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Uncoupling between noradrenergic and serotonergic neurons as a molecular basis of stable changes in behavior induced by repeated drugs of abuse

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ABSTRACT

A challenge in drug dependence is to delineate long-term behavioral and neurochemical modifications induced by drugs of abuse. In rodents, drugs of abuse induce locomotor hyperactivity, and repeating injections enhance this response. This effect, called behavioral sensitization, persists many months after the last administration, thus mimicking long-term sensitivity to drugs observed in human addicts. Although addictive properties of drugs of abuse are generally considered to be mediated by an increased release of dopamine in the ventral striatum, recent pharmacological and genetic experiments indicate an implication of α1b-adrenergic receptors in behavioral and rewarding responses to psychostimulants and opiates. Later on, it was shown that not only noradrenergic but also serotonergic systems, through 5-HT_{2A} receptors, were controlling behavioral effects of drugs of abuse. More recently, experiments performed in animals knockout for α1b-adrenergic or 5-HT_{2A} receptors indicated that noradrenergic and serotonergic neurons, besides their activating effects, inhibit each other by means of the stimulation of α1b-adrenergic and 5-HT_{2A} receptors and that this mutual inhibition vanishes in wild type mice with repeated injections of psychostimulants, opiates or alcohol. Uncoupling induced by repeated treatments with drugs of abuse installs a stable sensitization of noradrenergic and serotonergic neurons, thus explaining an increased reactivity of dopaminergic neurons and behavioral sensitization. We propose that noradrenergic/serotonergic uncoupling is a common stable neurochemical consequence of repeated drugs of abuse which may also occur during chronic stressful situations and facilitate the onset of mental illness. Drug consumption would facilitate an artificial re-coupling of these neurons, thus bringing a temporary relief.

The concept that specific neurons modulate information processing rather than they convey sensory or motor signals seems generally accepted. In any representation of the primary pathways responsible for the processing of sensory stimuli or motor outputs, it is notable that these pathways include neurons releasing GABA and excitatory amino acids, and possibly acetylcholine or neuropeptides, whereas noradrenergic (NE), dopaminergic (DA) or serotonergic (5-HT) neurons do not appear to be involved. The absence of this latter group of neurons in the main communication pathways of the central nervous system is both logical and surprising. It is logical if we consider that the entire group of monoaminergic cells represent less than 1% of the total brain neurons, and surprising, since therapeutic improvements obtained

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with most psychotropic drugs, such as neuroleptics and antidepressants, appear to act via modification of monoaminergic transmission. Drugs of abuse are also considered to act through a modification of monoaminergic transmission especially after the demonstration by Di Chiara and Imperato, in 1988, that drugs abused by humans, including psychostimulants and opiates, preferentially increase DA concentration in the mesolimbic system [1]. During the 20 years preceding this observation dopaminergic neurons had already received considerable attention because of their potential link with Parkinson's disease, schizophrenia and psychostimulants abuse. However, Di Chiara and Imperato's findings can be considered as the starting point of most of the neurochemical studies suggesting that DA release is responsible for the pleasure induced by drugs of abuse and for the ''craving'' that follows after repeated consumption. In this review, we will first analyze the place of DA in the central nervous system and try to indicate how dopaminergic neurons are recruited in addictive processes. This analysis will lead us to propose that DA may not be the critical factor in the development of pharmaco-dependence, and suggest that the role of other neuromodulators, such as NE and 5-HT, and the interactions between neurons releasing these transmitters should not be overlooked.

1. Dopamine dysfunction in the central nervous system: source of disease or its consequence?

In the neurobiological world, dopamine is kind of a star. One can read in a journal with one of the highest impact factor that ''these (dopaminergic) neurons have a central role in guiding our behaviour and thoughts. They are hijacked by every addictive drug; they malfunction in mental illness; and they are lost in dramatically impairing illnesses such as Parkinson's disease. If dopamine systems are overstimulated, we may hear voices, experience elaborate bizarre cognitive distorsions, or engage excessively in dangerous goal-directed behaviour. Dopamine function is also central to the way that we value our world, including the way we value money and other human beings'' [2].

Initially, DA was considered as the neurotransmitter regulating locomotor activity. Psychostimulants, such as amphetamine or cocaine, were thought to induce their locomotor activating effects through an increased release of DA in the nucleus accumbens, a subcortical structure. This was confirmed by Pijnenburg et al. in 1975. This team showed that locomotor response induced by the local injection of amphetamine into the nucleus accumbens was inhibited by neuroleptics, compounds known to block dopaminergic transmission [3]. However, in contrast to this finding, bilateral electrolytic destruction of the ventral tegmental area (VTA), where dopaminergic cell bodies lie, induces permanent behavioral deficits characterized by locomotor hyperactivity and disappearance of spontaneous alternation, the ''VTA-syndrome'' [4,5]. Moreover, in animals with a complete DA depletion in the nucleus accumbens, locomotor hyperactivity was shown to be proportional to the extent of destruction of the dopaminergic fibers innervating the prefrontal cortex [6]. This correlation was also found with the development of a cortical D1 receptor hypersensitivity, as displayed by adenylyl cyclase activity coupled to DA in the prefrontal cortex [7]. Interestingly, both the locomotor hyperactivity and the hypersensitivity of cortical D1 receptors were not obtained when dopaminergic cell bodies were destroyed chemically by a 6-hydroxy-dopamine (6-OHDA) injection. Moreover, it was even found that superimposing a 6-OHDA lesion into the VTA to a bilateral electrolytic lesion of the same area was blocking the development of associated locomotor hyperactivity and of cortical D1 receptors hypersensitivity [8]. Later on, this paradoxical finding was explained because electrolytic lesions of the VTA were shown to spare the cortical ascending NE innervation whereas 6-OHDA VTA lesions, because of the neurotoxin diffusion, destroy ascending NE fibers which pass near the DA cells [8,9].

Two conclusions can be drawn from these data: (1) ascending NE fibers exert a permissive role on the development of denervation supersensitivity of cortical D1 receptors and (2) ascending NE fibers are responsible for the development of a locomotor hyperactivity in absence of ascending DA fibers. This suggests that an increase in subcortical dopaminergic transmission is not necessary to obtain locomotor hyperactivity but rather an increased subcortical dopaminergic transmission is simply the consequence, among others, of an increased cortical noradrenergic transmission which would be, actually, responsible for a locomotor hyperactivity. The effects of cortical noradrenergic and dopaminergic innervations on cortical D1 receptors sensitivity suggest that interactions between cortical noradrenergic and subcortical dopaminergic transmissions may occur via the descending cortico-accumbens and cortico-VTA glutamatergic neurons [10]. In other words, modifications in DA transmission may not be the source of a dysfunction but rather its consequence.

2. Role of cortical α1b-adrenergic receptors in the interaction NE/DA

Because the destruction of ascending NE neurons was able to annihilate the behavioral deficits induced by the electrolytic lesion of the VTA, we tried to analyze whether the pharmacological blockade of either α- or β-adrenergic receptors could reverse these deficits. This was carried out in behavioral experiments in which animals, rendered hyperactive by an electrolytic lesion of the VTA, were treated with low doses of prazosin, yohimbine or propranolol, α1-adrenergic, α2-adrenergic or β-adrenergic receptor antagonists, respectively. Prazosin completely abolished the locomotor hyperactivity seen in lesioned rats for periods lasting up to 24 h following the injection, whereas no significant effects on locomotor activity could be observed when animals were tested in the presence of yohimbine or propranolol [11]. The role of cortical α1-adrenergic receptors on the descending cortico-accumbens and cortico-VTA glutamatergic fibers and their effects on subcortical extracellular DA levels was further confirmed by Darracq et al. [12,13]. First, it was shown that D-amphetamine is releasing DA in the nucleus accumbens following two modes; one is obtained by the local injection of D-amphetamine into the nucleus accumbens. Surprisingly, bilateral injections of D-amphetamine into the nucleus accumbens

do not induce locomotor hyperactivity even when increases in extracellular DA levels attain 15,000% of the basal DA levels. Therefore, this DA release triggered by the local, presynaptic to dopaminergic nerve terminals, effect of D-amphetamine was denominated "non-functional" [12]. The second mode, called functional, is in relation with locomotor activity and is obtained by a systemic D-amphetamine administration. When D-amphetamine is injected systemically, a +150% increase in extracellular DA values in the nucleus accumbens, when compared to basal DA values, is enough to induce a locomotor response. When prazosin is injected systemically before the intra-peritoneal injection of D-amphetamine, it blocks both the functional release of DA and the D-amphetamine-induced locomotor response. Same data are obtained when prazosin is injected, instead of systemically, bilaterally into the prefrontal cortex [12]. Later on, it was shown that the D-amphetamine-induced mesolimbic release of functional DA is impulse-dependent and controlled by the stimulation of glutamate metabotropic receptors located into the nucleus accumbens [13].

To identify the α1-adrenergic receptor subtype involved in this phenomenon, we used mice knockout for the α1b-adrenergic receptor subtype (α1b-AR KO) that were given by Susanna Cotecchia from the University of Lausanne (Switzerland) [14]. These KO mice have no apparent phenotype changes except a decreased phenylephrine-induced blood pressure response. Although neuro-developmental modifications may occur in α1b-AR KO mice [15], this model has the advantage to avoid the use of pharmacological compounds which may be unspecific or, as it is the case for prazosin in humans but not in rodents, may not cross readily the blood–brain barrier [16]. Following different biochemical and behavioral controls aimed at verifying that densities of dopaminergic innervation and dopaminergic receptors as well as basal motor behavior were similar in α1b-AR KO and wild type (WT) littermates, locomotor responses and behavioral sensitizations induced by D-amphetamine, cocaine and morphine were analyzed in both strains [17]. Acute locomotor effects of D-amphetamine, cocaine and morphine were dramatically reduced in α1b-AR KO mice. Although gene rescue experiments have not been performed yet, it is very likely that this decrease resulted from the absence of α1b-AR in KO mice since pre-treatment with prazosin abolished differences observed between WT and α1b-AR KO mice. Moreover, differences in locomotor responses were maintained when D-amphetamine, cocaine or morphine was administered repeatedly, only weak behavioral sensitization occurring in α1b-AR KO mice. Finally, in the two-bottle choice or the conditioned place preference paradigms, cocaine and morphine exerted rewarding properties in WT, but not in α1b-AR KO mice [17]. These data prompted us to test whether D-amphetamine still induced increases in extracellular DA levels in the nucleus accumbens of α1bAR-KO mice. First, we found that basal extracellular nucleus accumbens DA levels were almost 30% lower in α1bAR-KO mice when compared with that of WT littermates [18]. This suggested that stimulation of α1b subtype of adrenergic receptors exerts a tonic excitatory effect on subcortical DA release [17]. Second, it was observed that D-amphetamine fails to increase extracellular DA levels in the nucleus accumbens of α1bAR-KO mice. It is likely that this lack of D-amphetamine-induced release of DA in α1bAR-KO mice is related to their blunted locomotor response to D-amphetamine [17]. However, for the highest dose of D-amphetamine tested (6 mg/kg, i.p.), a significant locomotor hyperactivity was observed as well as a weak increase in extracellular DA levels in the nucleus accumbens [18], suggesting that an other locomotor component than the α1b-adrenergic one may exist. This will be shown below.

Because bursting activities of dopaminergic neurons in the VTA are either blocked by prazosin [19] or increased by a specific inhibitor of the noradrenergic transporter reboxetine [20], it can be proposed that D-amphetamine exerts, at least partly, its effects on subcortical DA release via the stimulation of α1b-adrenergic receptors. Such a stimulation can be attributable to an increased release of NE by D-amphetamine in the prefrontal cortex [21,22], a structure containing a high density of α1b-adrenergic receptors [17,22] and possibly responsible for the regulation of DA release in the nucleus accumbens [23].

Our data therefore indicate a critical role of α1b-adrenergic receptors in neurochemical and locomotor effects of psychostimulants; however, when analyzing the effects of opiates, we found that only part of the morphine-evoked behavioral locomotor response was affected by pharmacological or genetic blockade of α1b-adrenergic receptors [18,24]. Accordingly, the blockade of α1-adrenergic receptors by prazosin inhibits both the expression and development of behavioral sensitization to moderate doses of D-amphetamine and cocaine, but only reduces the expression of behavioral sensitization to morphine and leaves unaffected its development. This clearly indicates, as mentioned above with high doses of D-amphetamine, that at least another component than the α1b-adrenergic one could participate in the locomotor response to morphine. Numerous studies have shown that 5-HT neurons are implicated in the effects of morphine and an interaction between 5-HT_{2A} receptors and dopaminergic neurons has been proposed [25–30]. We therefore looked for a role of 5-HT_{2A} receptors in morphine-evoked locomotor responses.

3. Role of 5-HT_{2A} receptors in some behavioral and neurochemical responses to morphine: comparison with α1b-adrenergic receptors

To test the role of 5-HT_{2A} receptors on morphine-evoked locomotor responses, we used SR46349B, a specific antagonist of 5-HT_{2A} receptors [31], in α1b-AR KO and WT mice. We found that SR46349B completely blocked the locomotor response evoked by high doses of morphine in α1b-AR KO mice whereas this effect was only partial (−80%) in WT mice [32]. We also verified that, as expected, prazosin had no effect on morphine-induced locomotor response in α1b-AR KO mice whereas this α1-adrenergic receptor antagonist blocked by 80% the locomotor response induced by morphine in WT mice. Surprisingly, however, acute morphine induced a 3-fold higher locomotor response in α1b-AR KO mice, pre-treated or not with prazosin, than in WT mice pre-treated with prazosin. Same higher response in α1b-AR KO mice when compared with WT animals was obtained when extracellular DA levels were monitored in the nucleus accumbens. Finally, as mentioned above for locomotor response, morphine-induced increases of extracellular DA levels in the nucleus accumbens were completely

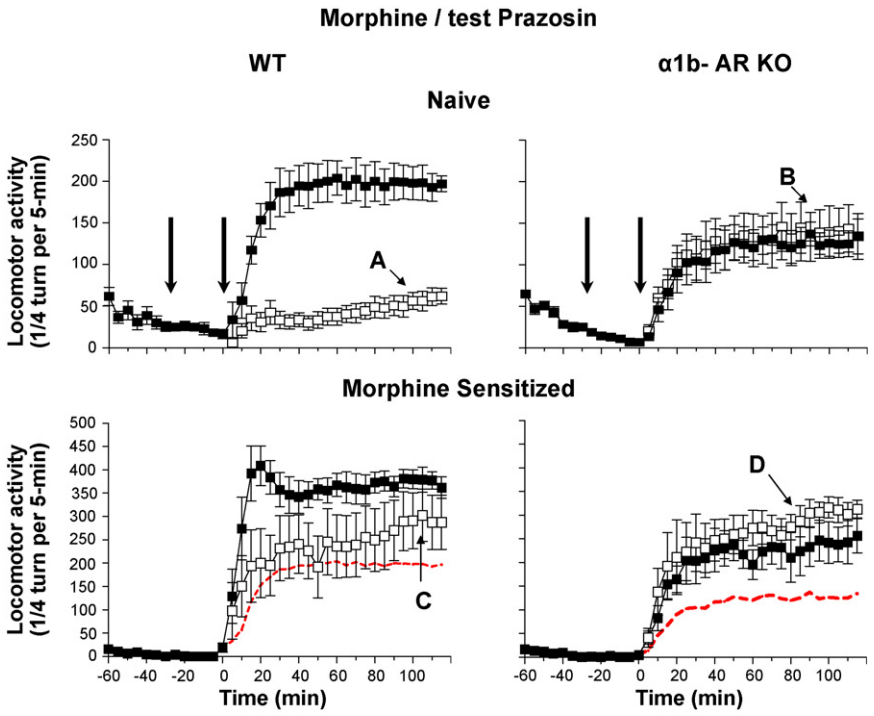

Fig. 1 – Locomotor response to morphine in WT and α1b-AR KO mice in presence of prazosin: animals received either saline (black squares) or prazosin (white squares) (first arrow, *t* = −30 min) and morphine (20 mg/kg second arrow *t* = 0) and locomotor activity was recorded for 120 min. Curve B indicates a 3-fold higher locomotor response than obtained in curve A, whereas curves B and D show similar locomotor activity (see text for explanations). Dotted lines correspond to locomotor response in naïve animals (adapted from ref. [32]).

blocked in α1b-AR KO mice by a SR46349B pre-treatment. Two suggestions may be done following these observations: first, because each antagonist, prazosin or SR46349B, is able to induce an 80% decrease in morphine-induced locomotor response, this suggests that each locomotor component, α1b-adrenergic or 5-HT_{2A}, is not independent. In other words, blockade by prazosin of the α1b-adrenergic receptor may affect not only the locomotor response induced by the α1b-adrenergic receptor stimulation but also the locomotor response induced by the 5-HT_{2A} receptor stimulation. Similarly, blockade of the 5-HT_{2A} receptor by SR46349B may also affect the locomotor response induced by the α1b-adrenergic receptor stimulation. This may explain why each antagonist has an 80% inhibitory effect making both effects more than additive (160%). Along this line, the existence of similarities between α1b-adrenergic and 5-HT_{2A} receptors can be noted. They exhibit analogous autoradiographic localization in the frontal cortex, are linked to the same Gq protein which activates phospholipase C and protein kinase C and their stimulations give identical electrophysiological responses [17,33–36].

Second, the genetic deletion of the α1b-adrenergic receptor has modified the morphine-induced 5-HT_{2A} receptor locomotor component which becomes 3-fold higher in α1b-AR KO mice than in WT littermates (Fig. 1). This is a further indication of the existence of an interaction between the effects of the stimulation of α1b-adrenergic and 5-HT_{2A} receptors, suggesting that the absence of the α1b-adrenergic receptor may remove its inhibitory action on the effects of the stimulation of 5-HT_{2A} receptors. This hypothesis became even more likely when we looked at the inhibitory effects of prazosin and SR46349B on the morphine-induced locomotor responses in animals sensitized to morphine: both components were additive, thus suggesting that both components had become independent in sensitized animals [32].

4. Repeating psychostimulants or opiates administrations induces behavioral sensitization and modifies the inhibitory effects of α1b-adrenergic and 5-HT_{2A} receptors antagonists

Psychostimulants and opiates produce locomotor stimulant effects that become enhanced with repeated intermittent injections. This enhanced behavioral response, named behavioral sensitization, is enduring and can last up to 1 year after drug exposure [37]. Studies of the neurobiological basis of behavioral sensitization have focused, despite conflicting data [38,39], on the midbrain DA system because of evidence suggesting that this system mediates locomotor-stimulation as well as the ability of drugs to elicit craving and to lead to abuse [40]. Indeed, extracellular DA levels in the nucleus accumbens following acute and repeated treatments with psychostimulants either increase, stay unchanged or even decrease with the development of behavioral sensitization [38–42]. For example, Segal and Kuckzenski, in 1992, have shown that increases in extracellular DA levels in the nucleus accumbens induced by D-amphetamine or cocaine were lower in animals previously sensitized to D-amphetamine or

cocaine, respectively, than in naïve animals [38,39]. When increases were observed, they generally occurred after 2 weeks or more of withdrawal. An absence of correlation between the development of behavioral sensitization and changes in subcortical extracellular DA levels can probably be explained by stimulant-induced non-functional DA release in the nucleus accumbens, as described above [12,13,18]. This non-functional DA release seems related to a blunted DA reuptake activity and a clearing of DA from vesicular stores [13]. Non-functional DA release may vary with time and hamper the determination of the functional part of the DA release during repeated treatments and early withdrawal.

Behavioral sensitization has been thought to underlie some aspects of drug addiction [43]. Indeed, it persists many months after the last administration, thus mimicking long-term sensitivity to drugs observed in human addicts. A conceptualization of the role of psychomotor sensitization has been proposed where a shift in an incentive-salience state described as *wanting*, as opposed to *liking*, was hypothesized to be progressively increased by repeated exposure to drugs of abuse and thus sustains drug addiction [44]. In contrast to this idea, others have argued that sensitization only impacts on the initial use of the drug but has little or nothing to do with the development of dependence [45]. However, in this latter case, evidence came from studies indicating that animals with prolonged access to cocaine escalate their intake of cocaine but lose their locomotor sensitization [46], studies which have not been replicated [47,48]. Whatever the hypothesis, we thought that behavioral sensitization is a suitable animal model to study the long-term neurochemical modifications induced by repeated treatments with drugs of abuse. To begin with, we aimed at understanding why, in α1b-AR KO mice, psychostimulants do not induce behavioral sensitization whereas the development of behavioral sensitization to morphine is only partly affected. Wild type and α1b-AR KO mice received therefore four once-a-day treatments of high doses of morphine (20 mg/kg, i.p.) and were tested for their locomotor response to morphine after a 4-day withdrawal. After having verified that this procedure induces behavioral sensitization in both strains, WT and α1b-AR KO mice locomotor responses to morphine were analyzed after a prazosin pre-treatment in naïve and sensitized animals. As previously mentioned, in naïve animals pre-treated with prazosin, locomotor response to morphine was 3-fold higher in α1b-AR KO mice than in WT mice. Interestingly, when the same experiments were performed in sensitized animals, locomotor responses to morphine were identical in WT and α1b-AR KO mice. This is illustrated on Fig. 1 where one can see that curve B (α1b-AR KO mice, prazosin, naïve) is 3-fold higher than curve A (WT mice, prazosin, naïve) and where curve D (α1b-AR KO mice, prazosin, sensitized [similar to α1b-AR KO mice, saline, sensitized]) is superimposable to curve C (WT mice, prazosin, sensitized). We made the hypothesis that, in naïve animals, prazosin is inhibiting the α1b-adrenergic locomotor component plus a significant part of the 5-HT_{2A} locomotor component (curve A). In sensitized animals, prazosin inhibits only the α1b-adrenergic locomotor component and leaves the 5-HT_{2A} locomotor component unaffected (Fig. 1, curve C). In α1b-AR KO mice, the locomotor activity corresponds only to the remaining 5-HT_{2A} locomotor component (curves B and D). Similar data were obtained when morphine-induced locomotor response was analyzed in presence of SR46349B. Briefly, SR46349B inhibited the 5-HT_{2A} component plus a significant part of the α1b-adrenergic locomotor component in WT naïve animals and only the 5-HT_{2A} component in WT sensitized animals [30]. Finally, same data were obtained when effects of prazosin and SR46349B were analyzed on D-amphetamine- and cocaine-induced locomotor response in WT mice.

Altogether, our data suggested that locomotor response induced by psychostimulants and opiates were co-activated by the stimulation of α1b-adrenergic and 5-HT_{2A} receptors in naïve animals. However, in animals sensitized by a repeated treatment with drugs of abuse, each component appears independent (*i.e.* uncoupled and not limited by the other locomotor component), thus explaining a higher reactivity to psychostimulants and opiates observed in sensitized animals. To test the interaction between both neurotransmitter systems, neurochemical and behavioral response to psychostimulants were analyzed in animals deprived of α1b-adrenergic or 5-HT_{2A} receptors.

5. Neurochemical and behavioral response to D-amphetamine or *para*-chloro-amphetamine in mice knockout for 5-HT_{2A} or α1b-adrenergic receptors, respectively

If, as proposed above, noradrenergic and serotonergic transmissions limit each other through α1b-adrenergic and 5-HT_{2A} receptors, it can be assumed that acute D-amphetamine-induced cortical extracellular NE levels would be higher in mice lacking 5-HT_{2A} receptors (5-HT_{2A}-R KO) than in WT mice. We have obtained 5-HT_{2A}-R KO mice from J. Gingrich (Columbia University, New York) and M. Hamon and L. Lanfumey (Hopital Pitié Salpêtrière, Paris, France). Animals were backcrossed for at least seven generations with C57Bl6 mice. We have found that, paradoxically, an acute injection of D-amphetamine (2 mg/kg) in 5-HT_{2A}-R KO mice induces both a higher locomotor response (+97%) and a higher increase in cortical extracellular NE levels (+101%) than in WT mice [22,49]. Then, extracellular prefrontal cortical 5-HT levels were determined in α1b-AR KO mice. However, because D-amphetamine did not modify cortical 5-HT extracellular levels in our experimental conditions, reactivity of serotonergic neurons was estimated following the injection of a 5-HT releaser, *p*-chloro-amphetamine (PCA), a compound analogous to ecstasy and known to induce locomotor hyperactivity and behavioral sensitization in mice [50]. We found that PCA (7 mg/kg) induces both a locomotor response and a +326% increase in extracellular cortical 5-HT basal levels in WT mice. Locomotor activity and increases in cortical extracellular 5-HT levels induced by acute PCA were, however, significantly higher in α1b-AR KO than in WT mice (+230% and +66%, for locomotor activity and cortical extracellular 5-HT levels, respectively) [22].

This clearly suggests the existence in naïve animals of a reciprocal interaction between α1b-adrenergic and 5-HT_{2A} receptors which would control serotonergic and noradrenergic transmissions, respectively. In α1b-AR KO or 5-HT_{2A}-R KO mice, the deletion of one component leaves the other one

without control and renders serotonergic or noradrenergic transmissions hyper-reactive, respectively.

6. Effects of repeated treatments with drugs of abuse on the reactivity of noradrenergic and serotonergic neurons

Previous experiments have shown that repeated treatments with amphetamine or morphine induce behavioral sensitization that is correlated with a decrease in the inhibitory effects of prazosin and SR46349B, indicating some change in a reciprocal relationship between noradrenergic and serotonergic neurons [32]. A microdialysis analysis of the noradrenergic response in 5-HT_{2A}-R KO mice and of the serotonergic one in α1b-AR KO mice has indicated that each system was disinhibited following the genetic deletion of the complementary receptor, 5-HT_{2A} one for NE and α1b-adrenergic one for 5-HT. Altogether this suggested that repeated treatments with amphetamine or morphine may modify the reactivity of noradrenergic and serotonergic neurons. Four days after exposure to a regimen of four once-daily injections of D-amphetamine (2 mg/kg), cortical noradrenergic and serotonergic responses to the respective injections of D-amphetamine or PCA were indeed enhanced when compared with animals repeatedly treated with saline. Increased responding remained elevated for at least 1 month [22]. Moreover, the time-course and the amplitude of the locomotor response and extracellular NE level appeared strikingly similar in the different experimental conditions in naïve as well as in sensitized animals (Fig. 2). Finally, both the D-amphetamine-induced increases in cortical NE levels and behavioral sensitization were not observed when animals were pre-treated, before each of the four once-daily injections of D-amphetamine, with prazosin and SR 46349B, suggesting that stimulation of both receptors was necessary for the development of this sensitization [22]. Same experiments were then performed 4 days after exposure to a regimen of four once-daily injections of morphine (20 mg/kg), cocaine (20 mg/kg) or alcohol (2 g/kg), three compounds belonging to main groups of drugs of abuse. We also found an increased responding of cortical noradrenergic and serotonergic neurons to the respective injections of D-amphetamine or PCA which was also in correlation with a cross-sensitization with D-amphetamine and PCA for the three compounds [51,52]. Moreover, as observed with repeated injections of D-amphetamine, the induction of cross-sensitization as well as the development of a sensitization of noradrenergic and serotonergic neurons was blocked by a pre-treatment with prazosin and SR46349B, suggesting, here again, a prominent role of α1b-adrenergic and 5-HT_{2A} receptors in sensitization and that the mechanisms responsible for this phenomenon are similar whatever the drug of abuse. Our data seem particularly interesting when one considers the different pharmacology of the four substances tested thus far. D-Amphetamine is a catecholamine releaser and cocaine blocks 5-HT, NE and DA reuptake activity. Morphine inhibits noradrenergic neurons [53] and disinhibits midbrain dopamine and serotonin cell firing via the stimulation of μ-opiate receptors located on GABAergic interneurons [25,26,54]. Finally, ethanol enhances GABAA receptor-mediated inhibitory post-synaptic currents and reduces NMDA receptor-mediated excitatory post-synaptic currents [55,56]. It possesses monoamine oxidase inhibiting properties via its metabolite acetaldehyde and increases extracellular 5-HT and DA levels in the nucleus accumbens [1,57]. Once again, this strongly suggests that, although neurochemical mechanisms of drugs of abuse are obviously different, NE-5-HT uncoupling is a central phenomenon in the effects of the repeated consumption of drugs of abuse.

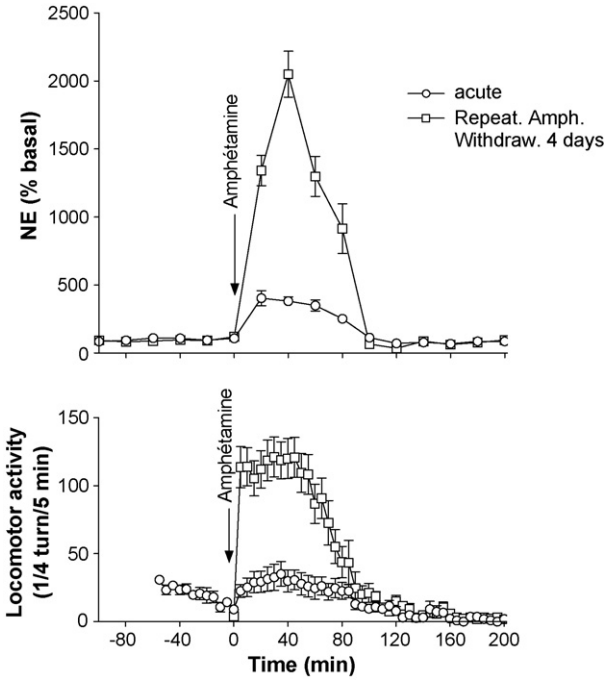

Fig. 2 – Cortical extracellular NE levels and locomotor response to D-amphetamine in naïve mice and in those sensitized to D-amphetamine: animals received an injection of D-amphetamine (2 mg/kg) at t = 0 and their locomotor response and cortical extracellular NE levels were monitored for 200 min. Data indicate dramatic increases both in NE levels and in locomotor response in animals sensitized to D-amphetamine. Moreover, a good correspondence between duration of locomotor and neurochemical responses can be observed (adapted from ref. [22]).

Because of the effects of the α1b-adrenergic and 5-HT_{2A} receptors antagonists, other compounds, which facilitate noradrenergic and serotonergic transmissions but do not induce addiction, such as certain antidepressants, were tested in our model.

7. Two antidepressants, clorimipramine and venlafaxine, do not sensitize noradrenergic and serotonergic neurons

Clorimipramine and venlafaxine are two antidepressants which share the characteristics of inhibiting both NE and 5-HT reuptake activity. They seem interesting because, although they facilitate noradrenergic and serotonergic transmissions, they do not induce addiction. In agreement with this latter

point, when noradrenergic and serotonergic neurons reactivity to D-amphetamine or PCA, respectively, was tested 4 days after a regimen of four once-daily injection of either clorimipramine (20 mg/kg) or venlafaxine (20 mg/kg) no sensitization was observed [51,52]. Similarly, we did not find any behavioral cross-sensitization following D-amphetamine or PCA injections. It is tempting to propose that differences between the two groups of addictive and non-addictive substances are related to differing abilities to increase extracellular DA levels. Venlafaxine, however, was shown to increase by more than 300% basal extracellular DA levels in the rat frontal cortex [58]. This may signify either that drugs of abuse possess a yet unknown common property necessary to induce uncoupling or that non-addictive antidepressants, or their metabolites, exhibit intrinsic pharmacological characteristics which limit noradrenergic and/or serotonergic transmission. For example, the occupation by antidepressants of α1-adrenergic, 5-HT_{2A} or 5-HT_{2C} receptors [59,60] may block, as shown above, the development of uncoupling and behavioral sensitization. It is also possible that noradrenergic and serotonergic neurons sensitization only occurs following a short and intense stimulation of α1b-adrenergic and 5-HT_{2A} receptors, whereas antidepressants induce slow and moderate increase in noradrenergic and serotonergic transmissions.

In any case, it seems that uncoupling between noradrenergic and serotonergic neurons may represent an interesting neurochemical candidate to differentiate between effects of drugs of abuse, which induce addiction, and those of other compounds which do not. At that point, however, the mechanism of uncoupling stays unknown and the fact that venlafaxine does not uncouple although it increases cortical extracellular DA levels is obviously not sufficient to completely exclude the role of DA in uncoupling.

8. Dopamine is not involved in the sensitization of noradrenergic and serotonergic neurons by repeated drugs of abuse

To test whether an increase in extracellular DA levels could induce a sensitization of noradrenergic and serotonergic neurons, animals were treated by a regimen of four once-daily injections of a high dose of GBR12783 (20 mg/kg), a specific inhibitor of DA reuptake. After each injection animals exhibited a high locomotor hyperactivity which did not, however, increase with the repeated injections. Four days after the last injection, animals received D-amphetamine or PCA to measure their noradrenergic and serotonergic neurons reactivity. It was found that both noradrenergic and serotonergic response were not different from those of animals repeatedly treated with saline [51,52]. This finding is in agreement with our previous data indicating that, in the rat, GBR12783 induces locomotor hyperactivity but no behavioral sensitization [61]. Therefore, an increase in extracellular DA levels may be important to trigger a locomotor activation but not sufficient to induce NE/5-HT uncoupling and behavioral sensitization. Actually, we found that even this high dose of GBR12783 (20 mg/kg) did not increase acutely cortical extracellular NE levels, suggesting that NE may be one of the limiting factor to obtain a NE/5-HT uncoupling.

These data prompted us to verify that DA release was not the limiting factor explaining why venlafaxine did not induce NE/5-HT uncoupling. Therefore, animals received a regimen of four once-daily injections of a mixture of venlafaxine (20 mg/kg) and GBR12783 (20 mg/kg) and were tested 4 days after the last injection. Once again, uncoupling of noradrenergic and serotonergic neurons did not occur (Lanteri, personal communication).

As previously mentioned, the role of DA in long-term neurochemical modifications induced by drugs of abuse is a source of controversies. It is generally accepted that D1 or/and D2 receptors antagonists do not block the development of behavioral sensitization to cocaine [62] and morphine [63] but the blockade of D1 receptors by SCH23390 was repeatedly shown to inhibit the development of behavioral sensitization to D-amphetamine [64]. Accordingly, when we have pre-treated animals with systemic SCH23390 before D-amphetamine repeated injections, we found that, after a 4-day withdrawal period, both cortical D-amphetamine-induced extracellular NE levels and PCA-induced extracellular 5-HT levels were identical to those observed in naïve animals [22]. This finding agrees with the possibility of an association between NE/5-HT uncoupling and behavioral sensitization but disagrees with our proposition that uncoupling is not dependent upon DA release. Actually, we found that SCH23390 blocks the acute increase of cortical extracellular NE levels induced by D-amphetamine and leaves unaffected the PCA-induced increase of cortical extracellular 5-HT levels [51,52]. Later on, it was demonstrated that the effects of SCH23390 on D-amphetamine-induced behavioral sensitization and uncoupling were due to its potent 5-HT_{2C} receptor agonist property [65,66]. Indeed, a 5-HT_{2C} receptor antagonist, RS102221, reverses the blockade by SCH23390 of the development of behavioral sensitization to D-amphetamine as well as the acute NE release and noradrenergic sensitization [51]. However, it should be emphasized that, although RS102221 is able to reverse the effects of SCH23390 on the induction of D-amphetamine behavioral sensitization, it does not reverse the expression of behavioral sensitization. This confirms that D1 receptors stimulation is not required for the development of behavioral sensitization to D-amphetamine but is nevertheless necessary for the expression of D-amphetamine behavioral sensitization [64], as it is for the expression of cocaine and morphine sensitization. Altogether, it seems that it is the stimulation of noradrenergic transmission, rather than that of DA, which is related to the development of NE/5-HT uncoupling and/or behavioral sensitization, a remark which disagrees with the role generally assigned to DA in the addictive process.

9. And what about nicotine and tobacco?

Tobacco is a potent reinforcing agent in humans, and nicotine is generally considered to be the major compound responsible for its addictive properties [67–69]. However, animal experiments indicate some discrepancies between the effects of nicotine and those of other drugs of abuse. For example, the capacity of repeated nicotine to elevate DA levels in the nucleus accumbens is controversial [69–71], and repeated nicotine treatments in rats induce a behavioral sensitization which vanishes more quickly than that for other drugs of

abuse [72,73]. Furthermore, although psychostimulants and opiates induce a substantial locomotor hyperactivity both in rats and mice, nicotine is a weak locomotor stimulant in rats and generally fails to induce locomotor hyperactivity in mice at any dose [74–76]. These differences could suggest that the addictive effects of tobacco are not only due to nicotine.

Tobacco and tobacco smoke are known to contain a number of compounds, among which monoamine oxidase inhibitors (MAOIs), such as harmane, norharmane or acetaldehyde, have been the focus of special interest [77–79]. It was also verified that inhibition of monoamine oxidases by tobacco smoke does not result from the actions of nicotine [80], but from that of other compounds also present in other psychotropic plants [79,81]. We have recently shown that MAOI pre-treatment allows the maintenance of behavioral sensitization to nicotine in rats [73], thus suggesting a role of MAOIs in the addictive properties of tobacco. More recently, we used tranylcypromine, a cyclized amphetamine five thousand times as potent an MAOI as amphetamine [82], in association with nicotine. It was found that a pre-treatment with tranylcypromine could trigger a locomotor response to nicotine in mice [83] and nicotine self-administration in rats [83,84] and that 5-HT was crucial for these effects [85]. Nicotine is commonly considered as a monoamine releaser [86,87] that increases serotonergic neurons firing [88–92]. This increased release of 5-HT – in absence of MAOI – is, however, transient. Indeed, an immediate inhibitory retro-control blocking the firing of serotonergic raphe neurons through the stimulation of somato-dendritic 5-HT1A receptors has been described [88,93,94]. This may explain why injection of nicotine alone transiently decreases the extracellular 5-HT levels in the ventral striatum [85]. We therefore propose that MAOIs, because of their enhancing effects on extracellular 5-HT levels, compensate the consequences of the indirect inhibition of serotonergic cells by nicotine. Our data suggest a mechanism by which MAOI's contained in tobacco smoke could act in synergy with nicotine to induce addiction. To confirm this hypothesis, animals received regimen of four once-daily injections of nicotine (1 mg/kg), tranylcypromine (6 mg/kg) or a mixture of nicotine and tranylcypromine. After 4 days withdrawal, data indicate that noradrenergic and serotonergic neuronal sensitization, *i.e.* increases in NE and 5-HT cortical responses to D-amphetamine and PCA, respectively, only occurs when both compounds, nicotine and tranylcypromine, are co-injected. Moreover, as shown previously for other drugs of abuse, these neuronal sensitizations are blocked when animals are pre-treated with prazosin and SR46349B (Salomon et al., unpublished data). These data confirm that uncoupling between noradrenergic and serotonergic neurons, and the neuronal sensitization which follows, may be a critical factor in the consequences of drug abuse.

10. What could be the anatomical and functional substrates for the NE/5-HT coupling and uncoupling?

Our central hypothesis is that the sensitization of noradrenergic and serotonergic neurons is the consequence of the NE/5-HT uncoupling. This suggests that, in naïve animals, it exists a coupling between these two neuronal groups. Anatomical and functional studies between the locus coeruleus and the raphe nuclei indicate that the discharge rate of serotonergic neurons is under the excitatory control of α1-adrenergic receptors [95,96] and, conversely, that serotonergic cells in raphe nuclei hyperpolarize noradrenergic cells in the locus coeruleus by stimulating 5-HT_{2A} receptors on GABAergic interneurons [97] (Fig. 3). It is also possible that coupling between both neurotransmitter systems occurs in the prefrontal cortex where α1b-adrenergic and 5-HT_{2A} receptors are co-localized [22,36] and thus control noradrenergic and serotonergic mesencephalic nuclei [98,99]. Increased cortical extracellular NE and 5-HT levels would stimulate glutamatergic pyramidal cells [10] which excite VTA dopaminergic neurons or project directly to the nucleus accumbens [100] and thus increase locomotor activity. However, although local injection of prazosin into the prefrontal cortex blocked amphetamine-induced locomotor response [12,101], local bilateral injections of SR46349B into the prefrontal cortex or into the VTA have indicated that only those injections done into the latter structure could counteract D-amphetamine-induced locomotor activity [30]. These data suggest that the 5-HT_{2A} receptors implicated in the effects we observe are preferentially located in the VTA. Altogether, this indicates

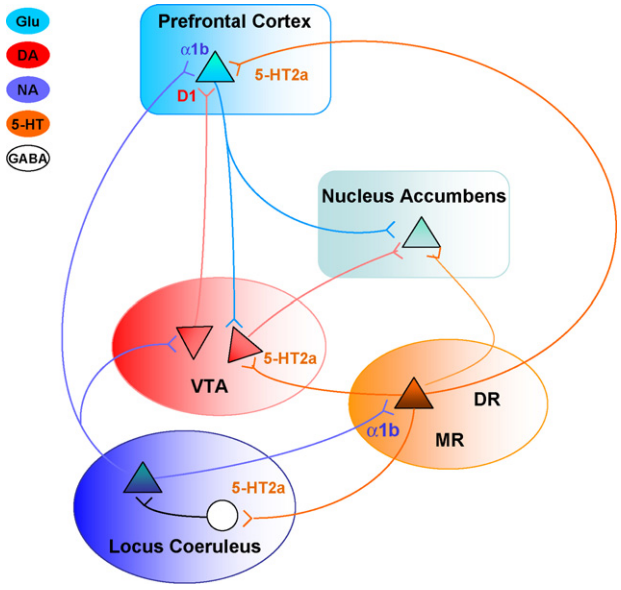

Fig. 3 – Schematic diagram of interactions between noradrenergic and dopaminergic neurons and between noradrenergic and serotonergic neurons. Noradrenergic neurons are coupled to mesolimbic dopaminergic cells through the descending glutamatergic pathways. Noradrenergic and serotonergic neurons activate dopaminergic neurons via cortical α1b-adrenergic receptors and VTA-5-HT_{2A} receptors. Uncoupling of noradrenergic and serotonergic neurons occurs following repeating stimulations of α1b-adrenergic and 5-HT_{2A} receptors by drugs of abuse. These receptors implicated in the coupling/uncoupling can be located in the raphe nuclei and the locus coeruleus, respectively. Abbreviations: VTA, ventral tegmental area; DR and MR, dorsal and median raphe nuclei.

that the effects we observe on noradrenergic and serotonergic neurons are located upstream to the VTA-DA neurons; the noradrenergic activation would be due to the stimulation of cortical α1b-adrenergic receptors, while the serotonergic activation could be due to 5-HT_{2A} receptors located in the VTA.

If we come back to our first hypothesis, *i.e.* the existence of a reciprocal control of locus coeruleus noradrenergic cells by serotonergic neurons arising from the raphe nuclei and, conversely, a noradrenergic control of raphe cells, uncoupling may be the consequence of a dendritic regression. Repeated drugs of abuse have been shown to induce morphological modifications in the nucleus accumbens and the prefrontal cortex [102–104] and, although the effects of psychostimulants and opiates on dendritic spines are different, the reciprocal controls between noradrenergic and serotonergic neurons may be disrupted.

The regulation of gene expression has also been proposed as one molecular mechanism that could mediate stable modifications in the brain [105,106]. However, up to now, it has been difficult to correlate changes in behavior, such as behavioral sensitization, with stable changes in gene expression. Almost all reported changes in transcription factors or nuclear regulatory proteins return to normal within hours or days of perturbation. Recently, the notion that epigenetic mechanisms could be responsible for stable changes has emerged. Without altering the genetic code, epigenetic mechanisms which modify histones by methylation, ubiquitination or acetylation, could mediate stable changes in brain function (see review [107]). Common enzymatic modifications to chromatin structure can regulate gene expression and, as most neurons do not divide, chromatin modifications are sustained within individual cells. In our case, it cannot be excluded that the expression of 5-HT_{2A} and α1b-adrenergic receptors is chronically modified in certain brain structures.

11. Is noradrenergic and serotonergic neuronal sensitization related to drug addiction?

The consequence of uncoupling, as displayed by D-amphetamine and PCA, is an increased reactivity of noradrenergic and serotonergic neurons. Although this has not been demonstrated yet, it is tempting to speculate that the hyper-reactivity of these two neuronal systems also exists when the brain is submitted to external stimuli. In naïve animals, non-specific visual or auditory stimuli lead to an excitation, followed by an inhibition of the unit activity of 5-HT cells of the anterior-dorsal and centralis superior-raphe nuclei [108,109]. There is no habituation, since the repetition of identical stimulations will still trigger the same neuronal responses. In freely moving animals, NE cells of the locus coeruleus also respond to a variety of stimuli, including auditory, visual and tactile, with an excitation–inhibition pattern [110]. However, unlike 5-HT neurons, the response rapidly habituates with repetitive stimuli, with the desensitization developing in parallel with the animal's disinterest in the stimulus. Post-synaptic events due to release of these neuromodulators are also different; it is well known that NE decreases the spontaneous activity of target cells without changing the amplitude of evoked responses, the final effect being an increase of the signal-to-noise ratio. Some experiments have also indicated that NE could potentiate responses to both excitatory and inhibitory inputs with only minimal change in background firing rate [111]. However, 5-HT, at least in the primary cortical somatosensory areas, decreases the signal-to-noise ratio by inhibiting the spontaneous cell activity to a smaller extent than the evoked response [112]. It is clear that all these responses are those most frequently induced by noradrenergic and serotonergic neurons and depend upon the subtype of receptor stimulated. In any case, it seems that NE cells increase the vigilance and focusing of attention in order to optimize the processing of external stimuli, whereas 5-HT cells can protect the central nervous system from too intense external stimuli. Altogether, both systems appear complementary and the possible existence of a strong link between them seems particularly interesting. If we extrapolate the information obtained in naïve animals to human non-addicts, it can be assumed that, because of the mutual regulation between noradrenergic and serotonergic neurons, the amplitude of the activation of one set of neurons induced by peripheral stimuli would be limited by the other group. In human addicts, because of uncoupling, each group of neurons would react to external stimuli as if they were independent and undergo not only higher but also probably desynchronized activations, thus inducing discomfort and distress. Mechanism of uncoupling could be conceptualized as the physiological response of a neuronal network to the effects of repeated injections of drugs of abuse. If one assumes that this adaptation is a homeostatic response, drugs of abuse would drive the neuronal network into a state where the neural link between noradrenergic and serotonergic neurons is no longer functional. Therefore, human addicts would relapse in order to return to a state that, at least temporarily, relieve them from the consequences of the chronic absence of coupling they have endured, a condition that drug consumption would facilitate.

We propose that human addicts are vulnerable to relapse because of the NE/5-HT uncoupling. Although obviously different in most of its behavioral consequence, rodent's behavioral sensitization presents some analogy with the extreme sensitivity to external stimuli observed in human addicts. Behavioral sensitization can not only last up to 1 year [37] but also may decrease after withdrawal [72,73], suggesting that uncoupling is, as mentioned earlier, long-lasting but, in some cases, reversible; future therapeutic treatments of addiction should therefore aim at developing new compounds that accelerate re-coupling of noradrenergic and serotonergic neurons.

12. Conclusion

Our findings indicate that monoaminergic neurons are not independent when they react to environmental stimuli. An activation of noradrenergic neurons is coupled to an increased subcortical dopaminergic transmission while noradrenergic and serotonergic neurons are coupled in such a way that they limit or activate each other as a function of the external situation. We have found that, following repeated consumption of drugs of abuse, noradrenergic and serotonergic

neurons become uncoupled. Because of uncoupling, noradrenergic and serotonergic neurons in sensitized animals and probably in human addicts are autonomous and react independently to environmental stimuli. Taking drugs would allow an artificial re-coupling of these neurons, thus bringing a temporary relief and possibly explaining relapse.

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REFERENCES

[1] Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 1988;85:5274–8.
[2] Montague PR, Hyman SE, Cohen JD. Computational roles for dopamine in behavioural control. Nature 2004;431:760–7.
[3] Pijnenburg AJ, Honig WM, Van Rossum JM. Inhibition of D-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacologia 1975;41:87–95.
[4] Le Moal M, Stinus L, Cardo B. Ventral tegmental lesions in the rat: effects on emotional behavior and variations in the alertness threshold. J Physiol (Paris) 1969;61 (Suppl. 1):148.
[5] Le Moal M, Stinus L, Simon H, Tassin JP, Thierry AM, Blanc G, et al. Behavioral effects of a lesion in the ventral mesencephalic tegmentum: evidence for involvement of A10 dopaminergic neurons. Adv Biochem Psychopharmacol 1977;16:237–45.
[6] Tassin JP, Stinus L, Simon H, Blanc G, Thierry AM, Le Moal M, et al. Relationship between the locomotor hyperactivity induced by A10 lesions and the destruction of the fronto-cortical dopaminergic innervation in the rat. Brain Res 1978;141:267–81.
[7] Tassin JP, Simon H, Glowinski J, Bockaërt J. Modulations of the sensitivity of dopaminergic receptors in the prefrontal cortex and the nucleus accumbens. Relationship with locomotor activity. In: Collu R, et al., editors. Brain Peptides and Hormones. New York: Raven Press; 1982. p. 17–30.
[8] Tassin JP, Studler JM, Herve D, Blanc G, Glowinski J. Contribution of noradrenergic neurons to the regulation of dopaminergic (D_1) receptor denervation supersensitivity in rat prefrontal cortex. J Neurochem 1986;46:243–8.
[9] Taghzouti K, Simon H, Herve D, Blanc G, Studler JM, Glowinski J, et al. Behavioural deficits induced by an electrolytic lesion of the rat ventral mesencephalic tegmentum are corrected by a superimposed lesion of the dorsal noradrenergic system. Brain Res 1988;440:172–6.
[10] Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 1992;320:145–60.
[11] Trovero F, Blanc G, Herve D, Vezina P, Glowinski J, Tassin JP. Contribution of an alpha 1-adrenergic receptor subtype to the expression of the ventral tegmental area syndrome. Neuroscience 1992;47:69–76.
[12] Darracq L, Blanc G, Glowinski J, Tassin JP. Importance of the noradrenaline–dopamine coupling in the locomotor activating effects of D-amphetamine. J Neurosci 1998;18:2729–39.
[13] Darracq L, Drouin C, Blanc G, Glowinski J, Tassin JP. Stimulation of metabotropic but not ionotropic glutamatergic receptors in the nucleus accumbens is required for the D-amphetamine-induced release of functional dopamine. Neuroscience 2001;103: 395–403.
[14] Cavalli A, Lattion AL, Hummler E, Nenniger M, Pedrazzini T, Aubert JF, et al. Decreased blood pressure response in mice deficient of the alpha1b-adrenergic receptor. Proc Natl Acad Sci USA 1997;94:11589–94.
[15] Carson RP, Robertson D. Genetic manipulation of noradrenergic neurons. J Pharmacol Exp Ther 2002; 301:410–7.
[16] Stone EA, Rosengarten H, Lin Y, Quartermain D. Pharmacological blockade of brain alpha1-adrenoceptors as measured by ex vivo [^{3}H]prazosin binding is correlated with behavioral immobility. Eur J Pharmacol 2001;420:97–102.
[17] Drouin C, Darracq L, Trovero F, Glowinski J, Cotecchia S, Tassin JP. Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci 2002;22:2873–84.
[18] Auclair A, Cotecchia S, Glowinski J, Tassin JP. D-Amphetamine fails to increase extracellular dopamine levels in mice lacking alpha1b-adrenergic receptors: relationship between functional and non functional dopamine release. J Neurosci 2002;22:9150–4.
[19] Grenhoff J, Svensson TH. Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. Eur J Pharmacol 1993;233:79–84.
[20] Linner L, Endersz H, Ohman D, Bengtsson F, Schalling M, Svensson TH. Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. J Pharmacol Exp Ther 2001;297:540–6.
[21] Florin SM, Kuczenski R, Segal DS. Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. Brain Res 1994;654:53–62.
[22] Salomon L, Lanteri C, Glowinski J, Tassin JP. Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. Proc Natl Acad Sci USA 2006;103:7476–81.
[23] Murase S, Grenhoff J, Chouvet G, Gonon FG, Svensson TH. Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. Neurosci Lett 1993;157:53–6.
[24] Drouin C, Blanc G, Trovero F, Glowinski J, Tassin JP. Cortical alpha 1-adrenergic regulation of acute and sensitized morphine locomotor effects. Neuroreport 2001;12:3483–6.
[25] Tao R, Auerbach SB. Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. Neuroscience 1995;68:553–61.
[26] Jolas T, Aghajanian GK. Opioids suppress spontaneous and NMDA-induced inhibitory postsynaptic currents in the dorsal raphe nucleus of the rat in vitro. Brain Res 1997;755:229–45.

[27] Sorensen SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK, et al. Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J Pharmacol Exp Ther 1993; 266:684–91.
[28] Moser PC, Moran PM, Frank RA, Kehne JH. Reversal of amphetamine-induced behaviours by MDL 100,907, a selective 5-HT2A antagonist. Behav Brain Res 1996;73:163–7.
[29] McMahon LR, Filip M, Cunningham KA. Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. J Neurosci 2001;21:7781–7.
[30] Auclair A, Blanc G, Glowinski J, Tassin JP. Role of serotonin 2A receptors in the D-amphetamine-induced release of dopamine: comparison with previous data on alpha1b-adrenergic receptors. J Neurochem 2004;91:318–26.
[31] Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Neliat G, et al. Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine2 receptor antagonist. J Pharmacol Exp Ther 1992;262:759–68.
[32] Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin JP. 5-HT_{2A} and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. Eur J Neurosci 2004;20:3073–84.
[33] Pazos A, Cortes R, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 1985;346:231–49.
[34] Lucaites VL, Nelson DL, Wainscott DB, Baez M. Receptor subtype and density determine the coupling repertoire of the 5-HT2 receptor subfamily. Life Sci 1996;59:1081–95.
[35] Aghajanian GK, Marek GJ. Serotonin via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161–71.
[36] Marek GJ, Aghajanian GK. 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. Eur J Pharmacol 1999;367:197–206.
[37] Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology (Berl) 1991;103:480–92.
[38] Segal DS, Kuczenski R. In vivo microdialysis reveals a diminished amphetamine-induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. Brain Res 1992;571:330–7.
[39] Segal DS, Kuczenski R. Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain Res 1992;577:351–5.
[40] Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 1986;396:157–98.
[41] Kuczenski R, Segal DS. Regional norepinephrine response to amphetamine using dialysis: comparison with caudate dopamine. Synapse 1992;11:164–9.
[42] Cadoni C, Solinas M, Di Chiara G. Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. Eur J Pharmacol 2000;388:69–76.
[43] Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioural sensitization: a critical review of preclinical studies. Psychopharmacology 2000;151:99–120.
[44] Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 2003;18:247–91.
[45] Koob G, Le Moal M. Neurobiology of addiction. New York: Academic Press; 2006. p. 447.
[46] Ben Shahar O, Ahmed SH, Koob GF, Ettenberg A. The transition from controlled to compulsive drug use is associated with a loss of sensitization. Brain Res 2004;995:46–54.
[47] Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 2005;58:751–9 [epub August 11, 2005].
[48] Ahmed SH, Cador M. Dissociation of psychomotor sensitization from compulsive cocaine consumption. Neuropsychopharmacology 2006;31:563–71.
[49] Salomon L, Lanteri C, Godeheu G, Blanc G, Gingrich J, Tassin JP. Paradoxical constitutive behavioral sensitization to amphetamine in mice lacking 5-HT_{2A} receptors. Psychopharmacology (Berl), 2007 May 19; [Epub ahead of print] PMID: 17510759.
[50] Itzhak Y, Achat-Mendes CN, Ali SF, Anderson KL. Long-lasting behavioral sensitization to psychostimulants following *p*-chloroamphetamine-induced neurotoxicity in mice. Neuropharmacology 2004;46:74–84.
[51] Lanteri C, Salomon L, Torrens Y, Glowinski J, Tassin JP. Drugs of abuse specifically sensitize noradrenergic and serotonergic neurons via a non dopaminergic mechanism. Neuropsychopharmacology 2007 (in press).
[52] Lanteri C, Salomon L, Glowinski J, Tassin JP. Drugs of abuse may be addictive because they disrupt a mutual regulation between noradrenergic and serotonergic neurons. In: SFN Meeting; 2006 [482.15/NN86].
[53] Korf J, Bunney BS, Aghajanian GK. Noradrenergic neurons: morphine inhibition of spontaneous activity. Eur J Pharmacol 1974;25:165–9.
[54] Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 1992;12:483–8.
[55] Nestoros JN. Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. Science 1980;209:708–10.
[56] Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 1989;243:1721–4.
[57] Yoshimoto K, McBride WJ, Lumeng L, Li TK. Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. Alcohol 1992;9:17–22.
[58] Millan MJ, Dekeyne A, Papp M, La Rochelle CD, MacSweeny C, Peglion JL, et al. S33005, a novel ligand at both serotonin and norepinephrine transporters. II. Behavioral profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. J Pharmacol Exp Ther 2001;298:565–80.
[59] Dekeyne A, Millan MJ. Discriminative stimulus properties of antidepressant agents: a review. Behav Pharmacol 2003;14:391–407.
[60] Van Oekelen D, Luyten WH, Leysen J. 5-HT2A and 5-HT2C receptors and their atypical regulation properties. Life Sci 2003;72:2429–49.
[61] Drouin C, Blanc G, Villégier AS, Glowinski J, Tassin JP. Critical role of alpha1-adrenergic receptors in acute and sensitized locomotor effects of D-amphetamine, cocaine, and GBR 12783: influence of preexposure conditions and pharmacological characteristics. Synapse 2002;43:51–61.

[62] White FJ, Joshi A, Koeltzow TE, Hu XT. Dopamine receptor antagonists fail to prevent induction of cocaine sensitization. Neuropsychopharmacology 1998;18:26–40.
[63] Jeziorski M, White FJ. Dopamine receptor antagonists prevent expression, but not development, of morphine sensitization. Eur J Pharmacol 1995;275:235–44.
[64] Vezina P. D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. J Neurosci 1996;68:259–67.
[65] Bischoff S, Heinrich M, Sonntag JM, Krauss J. The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT2) receptors. Eur J Pharmacol 1986;129:367–70.
[66] Millan MJ, Newman-Tancredi A, Quentric Y, Cussac D. The "selective" dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin2C receptors. Psychopharmacology (Berl) 2001;156:58–62.
[67] Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. Neuron 1996;16:905–8.
[68] Balfour D, Benowitz N, Fagerstrom K, Kunze M, Keil U. Diagnosis and treatment of nicotine dependence with emphasis on nicotine replacement therapy. A status report. Eur Heart J 2000;21:438–45.
[69] Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. Eur J Pharmacol 2000;393:295–314.
[70] Vezina P, Blanc G, Glowinski J, Tassin JP. Nicotine and morphine differentially activate brain dopamine in prefrontocortical and subcortical terminal fields: effects of acute and repeated injections. J Pharmacol Exp Ther 1992;261:484–90.
[71] Balfour DJ, Benwell ME, Birrell CE, Kelly RJ, Al-Aloul M. Sensitization of the mesoaccumbens dopamine response to nicotine. Pharmacol Biochem Behav 1998;59:1021–30.
[72] Ksir C, Hakan R, Hall Jr DP, Kellar KJ. Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 1985;24:527–31.
[73] Villégier AS, Blanc G, Glowinski J, Tassin JP. Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. Pharmacol Biochem Behav 2003;76:267–74.
[74] Marks MJ, Burch JB, Collins AC. Genetics of nicotine response in four inbred strains of mice. J Pharmacol Exp Ther 1983;226:291–302.
[75] Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. Psychopharmacology (Berl) 1999;141:145–53.
[76] Gaddnas H, Pietila K, Ahtee L. Effects of chronic oral nicotine treatment and its withdrawal on locomotor activity and brain monoamines in mice. Behav Brain Res 2000;113:65–72.
[77] Poindexter E, Carpenter R. The isolation of harmane and norharmane from tobacco and cigarette smoke. Phytochemistry 1962;1:215–21.
[78] Breyer-Pfaff U, Wiatr G, Stevens I, Gaertner HJ, Mundle G, Marin K. Elevated norharman plasma levels in alcoholic patients and controls resulting from tobacco smoking. Life Sci 1996;58:1425–32.
[79] Rommelspacher H, Meier-Henco M, Smolka M, Kloft C. The levels of norharman are high enough after smoking to affect monoamine oxidase B in platelets. Eur J Pharmacol 2002;441:115–25.
[80] Carr LA, Basham JK. Effects of tobacco smoke constituents on MPTP-induced toxicity and monoamine oxidase activity in the mouse brain. Life Sci 1991;48:1173–7.
[81] Uelbelhack R, Franke L, Schewe HJ. Inhibition of platelet MAOB by kavapyrone-enriched extract from Piper Methysticum Foster (kava-kava). Pharmacopsychiatry 1998;31:187–92.
[82] Zirkle CL, Kaiser C. Monoamine oxidase inhibitors (nonhydrazines). In: Psychopharmacological agents - I, ed., New York: Academic Press; 1964. pp. 474–5.
[83] Villegier AS, Salomon L, Granon S, Changeux JP, Belluzzi JD, Leslie FM, et al. Monoamine oxidase inhibitors allow locomotor and rewarding responses to nicotine. Neuropsychopharmacology 2006;31:1704–13.
[84] Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M, et al. Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. J Neurosci 2005;25:8593–600.
[85] Villegier AS, Salomon L, Blanc G, Godeheu G, Glowinski J, Tassin JP. Irreversible blockade of monoamine oxidases reveals the critical role of 5-HT transmission in locomotor response induced by nicotine in mice. Eur J Neurosci 2006;24:1359–65.
[86] Summers KL, Giacobini E. Effects of local and repeated systemic administration of (−)nicotine on extracellular levels of acetylcholine, norepinephrine, dopamine, and serotonin in rat cortex. Neurochem Res 1995;20:753–9.
[87] Summers KL, Lippiello P, Giacobini E. A microdialysis study of the effects of the nicotinic agonist RJR-2403 on cortical release of acetylcholine and biogenic amines. Neurochem Res 1996;21:1181–6.
[88] Li X, Rainnie DG, McCarley RW, Greene RW. Presynaptic nicotinic receptors facilitate monoaminergic transmission. J Neurosci 1998;18:1904–12.
[89] Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature 1999;398:805–10.
[90] Olausson P, Engel JA, Soderpalm B. Involvement of serotonin in nicotine dependence: processes relevant to positive and negative regulation of drug intake. Pharmacol Biochem Behav 2002;71:757–71.
[91] Olausson P, Akesson P, Engel JA, Soderpalm B. Effects of 5-HT1A and 5-HT2 receptor agonists on the behavioral and neurochemical consequences of repeated nicotine treatment. Eur J Pharmacol 2001;420:45–54.
[92] Olausson P, Akesson P, Petersson A, Engel JA, Soderpalm B. Behavioral and neurochemical consequences of repeated nicotine treatment in the serotonin-depleted rat. Psychopharmacology (Berl) 2001;155:348–61.
[93] Mihailescu S, Palomero-Rivero M, Meade-Huerta P, Maza-Flores A, Drucker-Colin R. Effects of nicotine and mecamylamine on rat dorsal raphe neurons. Eur J Pharmacol 1998;360:31–6.
[94] Engberg G, Erhardt S, Sharp T, Hajos M. Nicotine inhibits firing activity of dorsal raphe 5-HT neurones in vivo. Naunyn Schmiedebergs Arch Pharmacol 2000;362:41–5.
[95] Baraban JM, Aghajanian GK. Suppression of serotonergic neuronal firing by alpha-adrenoceptor antagonists: evidence against GABA mediation. Eur J Pharmacol 1980;66:287–94.
[96] Bortolozzi A, Artigas F. Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. Role of alpha-adrenoceptors. Neuropsychopharmacology 2003;28:421–34.
[97] Szabo ST, Blier P. Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT(2A) receptor antagonism on the firing activity of norepinephrine neurons. J Pharmacol Exp Ther 2002;302:983–91.
[98] Gobert A, Millan MJ. Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and

noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 1999;38:315–7.
[99] Amargos-Bosch M, Adell A, Bortolozzi A, Artigas F. Stimulation of alpha1-adrenoceptors in the rat medial prefrontal cortex increases the local in vivo 5-hydroxytryptamine release: reversal by antipsychotic drugs. J Neurochem 2003;87:831–42.
[100] Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 1996;16:1550–60.
[101] Blanc G, Trovero F, Vezina P, Herve D, Godeheu AM, Glowinski J, et al. Blockade of prefronto-cortical alpha 1-adrenergic receptors prevents locomotor hyperactivity induced by subcortical D-amphetamine injection. Eur J Neurosci 1994;6:293–8.
[102] Robinson TS, Kolb B. Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 2004;47(Suppl. 1):33–46 [review].
[103] Szumlinski KK, Abernathy KE, Oleson EB, Klugmann M, Lominac KD, He DY, et al. Homer isoforms differentially regulate cocaine-induced neuroplasticity. Neuropsychopharmacology 2006;31:768–77.
[104] Kalivas PW, Hu XT. Exciting inhibition in psychostimulant addiction. Trends Neurosci 2006;29:610–6.
[105] Hyman SE, Malenka RC. Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2001;2:695–703.
[106] Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2001;2:119–28.
[107] Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 2007;8:355–67.
[108] Heym J, Trulson ME, Jacobs BL. Raphe unit activity in freely-moving cats: effects of phasic auditory and visual stimuli. Brain Res 1982;232:29–39.
[109] Rasmussen K, Heym J, Jacobs BL. Activity of 5-HT-containing neurons in nucleus centralis superior of freely moving cats. Exp Neurol 1984;83:302–17.
[110] Aston-Jones G, Bloom FE. NE-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious experimental stimuli. J Neurosci 1981;1:876–86.
[111] Woodward DJ, Moises HC, Waterhouse BD, Hoffer BJ, Freedman R. Modulatory actions of norepinephrine in the central nervous system. Fed Proc 1979;38:2109–16.
[112] Waterhouse BD, Hylan CM, Woodward DJ. Interaction of serotonin with somatosensory cortical neuronal responses to afferent synaptic inputs and putative neurotransmitters. Brain Res Bull 1986;17:507–18.