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Sensitization of the Mesoaccumbens Dopamine Response to Nicotine

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BALFOUR, D. J. K., M. E. M. BENWELL, C. E. BIRRELL, R. J. KELLY AND M. AL-ALOUL. *Sensitization of the mesoaccumbens dopamine response to nicotine*. PHARMACOL BIOCHEM BEHAV **59**(4) 1021–1030, 1998.—This article reviews the evidence that pretreatment with nicotine causes a regionally selective sensitization of its stimulatory effects on a pathway, the mesoaccumbens dopamine (DA) system, which has been implicated in the locomotor stimulant response to nicotine and its ability to reinforce self-administration. The sensitization evoked by daily injections of nicotine is associated with a regionally selective downregulation of the control of mesoaccumbens DA neurons by inhibitory autoreceptors and depends upon co-stimulation of NMDA glutamatergic receptors. It is suggested that the sensitization is related to enhanced burst firing of mesoaccumbens neurons, which results in an enhancement of DA release into the extracellular space between the cells where it acts upon putative extrasynaptic dopamine receptors. The studies with NMDA receptor antagonists revealed a dissociation between the expression of sensitized mesoaccumbens DA and locomotor responses to nicotine. It is proposed, therefore, that the sensitized mesoaccumbens DA responses to nicotine may be implicated in psychopharmacological responses to drug concerned more closely with nicotine dependence. © 1998 Elsevier Science Inc.

Nicotine<Dopamine Nucleus accumbens Dorsolateral striatum Raclopride NMDA Receptors Locomotor activity Dependence

IT is now widely accepted that a majority of the people who smoke tobacco do so to experience the psychopharmacological properties of the nicotine present in the smoke, and that a significant proportion of habitual smokers become dependent upon the drug (53). As a result, a number of different nicotine preparations have been examined for their ability to alleviate the effects of nicotine withdrawal in smoking cessation protocols (6). The neural mechanisms that mediate the effects of the drug are complex and not fully understood. However, the compound seems to share many of the properties of other psychostimulant drugs of abuse such as cocaine and D-amphetamine. In particular, nicotine has been shown to stimulate the dopamine (DA) neurons that project to the nucleus accumbens from the ventral tegmental area (VTA) of the midbrain, and that its stimulatory effects on these neurons mediate both the locomotor stimulant properties of the drug and its ability to serve as a reinforcer in self-administration experiments (17,20,21). Repeated or chronic exposure to cocaine or D-amphetamine results in sensitization of their effects on both locomotor activity and DA overflow in the nucleus accumbens, and it has been suggested that the ability to elicit sensitized responses in the mesolimbic DA system may be central to the mechanisms underlying the development of addiction to these drugs (49). Repeated exposure to nicotine also results in sensitization of its stimulatory effects on locomotor activity in the rat (18). Studies in our laboratory have shown that repeated daily injections of nicotine, using a protocol that results in sensitization to the locomotor stimulant response to the drug, also causes sensitization of its effects on DA overflow in the nucleus accumbens (8). This article will review the mechanisms that may be implicated in the development of the sensitized DA responses to nicotine and consider the possible psychopharmacological consequences of the sensitized response to the drug.

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THE ROLE OF CENTRAL NICOTINIC RECEPTORS

Nicotine exerts its effects in the brain by stimulating neuronal nicotinic receptors (55). The mesoaccumbens DA responses to nicotine are mediated by these receptors because both the acute and sensitized responses to the drug are attenuated by systemic injections of the nicotinic antagonist, mecamylamine, which crosses the blood–brain barrier, but not by the quaternary antagonist, hexamethonium, which penetrates less readily into the brain (11,30). This conclusion is supported by results that show that locomotor stimulation is also seen in rats given intracerebral injections of nicotine or cytisine (38,39,48). Mesoaccumbens DA neurons express nicotinic receptors on both their somatodendritic membranes in the VTA and presynaptically on the nerve terminal membranes in the terminal fields (19). However, in vivo microdialysis studies suggest that the DA responses evoked by systemic injections of the drug are mediated, predominantly at least, by the somatodendritic receptors in the VTA because they have been shown to depend upon the propagation of nerve impulses to the terminal field (12) and to be blocked by the local administration of a nicotinic receptor antagonist into the VTA (42).

Many isoforms of the neuronal nicotinic receptor readily desensitise when exposed to nicotine for a period of time. This has been demonstrated for the receptors that mediate the stimulatory effects of nicotine on DA overflow in the nucleus accumbens by exploring the responses to nicotine in rats in which the plasma nicotine levels, prior to the injection, have been raised by the constant infusion of nicotine from a subcutaneous minipump (11). In these rats, the DA response to a subcutaneous injection of the drug (0.4 mg/kg) was abolished by infusions that maintained the plasma nicotine concentration at level in excess of 25 ng/ml. However, if the rats were challenged with a nicotine injection 2 or 7 days following removal of the pump used to infuse the nicotine, a sensitized DA response was observed in the nucleus accumbens (11). Thus, it is possible that sensitization of the mesoaccumbens DA response to the drug is evoked by repeated or prolonged desensitization of the receptors that mediate the response rather than repeated stimulation of the receptors.

REGIONAL SPECIFICITY OF THE RESPONSE TO NICOTINE

Electrophysiological studies suggest that the DA neurons that innervate the nucleus accumbens are more sensitive to nicotine that those that innervate the caudate/putamen (35). Dialysis studies also suggest that nicotine exerts a greater effect on DA overflow in the accumbens than the caudate/putamen (30), and that the projections to the accumbens respond to lower doses of nicotine than those that are required to stimulate the neurons that innervate the caudate/putamen (10). The expression of sensitized DA responses to repeated nicotine also seems to be regionally selective to the extent that sensitized responses to the drug are not observed in the caudate/putamen in rats treated with nicotine using a schedule that elicits sensitized responses in the nucleus accumbens (10). The dose of nicotine (0.4 mg/kg) used to pretreat the rats in this sensitization study was clearly sufficient to stimulate the neurons that innervate the caudate/putamen. Thus, the failure to observe sensitized responses in this region of the brain could not be attributed to a failure to stimulate the neurons. Higher doses of nicotine, however, also seemed to be required to desensitize the DA response to nicotine observed in these neurons (10), and it is possible that this contributes to their resistance to sensitization. Alternatively, the ability to express sensitized responses to nicotine may be an intrinsic property of mesoaccumbens neurons that is not shared by the nigrostriatal DA neurons that innervate the caudate/putamen.

Pretreatment with nicotine also causes sensitization of the noradrenaline (NA)-secreting neurons that innervate the hippocampus from the locus coeruleus (10,36). Indeed the responses of these neurons to nicotine closely resemble those of the mesoaccumbens DA neurons both in their sensitivity to nicotine injections and to the desensitising effects of nicotine infusions (10). However, there appear to be significant differences in the biochemical mechanisms underlying the expression of the sensitized responses. Sensitization of the NA projections to the hippocampus is associated with increased formation of NA in the nerve terminals (37,52), whereas this does not seem to be the mechanism underlying the development of sensitized DA responses to nicotine in the nucleus accumbens (13). Nevertheless, it is clear that repeated exposure to nicotine results in a number of adaptive changes in the neural responses to the drug that may be implicated in the behavioral adaptation observed in rats treated chronically with nicotine.

THE MECHANISMS UNDERLYING THE DEVELOPMENT OF SENSITIZED MESOACCUMBENS DOPAMINE RESPONSES TO NICOTINE

The studies outlined in the sections above imply that systemic nicotine exerts its effects on mesoaccumbens DA neurons by stimulating nicotinic receptors located on the somatodendritic membranes of the cells. Therefore, to test the hypothesis that pretreatment with the drug caused sensitization of the responses mediated by these receptors, the effects of intra-VTA injections of nicotine on DA overflow in the nucleus accumbens were examined. The results of these experiments showed that pretreatment did, indeed, cause sensitization of the responses to nicotine delivered locally in this way (Fig. 1). The nicotine-pretreated animals responded more rapidly to intra-VTA nicotine and to doses of the drug up to 10 times lower than those need to elicit a response in control animals pretreated with saline. In the nicotine-pretreated animals, the peak response seemed to be evoked by intra-VTA nicotine when it was given at a dose of 6 μg. In saline-pretreated animals, no significant responses to intra-VTA nicotine were observed until it was given at a dose of 12 μg, and even at this dose the response appeared to be slower than that observed in nicotine-pretreated rats. These data, therefore, provide evidence that the pretreatment protocol does cause sensitization of the responses mediated by receptors located in the VTA, results that are also entirely consistent with the data (12,42) that suggest that nicotine exerts its effects predominantly by stimulating nicotinic receptors on or close to the cell bodies in the VTA. Unpublished studies in our laboratory have shown that pretreatment with nicotine does not result in sensitization of the mesoaccumbens DA response evoked by microinjections of a tachykinin Nk_3 receptor agonist, senktide, into the VTA. Thus, this pretreatment protocol appears to cause a sensitization that may be specific to nicotinic receptor agonists and not all drugs that stimulate DA-secreting neurons in the nucleus accumbens.

The experiments with senktide imply that it is unlikely that the pretreatment protocol influences the pool of DA available for release from the nerve terminals in the nucleus accumbens. This conclusion is further supported by the fact that pretreatment also has no significant effects on the amount of DA released in response to systemic injections of D-amphetamine or cocaine (Birrell and Balfour, in preparation) or the local

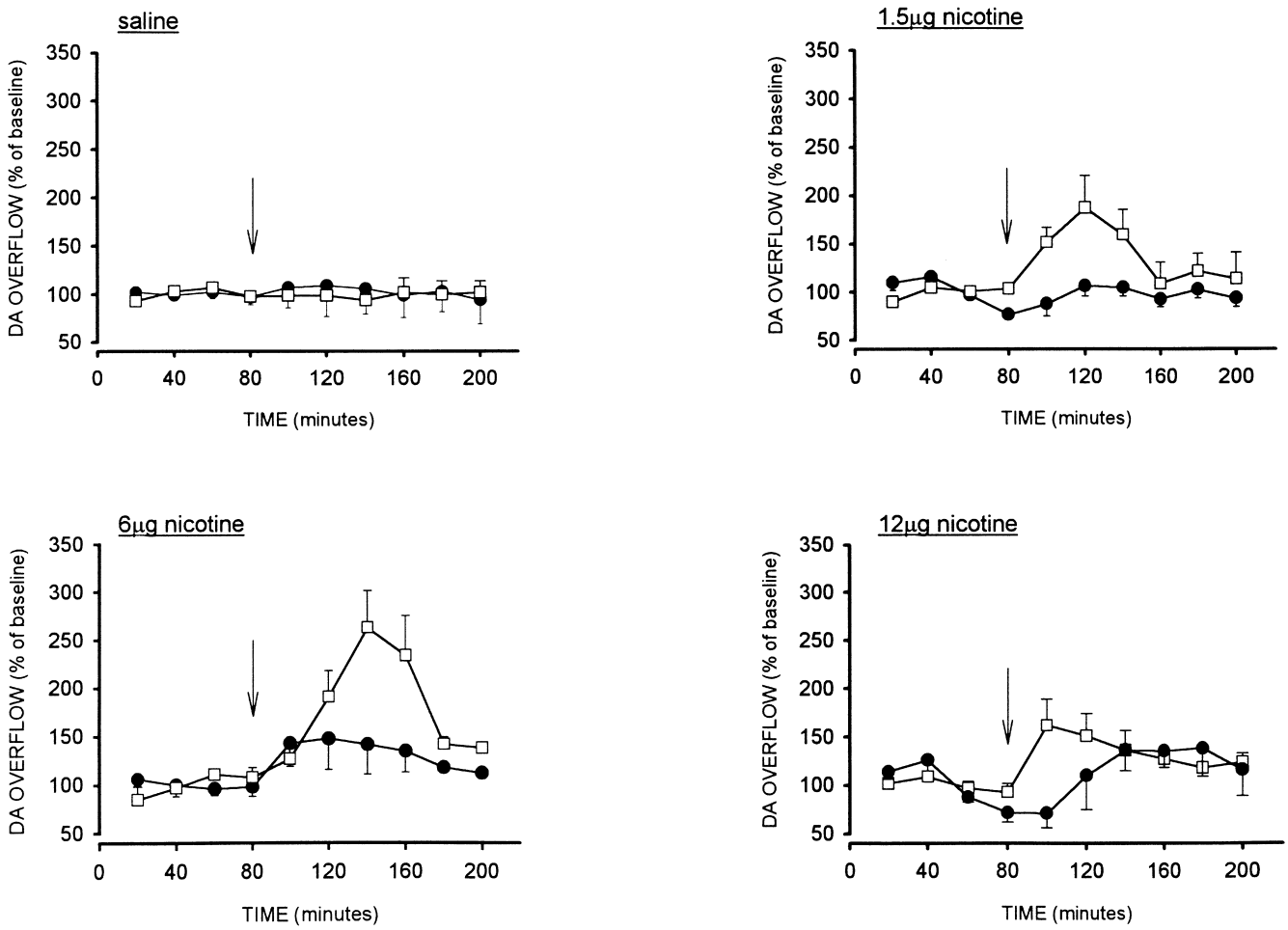

FIG. 1. The effects of nicotine pretreatment on mesoaccumbens dopamine responses to nicotine microinjected into the ventral tegmental area. Groups of adult male Sprague–Dawley rats ($n = 4$ per group), weighing 350–400 g, were pretreated with daily subcutaneous injections of saline (filled circles) or nicotine (0.4 mg/kg; open squares) for 5 days. Three hours after the last injection on day 5, the animals were anesthetized with Halothane (2% in oxygen) and dialysis probes were implanted into the nucleus accumbens using the procedure described by Benwell and Balfour (6). The coordinates for the probes were +1.7 mm from bregma in the AP plane, +1.5 mm in the lateral plane, and −7.5 mm vertically from the surface of the brain using the atlas of Paxinos and Watson (44). At the same time a 21-gauge guide cannula was inserted (stereotaxic coordinates; −5.0 mm in the AP plane, 0.9 mm laterally, and −6.0 mm from the surface of the brain). This terminated 2.0 mm above the ipsolateral ventral tegmental area. The probe and the guide cannula were held in place with dental cement anchored to the surface of the skull with two small screws. Dialysis studies were performed on the following day starting approximately 18 h after surgery. The animals were allowed to habituate to the test environment for 90 min before 4 × 20-min baseline samples of dialysate were collected and analyzed for DA by HPLC with electrochemical detection. Nicotine solutions or the saline vehicle (0.5 μl) were then microinjected over a 2-min period, at the points indicated by the arrows, through a 30-gauge needle, inserted through the guide cannula, which protruded 2.0 mm from the end of the guide cannula. The injection cannula was left in place for 1 min after the injection before being removed. Dialysate samples collected and analyzed for a further 2 h. Each rat was tested only once. At the end of the experiment, the animals were killed humanely and the brains carefully removed and frozen. The positions of the probes and the injection cannulae were confirmed histologically using sections cut from the frozen brains. The data are expressed as percentages of the mean baseline levels of DA measured in the three samples collected before the nicotine injections, and are presented as means ± SE mean. In the saline-pretreated rats, only the highest dose tested evoked a significant increase in DA overflow, $F(9, 45) = 2.38$, $p < 0.05$. Pretreatment with nicotine enhanced the responses to the microinjections nicotine [pretreatment × microinjection × time, $F(27, 270) = 2.24$, $p < 0.01$. Post hoc analysis showed that pretreatment with nicotine sensitized the responses to nicotine tested at all three doses, whereas the microinjections of the saline vehicle had no significant effects on DA overflow in the nucleus accumbens of the saline- or the nicotine-pretreated rats.

administration of depolarizing concentrations of K^+ delivered through the probe used to sample the extracellular DA in the accumbens (13). Other ex vivo studies in our laboratory have shown that the pretreatment also had no significant effects on the uptake and metabolism of DA in the accumbens (13). Chronic exposure to nicotine can result in an increase in the density of nicotine binding sites within the mammalian brain (55). However, other studies in our laboratory suggest that the pretreatment regimen used in our studies to elicit the sensitized DA responses does not influence the density of [^{3}H]-nicotine binding sites rat brain (7), although this study did not focus specifically on binding to neuronal membranes in the nucleus accumbens or VTA. Thus, it remains possible that the pretreatment procedure causes a localized change in the density of the

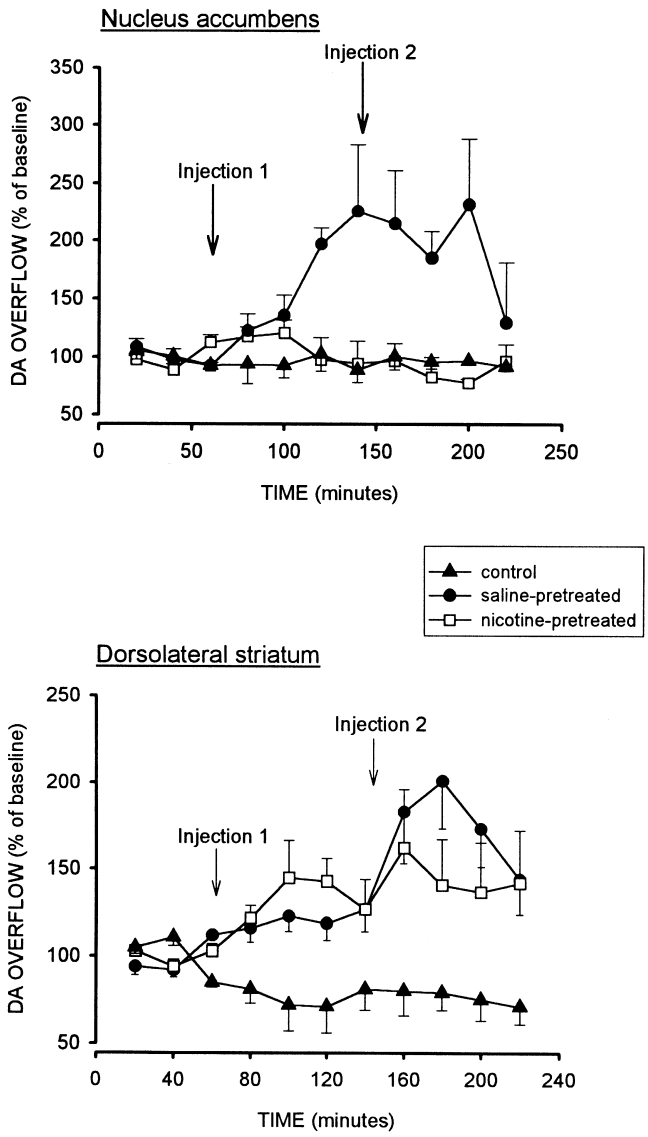

FIG. 2. The effects of nicotine pretreatment on dopamine responses to raclopride. Groups of adult male Sprague–Dawley rats ($n = 4$ per group) were pretreated with daily subcutaneous injections of saline (filled circles) or nicotine (0.4 mg/kg; open squares) for 5 days. Three hours after the last injection on day 5, dialysis probes were implanted, under Halothane anesthesia in the nucleus accumbens, using the coordinates described in the legend to Fig. 1, or the dorsolateral striatum. The coordinates for the probes located in the dorsolateral striatum were +1.00 mm from bregma in the AP plane, +3.0 mm laterally, and −5.4 mm vertically from the surface of the brain. Dialysis studies were begun approximately 18 h later. Following equilibration and the collection of three baseline samples, the rats were given intraperitoneal injections of raclopride (0.05 mg/kg) at the point indicated as injection 1 and raclopride (0.10 mg/kg) at the point indicated as injection 2. Control rats (filled triangles) were pretreated with daily injections of saline during the 5-day pretreatment phase of the experiment and with intraperitoneal injections of saline at injections 1 and 2. The data are expressed as percentages of the mean baseline levels of DA measured in the three samples collected before the raclopride injections, and are presented as means ± SE mean. In saline-pretreated animals, raclopride increased DA overflow in both the nucleus accumbens, $F(10, 60) = 2.64$, $p < 0.01$, and the dorsolateral striatum, $F(10, 90) = 2.30$, $p < 0.01$. In the nucleus accumbens, nicotine-pretreatment caused a significant attenuation, $F(2, 9) = 11.75$, $p < 0.01$, of the response to raclopride seen in rats pretreated with saline to the extent that the response was no longer significant when compared with the controls. Nicotine pretreatment had no significant effects on the response to raclopride in the dorsolateral striatum.

nicotinic receptors that mediate the mesoaccumbens DA responses to nicotine. Nevertheless, our results would appear to be most consistent with the hypothesis that the sensitized responses to nicotine, observed in the pretreated rats, reflect changes in the control of DA release from the neurons.

Two possible mechanisms that may account for the sensitization have been examined our laboratory. DA-secreting neurons in the brain are controlled by inhibitory DA autoreceptors located both on the somatodendritic membranes of the cells and on the nerve terminals. Systemic injections of the DA receptor antagonist, raclopride, caused an increase in DA overflow in both the nucleus accumbens and the caudate/putamen of saline-pretreated rats (Fig. 2). However, if the rats are pretreated with nicotine, the DA response to raclopride is abolished in the nucleus accumbens, which exhibits the sensitized response to nicotine, but not in the dorsolateral striatum, a structure that does not exhibit a sensitized response. Thus, the expression of sensitized responses to nicotine, seen in the accumbens, seem to be related to an attenuation of the control of the neurons by inhibitory DA autoreceptors. Further studies are necessary to establish if the DA autoreceptors involved are those that are located on the somatodendritic membranes of the cells in the ventral tegmental area that influence the activity of the cells or those that are located presynaptically and are concerned more with the regulation of DA formation and release in the terminal field. Interestingly, other studies have shown that the expression of sensitized responses to cocaine, seen in animals pretreated with the drug, are also associated with down-regulation of the somatodendritic DA autoreceptors (29). However, because no cross-sensitization was observed between the mesoaccumbens DA responses to nicotine and cocaine, it seems unlikely that the mechanisms involved are identical.

There is evidence that sensitization of responses to psychostimulants may be related to stimulation of NMDA glutamatergic receptors (33,34,50), and, therefore, in collaboration with Ian Stolerman's group at the Institute of Psychiatry in London, we investigated their putative role in the development of sensitized responses to nicotine. The co-administration of dizocilpine (MK801) during the pretreatment phase of the experiment attenuates the development of sensitized mesoaccumbens DA responses to nicotine and the locomotor stimulant response to the drug measured in the same animals (51). However, it is necessary to be cautious with results obtained with dizocilpine because the drug can also act as an antagonist at central nicotinic receptors, although it has less affinity for these receptors than NMDA receptors (3). Additional experiments, therefore, were performed with the competitive NMDA receptor antagonist, D-CPPene (3-(2-carboxypiperazin-4-yl)-1-propenyl-phosphonic acid; SDZ EAA 494). The administration of this antagonist, prior to each injection of nicotine administered during the pretreatment phase of the experiment, also attenuated the development of sensitized DA responses to nicotine (51). The administration of this compound during the pretreatment phase did not, however, attenuate the enhanced locomotor responses to nicotine seen in nicotine-pretreated rats. The interpretation of these data, however, is complicated by the fact that enhanced locomotor

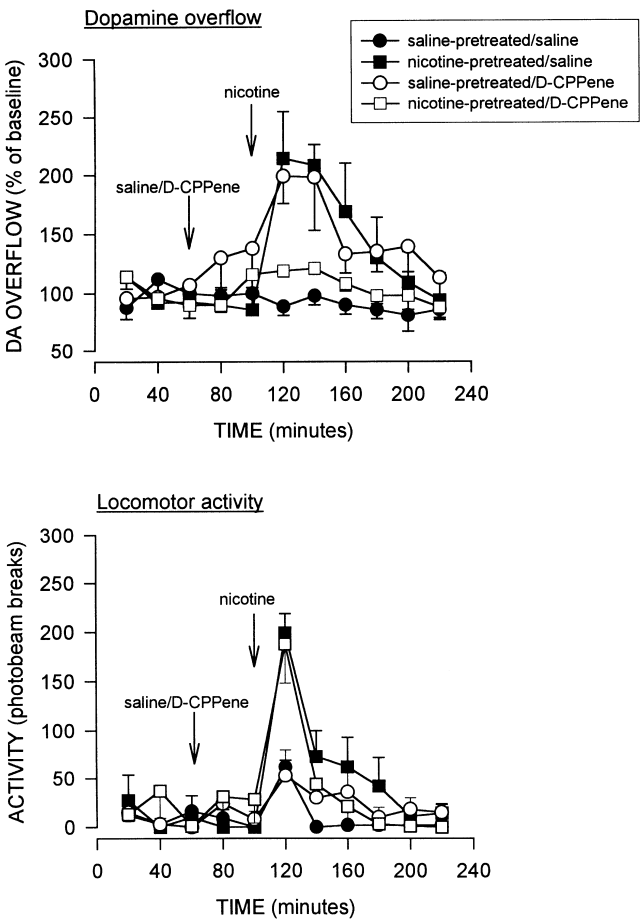

FIG. 3. The effects of acute D-CPPene on the expression of sensitized responses to nicotine. Groups of adult male Sprague–Dawley rats ($n = 6$ per group) were pretreated with daily injections of saline (open and closed circles) or nicotine (0.4 mg/kg; open and close squares) for 5 days. Dialysis probes were then implanted in the nucleus accumbens under Halothane anesthesia using the procedure outlined in the legend to Fig. 1. On day 6 the animals were transferred to an activity box and dialysis studies were performed using a procedure very similar to that described in the legend to Fig. 3. Following collection of three baseline samples, saline (filled symbols) or D-CPPene (2 mg/kg; open symbols) was injected intraperitoneally followed 40 min later by a subcutaneous injection of nicotine (0.4 mg/kg). The data are presented as means ± SE mean. DA overflow is expressed as a percentage of the baseline levels measured in the three samples collected before the first injections. The sensitized mesoaccumbens DA response, seen in the nicotine-pretreated animals, was attenuated significantly [pretreatment × D-CPPene × time, $F(10, 150) = 4.5$, $p < 0.001$] by the injection of D-CPPene. Nicotine pretreatment also caused sensitization of the locomotor response to the drug [pretreatment × time $F(10, 150) = 8.4$, $p < 0.001$]; this response was unaffected by the administration of D-CPPene prior to the challenge with nicotine. The data presented in this figure are reproduced from (Balfour, D. J. K.; Birrell, C. E.; Moran, R. J.; Benwell, M. E. M. Effects of acute D-CPPene on mesoaccumbens dopamine responses to nicotine in the rat. Eur. J. Pharmacol. 316, with kind permission of Elsevier Science–NL, Sara Burgerhartstraat 25, 1055KV Amsterdam, The Netherlands.

responses are also observed in rats pretreated D-CPPene alone when they are subsequently challenged with nicotine (51). It is entirely possible that the mechanisms underlying the expression of sensitized locomotor responses to nicotine following pretreatment with D-CPPene may be entirely different to those that mediate the development of the sensitized responses in nicotine-pretreated animals. Nevertheless, it seems reasonable to conclude that the development of sensitized mesoaccumbens DA responses to nicotine depends upon costimulation of NMDA receptors, whereas the role of NMDA receptors in the expression of enhanced locomotor responses are more complex and do not seem to be related directly to the effects of nicotine on DA overflow in the area of the accumbens sampled by the probe.

Because dizocilpine has some affinity for neuronal nicotinic receptors, studies on the effects of acute blockade of NMDA receptors focused on results obtained with the competitive antagonist, D-CPPene. In contrast to the experiments described in the previous paragraph, in these experiments a single injection of D-CPPene was given 40 min prior to the a challenge with nicotine. In the animals that had been pretreated with nicotine for 7 days prior to the test day, the administration of D-CPPene abolished the sensitized DA response to nicotine (Fig. 3). Surprisingly, however, an acute injection of D-CPPene to saline-pretreated rats elicited a sensitized DA response to nicotine. These data clearly imply that NMDA receptors play a complex role in the regulation of mesoaccumbens DA responses to nicotine that are difficult to interpret fully. They suggest, however, that the interactions between NMDA receptor stimulation and the response to nicotine is influenced significantly by prior exposure to nicotine. There is evidence that glutamate secretion in the brain may be stimulated by nicotine through an effect on nicotinic receptors located presynaptically on glutamate-secreting terminals (27). One possible explanation for the results, which clearly requires confirmation, is that in rats treated acutely with nicotine, the predominant effect of the increased glutamate release, evoked by nicotine, is to activate neurons that inhibit the effects of nicotine on mesoaccumbens DA neurons, whereas in animals that have become sensitized to the drug, the predominant effect of the glutamate is to facilitate the mesoaccumbens DA response to the drug. Although acute injections of D-CPPene exert significant effects on the DA responses to both acute and subchronic nicotine, they have no significant effects on the locomotor responses to the drug. Thus, the studies again reveal a clear dissociation between the locomotor responses to nicotine and its effects on DA overflow in the nucleus accumbens.

STUDIES WITH NOMIFENSINE

Much of the DA released from nerve terminals in the brain is thought to be cleared from the synaptic cleft by rapid reuptake into the nerve terminal cytoplasm by transporters located within the terminal membrane. The probes used to investigate the effects of drugs on neurotransmitter release are located in the interstitial space between cells. Thus, they do not measure neurotransmitter release directly into the synaptic cleft but overflow into the extracellular space sampled by the probe. A fundamental assumption of the approach is that neurotransmitter overflow into the extracellular space is derived from neurotransmitter released into the synaptic cleft and that the changes in overflow, measured in this way, provide an indirect measure of the effects of treatments on neurotransmitter release into the synaptic cleft (22). There is evidence that the density of DA transporters may vary sig-nificantly between terminal fields. In particular, it has been suggested that the DA transporters provide an efficient means of clearing DA from synapses in the nucleus accumbens and caudate/putamen, whereas they are less effective in the medial prefrontal cortex

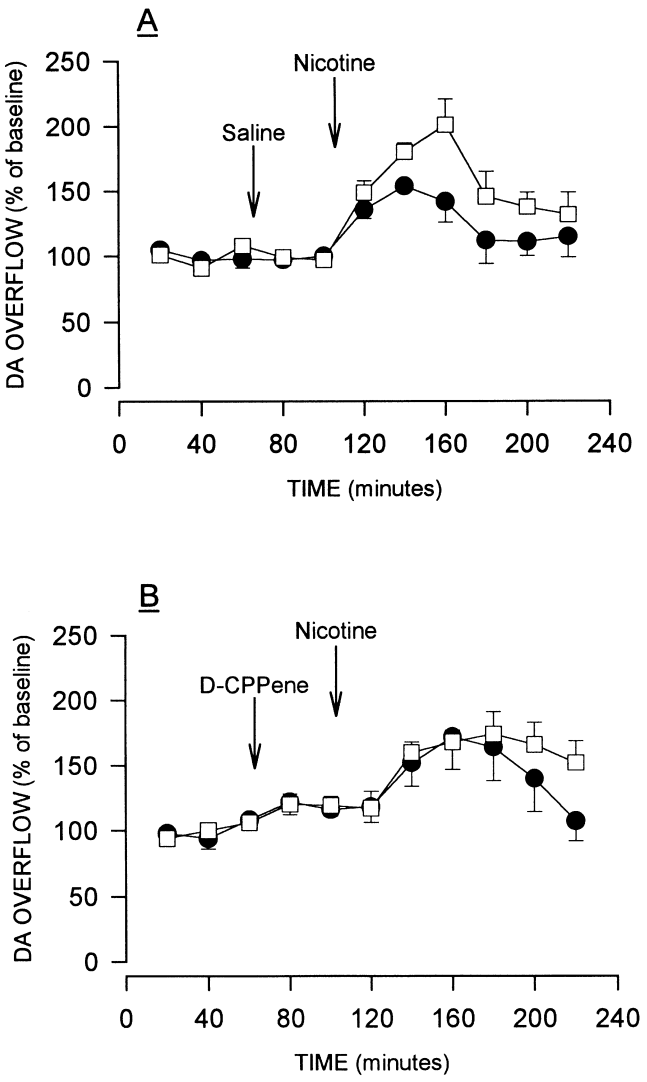

FIG. 4. The effects of acute D-CPPene on the expression of sensitized mesoaccumbens dopamine responses measured in the presence of nomifensine. Groups of adult male Sprague–Dawley rats ($n = 6$ per group) were pretreated with daily injections of saline (closed circles) or nicotine (0.4 mg/kg; open squares) for 5 days. Dialysis probes were then implanted in the nucleus accumbens under Halothane anesthesia using the procedure outlined in the legend to Fig. 1. Dialysis studies were performed on day 6 using a procedure very similar to that described in the legend to Fig. 3 with the exception that the Ringer solution used to perfuse the probes contained nomifensine (5 μM) to inhibit the presynaptic DA transporters. Following collection of three baseline samples, saline (A) or D-CPPene (2 mg/kg; B) was injected intraperitoneally followed 40 min later by a subcutaneous injection of nicotine (0.4 mg/kg). DA overflow is expressed as a percentage of the baseline levels measured in the three samples collected before the first injections and are presented as means ± SE mean. In the rats given an initial injection of saline (A), an injection of nicotine stimulated DA overflow [time, $F(10, 90) = 14.88$, $p < 0.001$]; this response was enhanced by pretreatment with nicotine prior to the test day [pretreatment × time, $F(10, 90) = 2.03$, $p < 0.05$]. In the rats given an initial injection of D-CPPene (B), nicotine also caused a significant increase in DA overflow [time, $F(10, 90) = 15.03$, $p < 0.001$); in these animals, nicotine pretreatment had no significant effect on DA overflow. Comparisons between the data presented in panels A and B showed that an injection of D-CPPene attenuated the response to the nicotine challenge in the animals pretreated with nicotine prior to the test day [D-CPPene × time, $F(10, 90) = 19.28$, $p < 0.001$] but had no significant effects on the response to acute nicotine measured in the animals pretreated with saline prior to the test day. Comparison of the data also showed that the modest increase in DA overflow evoked by the injection of D-CPPene prior to the nicotine injection was also significant [D-CPPene × time $F(4, 88) = 2.71$, $p < 0.05$].

(23). Even within the subcortical structures there is evidence for variation, the density of transporters being significantly higher in caudate/putamen than nucleus accumbens (23) and, within the nucleus accumbens, higher in the accumbens core than the shell (31). Thus, regional variations in the changes in DA overflow evoked by drugs, such as nicotine, which elicit their effects by influencing impulse flow to the terminal fields, could be associated with regional differences in the density of the presynaptic DA transporters rather than intrinsic differences in the responses of the neurons to the test drug. To explore this issue with respect to the effects of nicotine on DA overflow in the nucleus accumbens and caudate/putamen, our dialysis studies have been extended to include experiments in which responses to the drug have been investigated using probes perfused with a Ringer solution containing nomifensine to inhibit the presynaptic transporters.

The addition of nomifensine to the medium used to perfuse the probe causes a marked, fivefold increase in DA overflow in both the accumbens and the caudate/putamen (10), results that are entirely consistent with the hypothesis that much of the DA released by terminals in these areas of the brain never escapes from the synaptic cleft but is immediately recaptured and transported back into the nerve terminal cytoplasm. When nicotine was studied for its effects under these conditions, increased DA overflow was observed in response to acute nicotine in both the nucleus accumbens and striatum (10). However, whereas a near maximal response to nicotine was observed in the accumbens of rats treated with the drug at a dose of 0.1 mg/kg, DA overflow in the dorsolateral striatum was only increased significantly by the drug when it was given at a dose of 0.4 mg/kg. Thus, consistent with other electrophysiological data (35), the results suggest that mesoaccumbens DA neurons are more sensitive to nicotine than those that project to the dorsolateral striatum. It was also possible to detect the sensitized responses to nicotine using accumbens probes perfused with a solution containing nomifensine, whereas no sensitization of the response to nicotine was observed in the striatum of rats treated repetitively with nicotine. These results, therefore, provided further confirmation that the expression of sensitized DA responses to nicotine is regionally selective.

This approach was then used to explore further the effects of D-CCPene on the responses to subchronic nicotine. The results of these experiments showed that, in the absence of D-CPPene, the sensitized DA response to nicotine was still apparent in the nicotine-pretreated rats when the probes were perfused with a Ringer solution containing nomifensine (Fig. 4A). This response was attenuated in animals that were given D-CPPene on the test day prior to the injection of nicotine to the extent that there was no significant difference between the responses to nicotine in the saline and nicotine-pretreated animals (Fig. 4B). The magnitude of the responses to nicotine in the rats given D-CPPene were very similar to that measured in saline-pretreated (nonsensitized) rats challenged with nicotine alone (Fig. 4A). Thus, although there are a number of ways in which these data may be interpreted, they are consistent with the hy-

pothesis that the administration of D-CPPene on the test day may selectively or preferentially suppress the expression of sensitized mesoaccumbens DA responses to nicotine while leaving the acute response to the drug relatively intact.

ELECTROPHYSIOLOGICAL CORRELATES OF SENSITIZATION

The data clearly suggest that the expression of sensitized mesoaccumbens DA responses to nicotine involve the co-stimulation of NMDA receptors. Glutamate-secreting pathways are thought to innervate both the nucleus accumbens and the VTA, and it is possible, therefore, that increased glutamate secretion could influence DA release at the level of the nerve terminals or the electrophysiological responses to nicotine elicited by its effects on DA-secreting cells in the VTA. This issue remains to be resolved. However, there is evidence that the effects of nicotine on mesoaccumbens DA neurons are modulated by stimulation of NMDA receptors in the VTA. Mesoaccumbens DA neurons can fire in two modes: as irregular single spikes, or in a burst firing mode in which periods of rapid firing are interspersed with quiescent periods (25,26). DA overflow into the extracellular space is enhanced by increasing the proportion of neural activity that occurs as burst firing (24). The proportion of tegmental neurons that exhibit burst activity is increased by the local administration of NMDA receptor agonists into the VTA, whereas burst firing is diminished by the administration of NMDA receptor antagonists into this area of the brain (15,32). Repetitive treatment with nicotine, using a regime very similar to that used in our studies, also increases burst firing of ventral tegmental neurons in a manner that is antagonized by NMDA receptor antagonists (41). These data suggest that stimulation of NMDA receptors also play an important part in regulating the effects of nicotine on firing pattern of mesolimbic DA neurons.

This conclusion introduces the interesting possibility that the response of mesoaccumbens DA neurons to nicotine may reflect stimulation of nicotinic receptors located both on the somatodendritic membranes of the cells and on glutamate-secreting cells that synapse with them. There is evidence that nicotinic receptors are expressed on both cell types. Radioligand binding and in situ hybridization experiments suggest that DA-secreting neurons in the VTA express nicotinic receptors containing either α_3 or α_4 subunits (55). More recent studies suggest that glutamate-secreting nerve terminals in the hippocampus express the nicotinic receptor isoform thought to be composed of α_7 subunits (27). If this is also true of glutamate-secreting neurons in other areas of the brain, the data presently available are consistent with the hypothesis that the expression of sensitized responses to the drug may depend upon co-stimulation of at least two isoforms of the neuronal nicotinic receptor, one being located directly on the target cells, the other facilitating the release of glutamate onto the cells. This conclusion provides an explanation for some recent results in our laboratory (9) that have shown that sensitization to nicotine does not result in sensitization of the responses to other nicotinic agonists, such as ABT-418, which have been reported to act selectively on the isoform of the receptor (4). It may be the case, therefore, that the ability to elicit sensitized DA responses in the nucleus accumbens may be a property of relatively few nicotinic receptor agonists that exert the appropriate pattern of effects at all the isoforms of the receptor that contribute to the response. If this hypothesis is correct, it implies that it may be possible to develop novel therapeutic strategies using nicotinic drugs that are devoid of the addictive potential of nicotine itself.

The putative physiological role of burst firing remains unclear, although it has been suggested that its purpose may be to enhance neurotransmission at DA synapses because the overflow of neurotransmitter evoked by impulses presented as bursts is significantly greater than that evoked by single spikes delivered at the same overall rate (24,43). Thus, one explanation for burst firing may be that it provides a means of reinforcing synaptic transmission at dopaminergic synapses and that increased overflow of DA that occurs simply reflects saturation of the presynaptic transporters that normally limit DA overflow into the extracellular space sampled by a dialysis probe. It has been suggested, however, that DA may act on extrasynaptic receptors in the brain that respond to transmitter released into the interstitial fluid between the cells (1,56). Therefore, another possible role for burst firing may be to stimulate transmitter release into the extrasynaptic space between the cells to evoke responses mediated by the extrasynaptic receptors. This could be caused by increased DA overflow from the synaptic cleft following saturation of the presynaptic transporters. Alternatively, burst firing could cause a sufficiently large and generalized increase in the free calcium concentration within the terminals to evoke DA release directly into the extracellular space around the terminals from vesicles, such as those which co-store neuropeptide, which are relatively insensitive to the more localized influx of calcium evoked by relatively low frequency single spike discharges (40). This hypothesis is summarized in diagrammatic form in Fig. 5. If the latter is the correct explanation, then it seems reasonable to suggest that independent neural mechanisms may control the two firing modes and that each may subserve different functions. This hypothesis is consistent with the data reported here in which expression of the sensitized responses to nicotine appear to depend upon co-stimulation of NMDA receptors, whereas the acute responses to the drug do not.

PUTATIVE PSYCHOPHARMACOLOGICAL SIGNIFICANCE OF SENSITIZED DOPAMINE RESPONSES TO NICOTINE

Clarke and colleagues (19) have reported that the locomotor stimulant properties of nicotine are abolished if the mesoaccumbens DA system is lesioned by injecting 6-hydroxydopamine bilaterally into the nucleus accumbens. These and other observations led Clarke (16) to suggest that the locomotor stimulant properties of the drug are mediated by its stimulatory effects on the DA projections to the accumbens. This conclusion is consistent with the results of subsequent studies that have both confirmed the effects of the 6-hydroxydopamine lesions on the locomotor responses to nicotine (5) and shown that locomotor stimulation can be evoked by microinjecting nicotine directly into the VTA (48). It seemed reasonable to suggest, therefore, that the sensitization of mesoaccumbens DA overflow, evoked by repetitive injections of nicotine, mediates the enhanced locomotor responses observed in nicotine-pretreated animals (8). This conclusion is consistent with the fact that the dialysis probes, used in our experiments, are located predominantly in the core of the accumbens, a subdivision of structure that sends major projections to areas of the brain that have been implicated specifically in the control of motor activity (28). However, the results of the more recent experiments with NMDA antagonists have revealed a clear dissociation between the effects of nicotine on DA overflow in this area of the brain and its effects on locomotor activity. In particular, although pretreatment with acute D-CPPene elicits significant changes in the DA response to nicotine, it

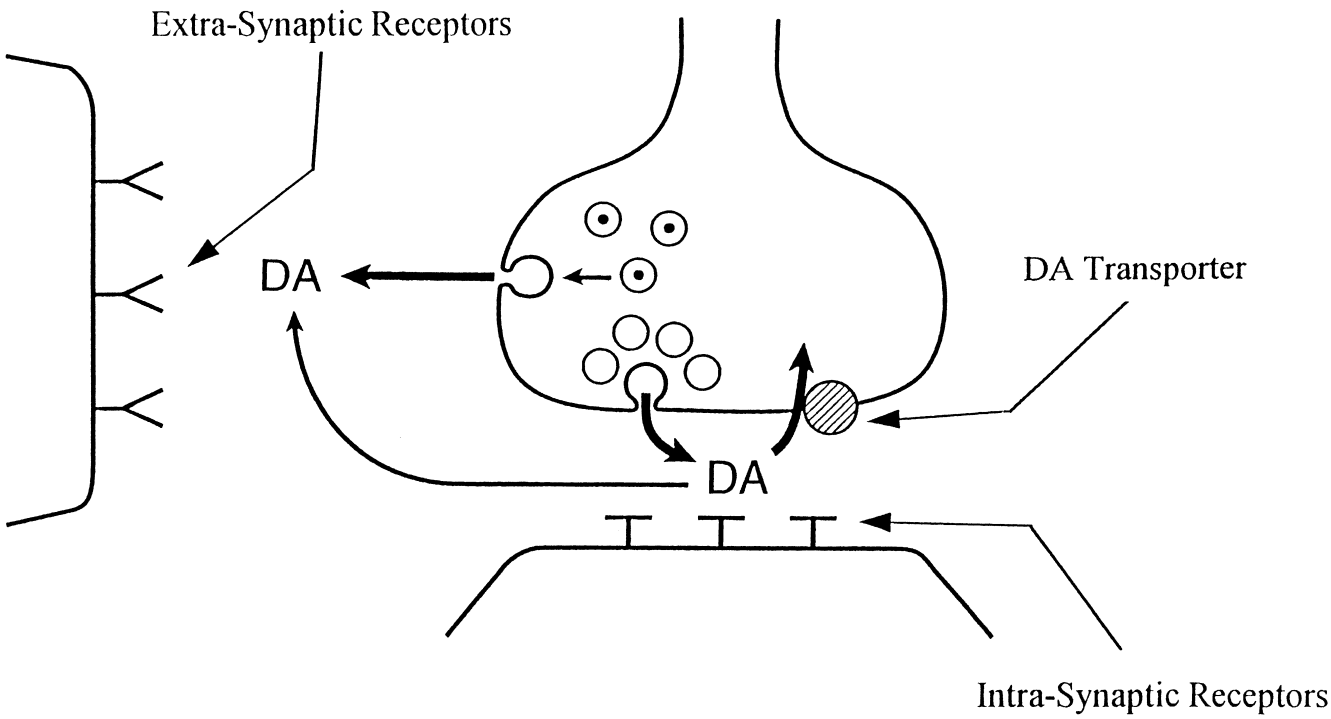

FIG. 5. Diagrammatic representation of a dopamine terminal in the nucleus accumbens This figure summarizes the two putative mechanisms by which DA may be released into the extrasynaptic space and gain access to extrasynaptic DA receptors. It may either escape from the synaptic cleft or be released directly into the extrasynaptic space from vesicles that release transmitter preferentially in response to burst firing.

has no effects on the locomotor responses to the drug measured following acute or subchronic nicotine administration (Fig. 4). In addition, in rats that have been allowed to habituate to their test environment, nicotine pretreatment causes sensitization to the locomotor stimulant properties of D-amphetamine, whereas the mesoaccumbens DA response to the drug is not significantly influenced by the pretreatment protocol (Birrell and Balfour, in preparation). Thus, these data do not provide support for the conclusion that the locomotor stimulant properties of nicotine are mediated by its effects on mesoaccumbens DA neurons, at least when they are measured using microdialysis. This conclusion is consistent with the results reported by Vezina et al. (54), which showed that lesions of the mesoaccumbens DA system, evoked by injecting 6-hydroxydopamine into the VTA, had no significant effects on the locomotor stimulant properties of nicotine in spite of the fact that the injections elicited a near complete reduction in accumbal DA. These results clearly cast doubt on the way in which Clarke et al. (17) and Balfour et al. (5) interpreted their results using intraaccumbal injections of toxin, and imply that the relationship between the stimulatory effects of nicotine on locomotor activity and DA release in the accumbens, if any, is more complex than was originally thought.

Recent studies suggest that many drugs of abuse preferentially increase activity in the DA neurons that project to the shell of the accumbens, a subdivision of the structure that seems to form an extension of the amygdala (2,46). As a result, it has been suggested that the rewarding properties of these drugs, which reinforce their self-administration, are related to their ability to stimulate the DA neurons that project to this subdivision of the accumbens. For cocaine at least, this conclusion is supported by the fact that the microinjection of DA receptor antagonists into the shell appears to attenuate the reinforcing properties of the drug in a self-administration paradigm (14). Although experiments of this complexity have not yet been performed with animals trained to self-administer nicotine, there is evidence that, like the other drugs of abuse, it preferentially stimulates neurons that project to the shell of the accumbens (47). However, a series of experiments reported by Pierce and Kalivas (45) have shown that pretreatment with cocaine causes a preferential sensitization of the locomotor response to amphetamine when it is delivered into the shell rather than the core of the accumbens. In addition, the sensitized locomotor responses observed when amphetamine is administered systemically to the rats pretreated with cocaine seems to correspond with an enhanced DA response in the shell rather than the core of the accumbens. Thus, in spite of the neuroanatomical evidence to the contrary, the expression of sensitized locomotor activity to amphetamine seems to correspond best with changes in DA overflow in the shell. The putative behavioral consequences of the enhanced DA response to nicotine, seen in the core of the nucleus accumbens, therefore, remains to be established. The hypothesis proposed by Robinson and Berridge (49) to explain the role of sensitized mesoaccumbens DA responses to stimulant drugs implies that they may be implicated in attributing 'incentive salience' to the stimuli associated with drug taking. It is tempting to suggest, therefore, that sensitization of the mesoaccumbens DA response to nicotine may serve a similar purpose. Agnati and colleagues (1,56) have suggested that DA released into the interstitial space may mediate 'volume transmission' between groups of cells that may be separated

anatomically. Thus, if this hypothesis is correct, it is tempting to speculate that the development of sensitized responses to drugs, such as nicotine, may provide a means of enhanced communication between different terminal fields within the accumbens concerned with different components of their psychopharmacological profile.

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