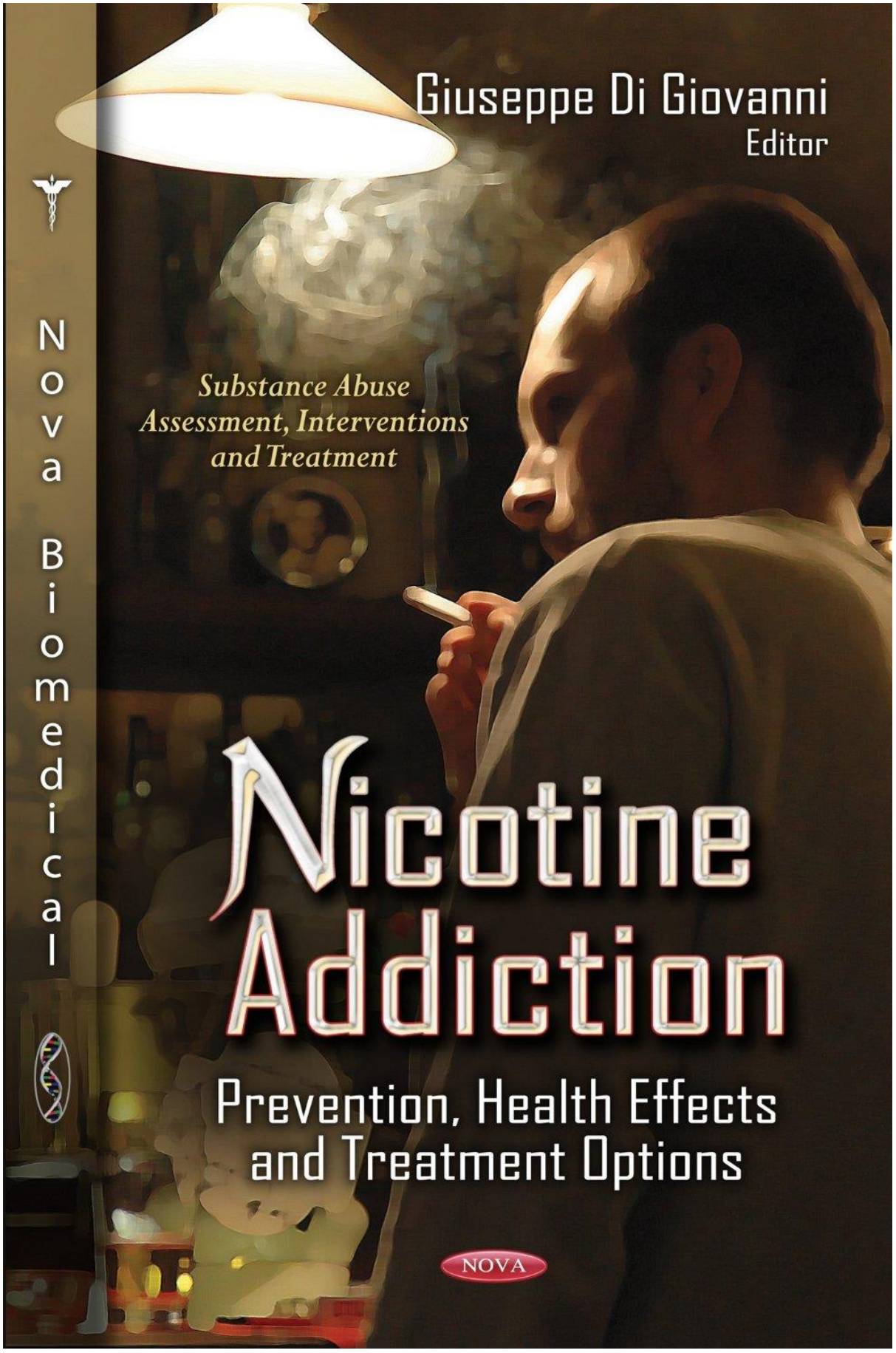
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Chapter 2

SEROTONIN-DOPAMINE INTERACTION IN NICOTINE ADDICTION: FOCUS ON 5-HT$_{2C}$ RECEPTORS

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ABSTRACT

Central dopaminergic systems play a critical role in the regulation of normal and abnormal behaviors. Recent evidence suggests that a dysfunction of dopamine (DA) and serotonin (5-HT) neurotransmitter systems contribute to various pathological conditions. Substantial evidence indicates that the mesolimbic pathway, particularly the DA cells

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innervating accumbal areas, is implicated in the reward value of both natural and drug reinforcers, such as sexual behavior or psychostimulants, respectively. Nicotine, the major psychoactive agent present in tobacco, acts as a potent addictive drug both in humans and laboratory animals. The locomotor activation and the reinforcing effects of nicotine may be related to its stimulatory effects on the mesolimbic dopaminergic function.

Thus, it is now well established that nicotine can increase *in vivo* DA outflow in the nucleus accumbens and the corpus striatum. The stimulatory effect of nicotine on DA release most probably results from its ability to excite neuronal firing rate and to increase bursting activity of DA neurons within the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), together with its facilitatory activity on DA terminal release in the corpus striatum and the nucleus accumbens. The neurochemical data are consistent with neuroanatomical findings showing the presence of nicotinic acetylcholine receptors (nAChRs) in the SNc, VTA, and in projection areas of the central dopaminergic system such as the corpus striatum and the nucleus accumbens. Several lines of evidence indicate that the reinforcing properties of drugs of abuse, including nicotine, can be affected by the serotonergic system which may act by modulating central dopaminergic function.

In this paper, the effects of 5-HT_{2C} receptors on DA function in relation to the neurobiological mechanisms underlying nicotine addiction will be reviewed, and the possible strategies with 5-HT_{2C} agents for new pharmacological treatments of nicotine dependence will be examined.

INTRODUCTION

There is now an extensive scientific literature regarding the functional interaction between serotonin (5-HT) and dopamine (DA) containing neurons in the brain [1-3]. In some cases, the nature of these interactions depends upon the baseline activity of DA and/or 5-HT systems, and whether they are activated. In addition, most of the effects of 5-HT on DA neurons are indirect, involving 5-HT modulation of complex neuronal circuitry, rather than direct effects on DA cell bodies. In recent years, research on this matter has been spurred by new important insights on the molecular biology of 5-HT receptor subtypes and by the availability of 5-HT receptor knockout mice [4, 5].

Central serotonergic and dopaminergic systems play an important role in regulating normal and abnormal behaviors [1, 6]. Dysfunctions of 5-HT and DA neurotransmission are involved in the pathophysiology of various neuropsychiatric disorders including schizophrenia, depression and drug abuse [1, 6, 7]. Thus, the development of a number of relatively selective pharmacological agents specific 5-HT receptor subtypes, has allowed investigators to better understand the functional role of these receptors in the control of central DA-ergic function [8-12].

In this chapter we will focus on the role of the 5-HT_{2C} receptor subtype in nicotine addiction. Therefore, the physiology, pharmacology and anatomical distribution of the 5-HT_{2C} receptors in the CNS, as well as experimental data regarding the effect of 5-HT_{2C} selective agents on the nicotine central effects, will be reviewed after a brief description of the functional neuroanatomy of dopaminergic and serotonergic systems. Finally, the potential use of 5-HT_{2C} agents in the treatment of nicotine addiction and as a tobacco smoking cessation aid will be also discussed.

DOPAMINE SYSTEMS

Dopamine-containing neurons of the ventral mesencephalon have been denominated as A8, A9 and A10 cell groups: these neurons collectively constitute the mesotelencephalic DA system [13]. Historically, the mesolimbic DA system was defined as originating in the A10 cells of the ventral tegmental area (VTA) and projecting to structures closely associated with the limbic system. This system was considered to be separated from the nigrostriatal DA system, which originates from the more lateral substantia nigra pars compacta (SNc, A9 cell group) [13-16].

The mesolimbic and mesocortical DA systems appear critically involved in modulation of the functions subserved by cortical and limbic regions such as motivation, emotional control and cognition [17]. Substantial evidence indicates that the mesolimbic pathway, particularly in the DA cells innervating accumbal areas, is implicated in the rewarding value associated with both natural and drug reinforcers, such as sexual behavior, food and psychostimulants [6, 18]. Animal studies have shown that lesion of DA terminals in the nucleus accumbens reduces the exploratory activity, enhances the latency to the initiation of motor responses, induces disturbances in organizing complex behaviors and in switching from one to another behavioural activity [17]. Hence the mesolimbic DA system seems important for acquisition and regulation of goal-directed behaviours, established and maintained by natural or drug reinforcers [17, 19].

The medial prefrontal cortex is generally associated with cognitive functions including working memory, planning and execution of behaviours, inhibitory control of responses and maintenance of focused attention [17]. In addition, the mesolimbic DA pathway is sensitive to a variety of physical and psychological stressors [20].

The nigrostriatal DA system, which originates from the substantia nigra (A9 cell group), is one of the best studied, because of its involvement in the pathogenesis of Parkinson's disease [21-23]. In mammals, the substantia nigra (SN) is a heterogeneous structure that includes two distinct compartments: the SNc and the substantia nigra pars reticulata (SNr). The SNc represent the major source of striatal DA and its degeneration causes PD. On the contrary, the SNr mainly contains GABA-ergic neurons which constitute one of the major efference of the basal ganglia circuitry [22].

SEROTONIN SYSTEMS

Virtually all parts of the central nervous system receive innervation from serotonergic fibers arising from cell bodies located in the the dorsal nucleus (DRN) and the median raphé nucleus (MRN), the two main subdivisions of the midbrain serotonergic nuclei [24-29]. Serotonin-containing neurons of the raphé nuclei send projections to dopaminergic cells of both the VTA and SN, and to their terminal fields in the nucleus accumbens, prefrontal cortex and striatum [25-29] (Figure 1). Electron microscopy demonstrates the presence of synaptic contacts of [^{3}H]5-HT labeled terminals with both dopaminergic and non-dopaminergic dendrites in all subnuclei of the VTA, and in the SNc and SNr [15, 26, 29].

The precise nature of the interaction between 5-HT and DA is difficult to elucidate, in that both inhibitory and excitatory roles for 5-HT have been suggested. However, these

discrepancies may be attributable to the differential distribution and to the diverse functional roles of 5-HT receptor subtypes within the dopaminergic systems [2, 30, 31]. Thus, much attention has been devoted to the role of 5-HT receptors in the control of central DA activity, because of their implication in the pathophysiology of the diseases that affect central DA systems, such as schizophrenia, depression, drug abuse and PD. In this review we will focus on the 5-HT_{2C} receptor subtype that seems to play an inhibitory role in many nicotine-addiction related behaviors in animals.

5-HT RECEPTORS LOCALIZATION

The diverse physiological effects of serotonin in the brain are mediated by a variety of distinct receptors. These receptors are presently divided into seven classes (5-HT_1 – 5-HT_7), which are then subdivided into subclasses based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms, with a total of at least 14 different receptors [5, 30, 31]. With the exception of the ionotropic 5-HT_3 receptor, 5-HT receptors are G-protein coupled receptors (metabotropic) and act through intracellular signaling pathways to hyperpolarize, in the case of 5-HT_1 receptors, or depolarize, in the case of the remaining 5-HT receptor classes, their host cells [31]. In this chapter we will limit to the description of 5-HT_2 receptor family.

The 5-HT_2 Receptor Family

5-HT_2 receptors form a closely related subgroup of G-protein-coupled receptors, functionally linked to the phosphatidylinositol hydrolysis pathway and currently classified as 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes [30-32]. There is a high sequence homology (> 80% in the transmembrane regions) between the mouse, rat and human 5-HT_{2C} receptors [31], and it is not surprising that many compounds bind with high affinity to all these three receptor subtypes.

5-HT_{2C} receptors are widely distributed throughout the brain and have been proposed to represent the main mediators of the different actions of 5-HT in the central nervous system [30-32]. High levels of 5-HT_{2C} mRNA or protein expression have been found in the choroid plexus, the frontal cortex, limbic structures such as hippocampus, septum and hypotalamus, and also in the striatum, nucleus accumbens, rhombencephalon and spinal cord. The presence of these receptors has also been demonstrated on DA and non-DA cells in the VTA, SNc and the SNr [33-37]. The regional and cellular distribution of 5-HT_{2C} receptors has also been investigated in the human brain. The main sites of mRNA 5-HT_{2C} receptors or protein expression, were the choroid plexus, cerebral cortex, hippocampus, amygdala, some components of the basal ganglia and other limbic structures [38, 39], suggesting that this receptor might be involved in the regulation of different human brain functions, as well as in the pathophysiology of several mental disorders [8-12, 40, 41]. There is now evidence that the 5-HT_{2C} receptor is mainly located postsynaptically within dopaminergic, GABA-ergic, cholinergic, substance P, dynorphin and other systems [31, 37, 42-44]. Interestingly, the studies by Eberle-Wang et al. [43] showed the presence of 5-HT_{2C} mRNA within inhibitory

GABA-ergic interneurons making direct synaptic contact with SNc and VTA dopaminergic cell bodies. Other immunohistochemical and electrophysiological studies demonstrated an important role of 5-HT_{2C} receptors, localized on non-DA neurons, presumably GABA-ergic, in the regulation of DA cells in the VTA [37, 45, 46], medial prefrontal cortex [47] and in the SNc [48, 49].

Recent studies found a somatodentritic localization of 5-HT_{2A} receptors on DA neurons in both the parabrachial and paranigral subdivisions of the VTA [50, 51], which project mainly to the prefrontal cortex and nucleus accumbens, respectively. In addition, 5-HT_{2A} immunoreactivity was also expressed on non-DA cells in the VTA, providing a potential anatomical basis for the modulation of DA neurons within this area, either directly, by 5-HT_{2A} receptors localized on DA cell, or indirectly, through receptors present on non–DA (presumably GABA-ergic) neurons [50, 51]. These receptors were also found at high concentrations in various cortical regions [34, 50]. It is likely that 5-HT_{2A} receptors could affect DA function by acting at the level of dopaminergic nerve terminals, although no direct evidence for the presence of 5-HT_{2A} receptors on such terminals has been provided so far.

Using sensitive techniques, several groups have also shown the presence of both 5-HT_{2B} receptor mRNA and protein [52] in the rat brain, including midbrain regions. Although there are regional differences in the distribution of these receptors, they are all expressed in the brain with extensive pharmacological and functional similarities, so that it is often difficult to ascribe particular functions to a specific receptor subtype.

EFFECTS OF NICOTINE ON CENTRAL DOPAMINERGIC FUNCTION

There is an extensive scientific literature indicating that nicotine can stimulate central dopaminergic function. Thus, it is now a paradigmatic assumption that the locomotor activation and the reinforcing effects of nicotine are related to its stimulatory effects on the mesolimbic dopaminergic function [53-59].

Although the exact mechanisms by which nicotine stimulates DA neuron activity are still not completely understood, there is evidence that it can directly depolarize DA-containing neurons by activating somatodendritic nicotinic receptors which, in this region, are mostly constituted by the assembly of $\alpha4\beta2$ subtypes [60-65]. Moreover, nicotine can indirectly increase DA activity by eliciting the release of glutamate (GLU) from nerve terminals synapsing on DA neurons, and by depressing the inhibitory GABA-ergic input to these neurons [64, 66]. Furthermore, various studies have suggested that nitric oxide (NO) functions as a neuronal messenger in nicotine effects subsequent to glutamatergic activation [67, 68]. Nevertheles, a direct NO/DA interaction is also plausible. Indeed, there is evidence that NO can be endogenously released by acute nicotine injection from nerve cells, acting at nAChRs increasing Ca^{2+} intracellular concentration [69] independently from the well known NMDA GLU receptor-mediated NO release [70]. NO seems to be necessary for the hedonic nicotine-induced increase in firing rate and pattern of DA cells and consequent DA release in taget areas [67, 71].

In addition to its stimulatory effects on neuronal DA firing rate, nicotine has been shown to increase DA release from DAergic terminals in the corpus striatum and the nucleus accumbens [72-75] (Figure 1).

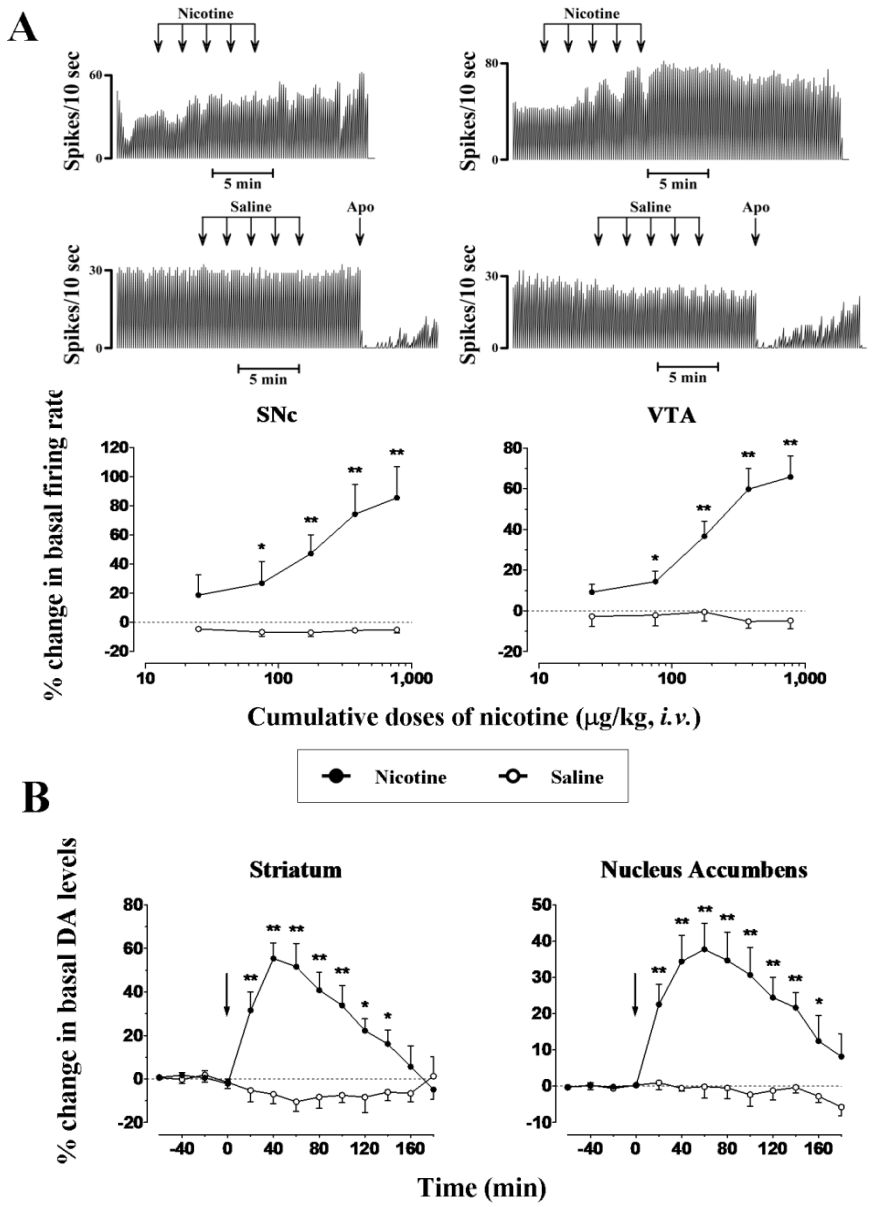

Figure 1. Systemic administration of nicotine increases the activity of the both nigro-striatal and meso-corticolimbic DAergic systems of drug-naïve rats *in vivo*. (A) The injection of cumulative doses of nicotine (25-775 μg/kg, i.v.) increased the firing rate of DA neurons recorded using *in vivo* single unit extracellular recording technique. The rate meters on top show the effect of nicotine administration on single DAergic neurons' firing rates compared to controls (saline, 100 μl i.v., at arrows), while the graphs below report the cumulative dose-response curves showing the mean percentage changes (±S.E.M.) in firing rate after either nicotine or saline administration. Arrows indicate the time of nicotine or saline injections; apomorphine (Apo), a D1/D2 receptors agonist, was injected (10-30 μg/kg, i.v.) at the end of most of experiments to confirm the DAergic identity of recorded neurons (adapted from [81]). (B) Time course of nicotine (1 mg/kg, i.p.) induced effect on DA terminal release in both the striatum and nucleus accumbens. Changes in DA extracellular levels were assessed using *in vivo* microdialysis on freely-moving animals. The arrows indicate the time of injection of either nicotine or saline (adapted from [78]).

It appears, however, that the action of nicotine on terminal areas of the mesolimbic DA system is less relevant as compared with the effect exerted by this drug in the VTA [74]. Thus, it has been shown that intra-VTA infusion of nicotine causes a more robust and prolonged increase of accumbal DA release as compared with intra-accumbens nicotine administration [74]. These neurochemical findings are consistent with behavioral data showing that nicotine-stimulated locomotor activity and self-administration are mediated by a direct action of this drug on DA neurons in the VTA [53, 56]. Although some studies claim no significant differences between the effects of nicotine on striatal and accumbal DA release, more compelling evidence shows that nicotine preferentially stimulates DA release in the nucleus accumbens [76, 77]. Nevertheless, we found that the peak effect of nicotine was more pronounced in the corpus striatum (+ 65%) than in the nucleus accumbens (+ 38%) [78] (Figure 1B). However, it is important to point out that nicotine was found to cause a preferential stimulation of DA release in the shell portion of the nucleus accumbens [79, 80], whereas in the Di Matteo et al. [78] study the microdialysis probes were mostly positioned at the transition border between shell and core. Neurochemical data are consistent with single-cell recordings showing that nicotine can increase the firing rate and the bursting activity of DA containing neurons in the SNc and the VTA [60-62, 81-85].

Several reports show that repeated nicotine exposure enhances its locomotor and reinforcing effects in rodents [54, 86-90], a phenomenon known as sensitization which is common to psychostimulant drugs [86, 91-93]. Moreover, repeated nicotine administration potentiates its stimulatory effects on DA overflow in the nucleus accumbens [86-88, 94] and increases the levels of mRNA for tyrosine hydroxylase (TH), the rate-limiting enzyme in DA biosynthesis, in the SN and the VTA [95]. This potentiation of nicotine effect might result, at least in part, from up-regulation of nAChRs following chronic exposure to nicotine [96-98]. Consistent with the data mentioned above, it has been shown that repeated administration of nicotine for 10 consecutive days caused a significant increase of basal DA outflow, which was particularly evident in the corpus striatum [78]. Those findings are in agreement with previously published data showing an enhanced basal DA outflow in the nucleus accumbens of rats treated daily with subcutaneous injections of nicotine (0.03–0.10 mg/kg) for 7 days [90].

These laboratory data might have clinical relevance since it has been shown that prior repeated nicotine exposure increases the control over behaviour by a conditioned reinforcer and enhances the ability of a conditioned stimulus to control behaviour in extinction [99]. Moreover, these findings may have important implications for studies investigating the consequences of repeated nicotine exposure on incentive motivational processes and expand our understanding of the mechanisms that mediate compulsive smoking behaviour and relapse, which may provide novel insight into treatments for smoking cessation [90]. In addition, repeated nicotine exposure augments the incentive salience of reward-associated cues in general. This effect of nicotine may be particularly important because nicotine is extensively co-abused with other addictive drugs and could thus promote the use of alcohol and illicit drugs by enhancing the vulnerability of cue-induced craving and relapse in general.

However, it does not seem that the potentiation of nicotine effect following its chronic administration is due to an increased firing activity of mesolimbic dopaminergic neurons inasmuch as no changes in nicotine-induced excitation of DA neurons in the VTA were found after repeated administration of this drug by Pierucci et al. [81] (Figure 2A).

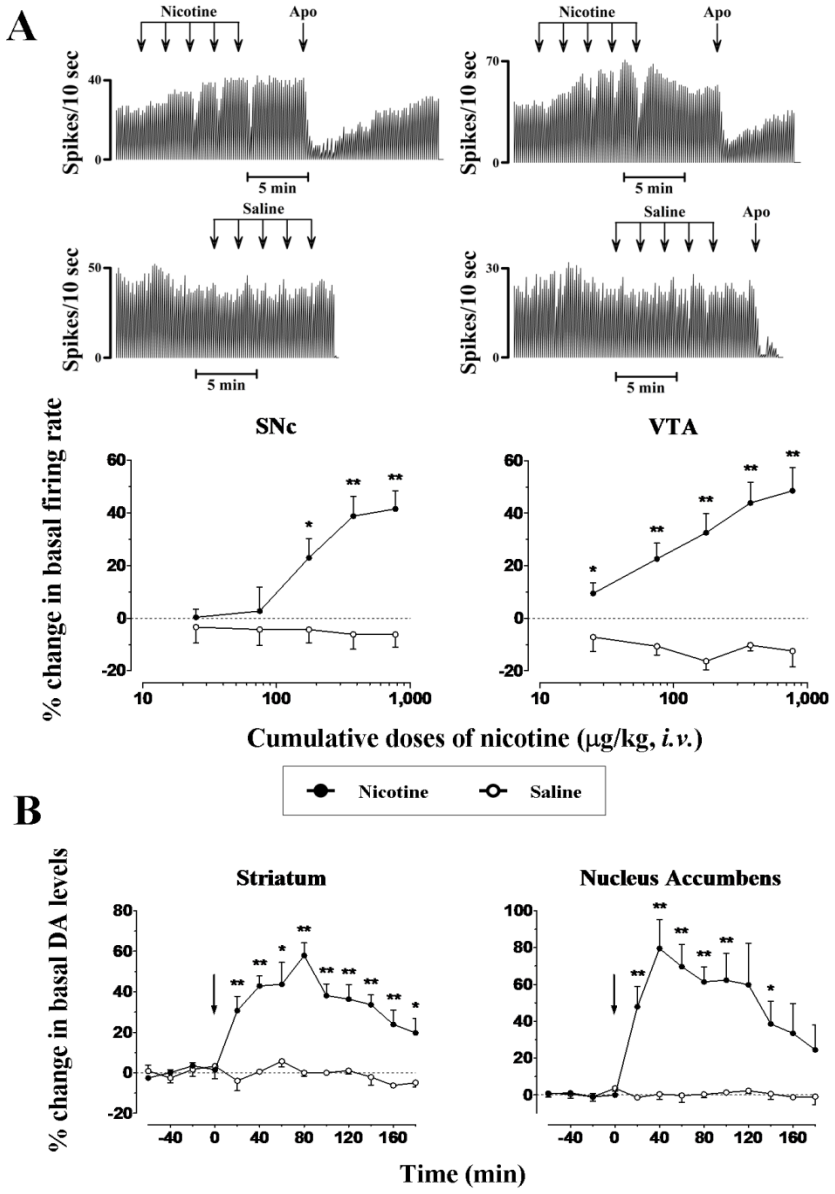

Figure 2. Systemic administration of nicotine increases the activity of the both nigro-striatal and meso-corticolimbic DAergic systems *in vivo* of rats chronically treated with nicotine (1 mg/kg, i.p.) for 10 consecutive days. (A) The injection of cumulative doses of nicotine (25-775 μg/kg, i.v.) increased the firing rate of DA neurons recorded using *in vivo* single unit extracellular recording technique. The rate meters on top show the effect of nicotine administration on single DAergic neurons' firing rates compared to controls (saline, 100 μl i.v., at arrows), while the graphs below report the cumulative dose-response curves showing the mean percentage changes (±S.E.M.) in firing rate after either nicotine or saline administration. Arrows indicate the time of nicotine or saline injections; apomorphine (Apo), a D1/D2 receptors agonist, was injected (10-30 μg/kg, i.v.) at the end of most of experiments to confirm the DAergic identity of recorded neurons (adapted from [81]). (B) Time course of nicotine (1 mg/kg, i.p.) induced effect on DA terminal release in both the striatum and nucleus accumbens. Changes in DA extracellular levels were assessed using *in vivo* microdialysis on freely-moving animals. The arrows indicate the time of injection of either nicotine or saline (adapted from [78]).

In addition, acute challenge with nicotine in rats treated repeatedly with this drug was still capable of significantly increasing bursting activity of DA neurons in the VTA but not in the SNc [81]. These data are consistent with the hypothesis that tolerance to the stimulatory effect of nicotine occurs in the nigrostriatal but not in the mesolimbic dopaminergic system after repeated nicotine administration.

EFFECTS OF NICOTINE ON CENTRAL SEROTONERGIC FUNCTION

Several lines of evidence indicate that the reinforcing properties of drugs of abuse, including nicotine, can be affected by a number of transmitter systems which may act by modulating central dopaminergic function. Among these, the role played by 5-HT seems particularly important. Serotonin contributes to mood, depression, and psychiatric illness, and several animal studies suggest that changes in serotonin transmission may also contribute to the affective symptoms of nicotine withdrawal [100]. Indeed, nicotine has an antidepressant action [101] and the prevalence of smoking is much higher amongst people suffering from depression [102]. Previous anatomic studies demonstrated the presence of α4, α7, and β2 subunit-containing nAChRs in the DRN [103-106]. The nAChR α7 subunit was found on large diameter DRN neurons (15–25 μm in diameter) that colocalized with tryptophan hydroxylase [107] and small-diameter neurons (5–10 μm), that are usually GABAergic [103]. The cholinergic input to the serotonergic DRN cells is substained by the pedunculopontine tegmentum [108]. Electrophysiological *in vitro* studies have revealed the influence of nicotine on 5-HT neuronal activity. The majority of serotonergic DRN neurons increase their action potential firing in response to nicotine administration, leading to an increased 5-HT release in target areas [109-111]. Nicotinic receptors also indirectly affect excitability of DRN neurons by modulating their synaptic drive. Nicotine enhances excitatory glutamatergic inputs to a subset of DRN-NAc projection neurons, while inhibitory GABAergic inputs are modulated either positively or negatively in a subset of these neurons. On the other hand, *in vivo* systemic nicotine was reported to decrease the firing rate in the majority of DRN neurons and increased firing in only a small subset [112]. Strikingly, nicotine failed to show any effect when locally applied within the DRN. The *in vivo* evidence does not agree with *in vitro* results, but it is important to note that serotonergic neurons are strongly influenced by the animal's arousal state (sleep-waking) [113]. Thus it is possible that the effect of nicotine on DRN neuron firing rate *in vivo* is influenced by anesthesia and/or the time of the day. Thus acute exposure to nicotine might excite DRN neurons by direct depolarization and indirect synaptic modulation. Given the excitatory effects of nicotine on DRN neurons, serotonin likely contributes to the rewarding effects of nicotine.

Moreover, there are also several behavioural actions of nicotine that seem to be mediated by the serotonergic system [100]. Thus, nicotine and the nAChRs agonist RJR-2403 significantly increased cortical 5-HT release in awake, behaving rats [114]. Nicotinic binding sites have not been observed on serotonergic axon terminals in the cortex [115]. It has thus been suggested that the increase in 5-HT release is likely due to stimulation of nAChRs on cortically projecting cell bodies in the DRN [114].

Acetylcholine, nicotine, and the potent nicotinic receptor agonists epibatidine and cytisine significantly increased [^{3}H]5-HT release from striatal synaptosomes, an effect

blocked by the non-competitive nicotinic receptor antagonist, mecamylamine [116]. Moreover, the nicotine-stimulated increase in 5-HT release from striatal slices was significantly enhanced after 10 days of nicotine treatment [117] and the effects of chronic nicotine were enhanced by exposure to stress [118]. The nicotinic receptor agonists, 1,1-dimethyl-4-phenylpiperarazinium (DMPP),lobeline and nicotine have been shown to increase [^{3}H]5-HT release from rat hippocampal slices, although cytisine, epibatidine, and nicotine had no effect [119, 120] while Reuben and Clarke [116] found that nicotine had no effect. Contrasting data also exists on the effect of chronic treatment with nicotine on 5-HT concentrations in the dorsal hippocampus; both decrease [121] and increase has been described [122]. It is likely that the effect of nicotine on the brain serotonergic system depends on the duration of treatment. Both acute and chronic systemic administration of nicotine in rats increase 5-HT levels in the hypothalamus, although the rates of 5-HT synthesis were unaffected [100]. A series of experiments suggest the existence of multiple populations of functional nAChRs controlling, directly or indirectly, 5-HT release in the spinal cord. The first, activated by agonist application, has an excitatory effect and could be directly located on serotonergic terminals; the second, tonically activated by endogenous ACh and inhibited by nicotinic receptor antagonists, has an inhibitory outcome and is present in inhibitory neurons [100].

Overall, there is good evidence that nicotine increases 5-HT neuronal activity and its release in several brain regions and a serotonergic approach may hold promise as a novel avenue for smoking cessation treatment.

SEROTONIN 2C (5-HT$_{2C}$) AND NICOTINE REWARDING PROPERTIES

Serotonin modulates the effect of many drugs of addiction on DAergic systems prevalently acting through the 5-HT$_{2C}$ receptor subtypes [123-125]. Thus, a series of studies has clearly shown that 5-HT$_{2C}$ receptors have a prominent role in the control of central DA function [11, 126, 127]. In fact, a series of *in vivo* electrophysiological and neurochemical studies showed that SB 206553, a selective 5-HT$_{2C/2B}$ receptor antagonist [128] and SB 242084, the most potent and selective 5-HT$_{2C}$ receptor antagonist available [129], increased the basal firing rate and the bursting activity of VTA DA neurons and enhanced DA release in both rat nucleus accumbens and prefrontal cortex [127, 130, 131]. On the other hand, RO 60-0175, a selective 5-HT$_{2C}$ receptor agonist [132] showed opposite effects on DA neurons activity [131, 133-135]. SB 242084 was also found to potentiate the phencyclidine-induced increase in accumbal DA release [136] and stress-stimulated DA outflow in the rat prefrontal cortex [137] while stimulation of 5-HT$_{2C}$ receptors by RO 60-0175 in the VTA suppressed it [137], suggesting a role of these receptors in controlling evoked DA release. In line with these data, it was recently found that SB 206553 administration potentiates both the enhancement of DA release in the nucleus accumbens and corpus striatum, and the increase in VTA and SNc DA neuronal firing rate induced by morphine [138]. Consistent with these findings, stimulation of central 5-HT$_{2C}$ receptors has been shown to inhibit morphine-induced increase in DA release in the nucleus accumbens of freely moving rats [139]. In addition, it was found that RO 60-0175 reduced cocaine-reinforced behaviour by stimulating 5-HT$_{2C}$ receptors [140]. Moreover, ketanserin [141], RO 60-0175 [89, 142] and lorcaserin [143], a relatively

selective 5-HT_{2C} agonist, reduced nicotine-induced self-administration and hyperactivity. Interestingly, RO 60-0175 not only reduced the operant responding for nicotine and the nicotine-induced hyperlocomotion in sensitized rats, but was also capable of blocking the sensitization to nicotine which occurs after repeated treatment with this drug [89]. Two recent studies found that WAY161503 [144] and WAY163909 [145] also blocked the effects of nicotine to stimulate locomotion in rats with prior nicotine exposure, and that these effect were reversed by SB242084.

An investigation conducted in our laboratory was designed as a neurochemical counterpart of Grottick et al. [89] study, with the aim of elucidating the possible involvement of DA in the behavioral effects of RO 60–0175 against nicotine-induced hyperlocomotion and reward [78]. Thus RO 60–0175, at doses of 1 and 3 mg/kg, was capable of preventing the increase in DA release induced by acute nicotine administration in the corpus striatum but not in the nucleus accumbens. On the other hand, 1 and 3 mg/kg RO 60–0175 dose-dependently prevented the enhancement of DA release induced by repeated nicotine administration, both in the corpus striatum and in the nucleus accumbens. The effects of RO 60–0175 were completely blocked by the selective 5-HT_{2C} antagonists SB 242084 and SB 243213 [78, 146]. Both SB 242084 and SB 243213 show high affinity for the cloned 5-HT_{2C} receptors (p*K*i 9.0) and a 100 to 160-fold selectivity over the closely related human 5-HT_{2B} and 5-HT_{2A} subtypes, respectively [129, 146]. In addition, SB 242084 and SB 243213 potently inhibit mCPP-induced hypolocomotion in rats, a model of *in vivo* central 5-HT_{2C} receptor function, with an ID_{50} of 0.11 mg/kg i.p. for SB 242084 [129] and an ID_{50} of 0.7 mg/kg p.o. for SB 243213 [146]. Therefore, it is very likely that both SB 242084 and SB 243213 specifically block 5-HT_{2C} receptors in the brain at the dose used. Thus, it is conceivable that the blockade by RO 60–0175 of the hyperlocomotive and rewarding effects of nicotine [140] are most probably mediated by its ability to inhibit mesolimbic DA function. The reasons for the differential effects of RO 60–0175 on acute and repeated nicotine-induced DA release in the nucleus accumbens are not readily apparent, but might be explained by the effect of nicotine on central 5-HT system [100]. Thus, acute nicotine administration has been shown to stimulate central 5-HT function increasing the firing rate of 5-HT-containing neurons in the DRN [147] and the 5-HT terminal release [116, 147, 148]. In contrast, repeated nicotine treatment was found to reduce brain 5-HT turnover [101, 149, 150], and hippocampal 5-HT concentration and release [100, 121]. Moreover, postmortem studies conducted on human brains showed a significant reduction in the concentrations of 5-HIAA (5-hydroxyindoleacetic acid), the main metabolite of 5-HT, in the hippocampus and the MRN of smokers [151]. Consistent with the above mentioned data, recent evidence indicates that repeated nicotine administration causes a reduced 5-HT turnover and increased 5-HT_2 receptor sensitivity [152]. Therefore, it is tempting to speculate that an up-regulation of 5-HT_2 might explain the finding described by Di Matteo et al [78] and indicating that RO 60–0175 can block nicotine-induced accumbal DA release only after repeated treatment with this alkaloid. Consistent with this hypothesis is the evidence that the unselective 5-HT_2 receptor agonist DOI can reduce nicotine-stimulated locomotor activity and accumbal DA release in nicotine-sensitized rats but not in animals treated acutely with nicotine [153] Moreover, repeated administration of the selective serotonin reuptake inhibitor citalopram counteracts the expression of nicotine-induced locomotor sensitization [54]. It thus seems that activation of the serotonergic system can counteract the development and expression of the mesolimbic DA system sensitization to nicotine which is typically induced by its repeated administration. This action can ultimately

lead to the extinction of nicotine-induced rewarding effect on the DAergic system, an effect that would be beneficial to smoking cessation therapies. In this respect, it is interesting to note that bupropion, which is an effective drug in the treatment of nicotine dependence [154], causes a sustained increase in the basal firing activity of 5-HT neurons in the DRN [155]. Finally, there is evidence that gamma-vinyl GABA, which increases endogenous GABA, reduces nicotine-stimulated DA overflow in the nucleus accumbens and blocks the acquisition and expression of place preference induced by nicotine [156]. Consistent with these data, it has been also found that muscimol and baclofen, two GABA agonists, significantly reduce nicotine self-administration when infused into the VTA [157]. It is thus tempting to speculate that the inhibitory action of RO 60–0175 on nicotine-induced DA release reported in the present study might be mediated, at least in part, by stimulation of GABA-ergic neurons impinging on DA-containing neurons in the SN pars compacta and in the VTA, an effect which was previously associated with activation of 5-HT_{2C} receptors [158].

SEROTONERGIC DRUG TREATMENT FOR SMOKING CESSATION

Within the USA and Europe, 70% of all smokers have tried to quit smoking at least once, but only about 6% of these succeded in maintaining abstinence [159]. Thus, one of the major problems limiting the success of smoking cessations aids is that the great majority of abstinent people relapse [159]. Most relapses occur within three months of quitting, and half within the first two days [160]. Thus, it is important to consider that environmental and sensory stimuli associated with smoking can act as conditioned reinforcers in humans [161], so the exposure to such cues increase the motivation to smoke (i.e., produce cue-induced craving) both in active or former smokers [162, 163].

Since it has been estimated that smoking leads to an average loss of 12 healthy years and reduces the lifespan by 8 years [159], there is great interest in developing effective therapies that will help in facilitating smoking cessation and assist in maintaining abstinence. Two types of pharmacological therapies have been approved for smoking cessation by the US Food and Drug Administration. The first is nicotine replacement therapy (NRT), which enables the smoker to substitute the nicotine from cigarettes from other nicotine formulations that are safer than tobacco, such as chewing gum, transdermal patches or inhalers [164]. The second therapy is non-nicotine based represented mostly by the atypical antidepressant bupropion [165, 166] and the alpha4beta2* nAChRs partial agonist varenicline. Between these two, varenicline has been shown to be the most effective for smoking cessation, followed by bupropion and nicotine replacement therapies [167]. The improved varenicline's efficacy is likely due to its dual mechanism of action: when quitting smoking, varenicline can reproduce the subjective effects of smoking by partially activating α4β2* nAChRs, and can prevent full activation of these receptors by nicotine when smoking again [168].

The phenylaminoketone atypical antidepressant agent buproprion, in the sustained-release formulation, was approved by Food and Drug Administration (FDA) in the USA in 1997 as the first non-nicotine pharmacotherapy for smoking cessation. Although the mechanism of action of bupropion in the treatment of nicotine dependence is still unclear, it is probably related to its modest capability to block DA and noradrenaline (NA) reuptake [166].

Moreover, the antagonistic activity of bupropion on nAChRs might also be relevant for its clinical efficacy as a smoking cessation aid [169]. The major metabolites of bupropion, hydroxybupropion and threohydroxybupropion produce equal or weaker inhibition of DA and NA reuptake than does bupropion [166]. Similar to tricyclic antidepressants, bupropion and hydroxybupropion were found to reduce the firing rate of noradrenergic neurons in the locus coeruleus in a dose-dependent manner – an action that is reversed by the α_2-adrenergic antagonist yohimbine [170]. Bupropion dramatically reduced the effects of nicotine on DA neuron excitability and alone reduced GABAergic transmission to DA neurons, thereby diminishing tonic inhibition of these neurons *in vitro* [171]. This increased DA neuron excitability during bupropion treatment in the absence of nicotine may contribute to bupropion's antidepressant actions [171]. These findings suggest that a noradrenergic and dopaminergic component may contribute to the antidepressant effect of bupropion. Bupropion was also found to cause a sustained increase in the basal firing activity of 5-HT neurons in the DRN [155]. However, the relevance of the latter effect in the mechanism of clinical action of bupropion is presently unknown.

Varenicline (trade name Chantix in the USA and Champix in Canada, Europe and other countries) by Pfizer was introduced in 2006 as a novel efficacious smoking cessation aid that acts as an α4β2 nAChR partial agonist [168, 172]. Varenicline affects monoaminergic neurotransmission modulating release of DA, NE, and 5-HT. Varenicline binds selectively with sub-nanomolar affinity to α4β2* nAChRs and the varenicline-induced increase in DA release from rat nucleus accumbens [168, 172] is mediated via interactions with α4β2* nAChRs in the VTA, similar to nicotine-evoked DA release [173]. Thus varenicline does not greatly increase the downstream release of DA. Due to its competitive binding on these receptors, varenicline blocks the ability of nicotine to bind and stimulate the mesolimbic DA system.

On the other hand, varenicline causes no significant changes in extracellular levels of 5-HT, NE, or DA in rat prefrontal cortex [174], while it produces robust increases in cortical DA and NE release after a high dose of 10 mg/kg [175]. This dose is associated with very high brain concentrations of >1 μM, which can interact with several other nAChR subtypes other than α4β2 nAChRs. The lack of effects of varenicline on cortical monoamine release is also consistent with its *in vitro* properties, since varenicline has very low affinity for the 5-HT transporter or 5-HT receptors and thus cannot increase 5-HT either by blocking 5-HT reuptake or by interacting with central 5-HT receptors. Although varenicline has modest affinity for 5-HT_{3A} receptors, at which it is a full agonist, the therapeutic unbound varenicline brain concentrations are predicted to be insufficient for activating central 5-HT_{3A} receptors. Furthermore, varenicline does not bind to DA receptors that can modulate 5-HT release, and does not inhibit the enzyme that metabolizes 5-HT, MAO-A [174].

In recent meta-analyses varenicline resulted superior in effectiveness to bupropion and NRT [167, 176]. Nevertheless, the US FDA issued an alert concerning an the observed increase in serious neuropsychiatric symptoms in patients taking varenicline [177]. Such events can include changes in behavior, hostility, agitation, depressed mood, and suicidal thoughts or actions. FDA is continuing to evaluate the risk of neuropsychiatric events with Chantix. Pfizer is conducting a large safety clinical trial of Chantix to assess neuropsychiatric adverse events, and results from this study are expected in 2017.

Several tricyclic antidepressants (TCAs) which inhibit NA and 5-HT, such as nortriptyline [178-180] and doxepin [181], might facilitate smoking cessation in combination

with behavioural treatment. Indeed, as a class, antidepressants are the most common non-nicotine drugs which have been shown to be of benefit in smoking cessation.

As already mentioned above, smokers are more likely than non-smokers to have symptoms of depression. In addition, smokers with a history of depression are more likely to be more dependent on nicotine and have a lower likelihood of successfully quitting. When they do quit, depression is more apt to be a prominent withdrawal. Cases of psychotic depression after smoking cessation have been reported.

Since TCAs have significant side-effects, such as anticholinergic activity and frequent lethality in overdose, a better tolerated and safer class of antidepressants, selective serotonin reuptake inhibitors (SSRIs), have also been studied, given the role of 5-HT in modulating nicotine reward and smoking behavior [78, 81, 89, 100]. Studies with the prototypical SSRI fluoxetine yielded equivocal results.

Fluoxetine might ameliorate of the most bothersome symptoms experienced during smoking cessation, in smokers with a history of major depression [182]. Another serotonergic agent used to treat nicotine addiction is buspirone, a 5-HT_{1A} partial agonist which is provided with anxiolytic activity. Thus stimulation of 5-HT_{1A} receptors by buspirone leads to a reduction in presynaptic release of 5-HT, which appears to mediate its anxiolytic effects. However, a placebo controlled clinical trial failed to support its efficacy in smoking cessation [183].

CONCLUSIONS

It is now well established that nicotine can be considered an extraordinary psychotropic drug in that, by acting on nAChRs, it can stimulate the release of a number of neurotransmitters including ACh, GLU, DA, NA, 5-HT, and GABA. It is indeed difficult to find in the official pharmacopeia a psychotropic drug which is capable to exert such a wide range of effects on multiple neurotransmitters such as nicotine. The serotonin pathway has been implicated in nicotine dependence and may influence smoking cessation. The most promising receptor seems to be the 5-HT_{2C}. As far as the other 5-HT receptors are concerned, there is some evidence that 5-HT_{1A} receptor stimulation inhibits behavioral sensitization to nicotine [54, 184] and may mediate nicotine withdrawal symptoms [184, 185]. Moreover, an inhibitory role of the 5-HT_6 and a facilitating role of the 5-HT_3 receptors have been described. However, 5-HT_3 receptor antagonism did not prove to be an effective treatment strategy in human smokers. Thus, these compounds are still at the stage of pre-clinical investigation and deserve further experimentantion to evaluate their potential usefulness as effective smoking cessation aids. Antidepressants such as nortriptyline, doxepin, fluoxetine, the reversible MAO-A inhibitor moclobemide, and the anxiolytic agent buspirone have been found to be helpful in the therapy of nicotine addiction. However, their efficacy as smoking cessation agents appears to be limited and it is still unclear if their clinical action in smoking cessation is specific or if it is simply linked to their therapeutic effect on the underlying depression and anxiety which are frequently associated with cigarette smoking.

Although basic research has provided a great deal of data on the neurobiological bases of nicotine action, dependence, and withdrawal, the pharmacotherapy of nicotine addiction can be still considered unsatisfactory. Serotonergic drugs may have some efficacy in supporting

discontinuation of smoking. There is mounting evidence indicating that 5-HT neurotransmission is a critical neurological substrate for the process of smoking cessation per se or for a putative role in suppressing nicotine withdrawal in smokers administered NRT.

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