamine dietary-depletion strategy that helped establish the pivotal role of 5-HT and NE in the antidepressant action of SSRIs and NE reuptake inhibitors, respectively,[38,39] could help elucidate the role of these 2 neurotransmitters in patients who respond to NK1 antagonists.

It is nevertheless surprising that only 3 positive trials (the third one being on the efficacy of CP-122,721 in a placebo-controlled and fluoxetine-controlled trial; presented at the 2002 New Clinical Drug Evaluation Unit meeting [www.nimh.nih.gov/ncdeu/Index.cfm]) have been reported with such agents given the considerable efforts in the pharmaceutical industry dedicated to the development of this type of therapeutic agent for depression over the past 5 years. There is no doubt that there can be significant hurdles to the optimal use of the nonpeptide NK1 antagonists, such as bioavailability, brain penetration and receptor occupancy. Although NK1 antagonists may be endowed with intrinsic, but delayed, antidepressant action on their own, they may end up revealing their greatest potential as adjuncts to accelerate or potentiate, or both, other antidepressant drugs because of their capacity to promptly decrease the negative feedback actions exerted by the cell-body autoreceptors on 5-HT and NE neurons. The ongoing research endeavour regarding substance P and NK1 antagonists has provided increasing evidence of important physiologic links between neuronal systems, and the development of NK1 antagonists still has the potential to provide the means to help fulfill unmet needs in antidepressant therapy, namely, rapid onset, greater effectiveness and minimal side effects.

**Competing interests:** None declared by Drs. Gobbi, Haddjeri, Santarelli and Hen and by Ms. Mathew. Dr. Blier has been a consultant for Pfizer, Merck Frosst and Roche and has received an honorarium from Merck Frosst for research on an unnamed drug and an educational grant from Merck Frosst.

## References

1. Thase ME, Entsuah AR, Rudolph RL. Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *Br J Psychiatry* 2001;178:234-41.
2. Thase ME, Ninan PT. New goals in the treatment of depression: moving toward recovery. *Psychopharmacol Bull* 2002; 36(Suppl 2):24-35.
3. Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, et al. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 1998; 281:1640-5.
4. Guyenet PG, Aghajanian GK. Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. *Brain Res* 1977;136:178-84.
5. McLean S, Ganong AH, Seeger TF, Bryce DK, Pratt KG, Reynolds LS, et al. Activity and distribution of binding sites in brain of a nonpeptide substance P (NK1) receptor antagonist. *Science* 1991;251:437-9.
6. Sergeyev V, Hokfelt T, Hurd Y. Serotonin and substance P coexist in dorsal raphe neurons of the human brain. *Neuroreport* 1999;10:3967-70.
7. Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K. Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. *Brain Res* 1996;739:70-8.
8. Szabo ST, Blier P. Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. *Brain Res* 2001;922:9-20.
9. Chen LW, Wei LC, Liu HL, Rao ZR. Noradrenergic neurons expressing substance P receptor (NK1) in the locus coeruleus complex: a double immunofluoresence study in the rat. *Brain Res* 2000;873:155-9.
10. Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, et al. Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci U S A* 2001; 98:1912-7.
11. Liu R, Ding Y, Aghajanian GK. Neurokinins activate local glutamatergic inputs to serotonergic neurons of the dorsal raphe nucleus. *Neuropsychopharmacology* 2002;27:329-40.
12. Haddjeri N, Blier P. Effect of neurokinin-1 receptor antagonists on the function of 5-HT and noradrenaline neurons. *Neuroreport* 2000;11:1323-7.
13. Haddjeri N, Blier P. Effects of neurokinin 1 antagonists on the firing of locus coeruleus norepinephrine neurons. *Abstr Soc Neurosci* 2001;31:749.
14. Maubach KA, Martin K, Chicchi G, Harrison T, Wheeldon A, Swain CJ, et al. Chronic substance P (NK1) receptor antagonist and convention: antidepressant treatment increases burst firing of monoamine neurons in the locus coeruleus. *Neuroscience* 2002;109:609-17.
15. Gobbi G, Santarelli L, Hen R, Blier P. Neurokinin 1 (NK1), serotonin (5-HT) and norepinephrine (NE) receptors interactions: *in vivo* electrophysiological evidence. *Abstr Soc Neurosci* 2001;31:239.
16. Bert L, Rodier D, Bougault I, Allouard N, Le Fur G, Soubrie P, et al. Permissive role of neurokinin NK(3) receptors in NK(1) receptor-mediated activation of the locus coeruleus revealed by SR 142801. *Synapse* 2002;43:62-9.
17. Millan MJ, Lejeune F, De Nanteuil G, Gobert A. Selective blockade of neurokinin (NK)(1) receptors facilitates the activity of adrenergic pathways projecting to frontal cortex and dorsal hippocampus in rats. *J Neurochem* 2001;76:1949-54.
18. Svensson TH, Bunney BS, Aghajanian GK. Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res* 1975;92:291-306.
19. Blier P, Lavoie N, Haddjeri N. Electrophysiological evidence for the tonic activation of the 5-$HT_{1A}$ autoreceptor *in vivo* in the rat brain. *Abstr Soc Neurosci* 2001;31:124.
20. Wang RY, Aghajanian GK. Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science* 1977;197:89-91.

21. Nishikawa T, Fage D, Scatton B. Evidence for, and nature of, the tonic inhibitory influence of habenulointerpeduncular pathways upon cerebral dopaminergic transmission in the rat. *Brain Res* 1986;373:324-36.

22. Conley RK, Cumberbatch MJ, Mason GS, Williamson DJ, Harrison T, Locker K, et al. Substance P (neurokinin 1) receptor antagonists enhance dorsal raphe neuronal activity. *J Neurosci* 2002;22:7730-6.

23. Froger N, Gardier AM, Mratalla R, Alberti I, Lena I, Boni C, et al. 5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. *J Neurosci* 2001;21:8188-97.

24. Blier P, Lista A, de Montigny C. Differential properties of pre- and postsynaptic 5-hydroxytryptamine$_{1A}$ receptors in the dorsal raphe and hippocampus: I. Effect of spiperone. *J Pharmacol Exp Ther* 1993;265:7-15.

25. Gardier AM, David DJ, Jego G, Przybylski C, Jacquot C, Durier S, et al. Effects of chronic paroxetine treatment on dialysate serotonin in 5-HT$_{1B}$ receptor knockout mice. *J Neurochem* 2003;86:13-24.

26. Haddjeri N, Blier P. Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. *Biol Psychiatry* 2001;50:191-9.

27. Richer M, Hen R, Blier P. Modification of serotonin neuron properties in mice lacking 5-HT$_{1A}$ receptors. *Eur J Pharmacol* 2002;435:195-203.

28. Haddjeri N, Szabo ST, de Montigny C, Blier P. Increased tonic activation of rat forebrain 5-HT(1A) receptors by lithium addition to antidepressant treatments. *Neuropsychopharmacology* 2000;22:346-56.

29. Blier P, Ramdine R, Galzin AM, Langer SZ. Frequency-dependence of serotonin autoreceptor but not alpha adrenoceptor inhibition of [$^3$H]-serotonin release in rat hypothalamic slices. *Naunyn Schmiedebergs Arch Pharmacol* 1989;339:60-4.

30. Gonon FG. Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by *in vivo* electrochemistry. *Neuroscience* 1988;24:19-28.

31. Gartside SE, Hajos-Korcsok E, Bagdy E, Harsing LG Jr, Sharp T, Hajos M. Neurochemical and electrophysiological studies on the functional significance of burst firing in serotonergic neurons. *Neuroscience* 2000;98:295-300.

32. Dawe GS, Huff KD, Vandergriff JL, Sharp T, O'Neill MJ, Rasmussen K. Olanzapine activates the rat locus coeruleus: in vivo electrophysiology and c-Fos immunoreactivity. *Biol Psychiatry* 2001;50:510-20.

33. Blier P, de Montigny C. Serotonin and drug-induced therapeutic responses in major depression, obsessive–compulsive and panic disorders. *Neuropsychopharmacology* 1999;21(2 Suppl): 91S-98S.

34. Haddjeri N, Blier P, de Montigny C. Effect of the alpha-2 adrenoceptor antagonist mirtazepine on the 5-hydroxytryptamine system in the rat brain. *J Pharmacol Exp Ther* 1966; 277:861-71.

35. Dong J, Blier P. Modification of norepinephrine and serotonin, but not dopamine neuron firing by sustained bupropion treatment. *Psychopharmacology* 2001;155:52-7.

36. Bondy B, Baghai B, Minov C, Schule C, Schwarz MJ, Zwanzger P, et al. Substance P serum levels are increased in major depression: preliminary results. *Biol Psychiatry* 2003; 15;53:538-42.

37. Stockmeier CA, Shi X, Konick L, Overholser JC, Jurjus G, Meltzer HY, et al. Neurokinin-1 receptors are decreased in major depressive disorder. *Neuroreport* 2002;12:1223-7.

38. Miller HL, Delgado PL, Salomon RM, Berman R, Krystal JH, Heninger GR, et al. Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. *Arch Gen Psychiatry* 1996;53:117-28.

39. Moreno FA, Gelenberg AJ, Heninger GR, Potter RL, McKnight KM, Allen J, et al. Tryptophan depletion and depressive vulnerability. *Biol Psychiatry* 1999;46:498-505.