European Journal of Neuroscience, Vol. 15, pp. 1810–1818, 2001

Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections

R. Ferrari,[1] N. Le Novère,[2] M. R. Picciotto,[3] J. P. Changeux[2] and M. Zoli[1,2]
[1]Department of Biomedical Sciences, Section of Physiology, and Interuniversity Center for the Study of Ageing, University of Modena, Italy
[2]CNRS UA 2182 – 'Récepteurs et Cognition', Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cédex 15, France
[3]Department of Psychiatry, Yale University School of Medicine, 34 Park St., New Haven, CT 06508, USA

Keywords: GluR1, *in situ* hybridization, intracerebral microdialysis, locomotor activity, rat, tyrosine hydroxylase

Abstract

We have examined several neurochemical and behavioural parameters related to the function of the mesolimbic dopamine (DA) pathway in animals treated with nicotine following three modes of drug administration, i.e. systemic intraperitoneal injection, intra-accumbens (Acb) infusion or intraventral tegmental area (intra-VTA) microinjection. The present modes of systemic, intra-Acb and intra-VTA nicotine administration elicited comparable acute increases in dialysate DA levels from the Acb. The increase in extracellular DA levels was paralleled by a significant enhancement of locomotion in a habituated environment in the case of systemic or intra-VTA nicotine administration, whereas unilateral or bilateral intra-Acb nicotine infusion was ineffective, showing that accumbal DA increase is not sufficient to elicit locomotion in this experimental paradigm. Intra-VTA, but not systemic or intra-Acb, nicotine administration caused a long-term (at least 24-h) increase in basal dialysate DA levels from the Acb. In addition, significant increases in tyrosine hydroxylase (TH) and GluR1 (but not dopamine transporter or NR1) mRNA levels in the VTA were detected 24 h after intra-VTA nicotine administration. Systemic nicotine injection caused only an increase in TH mRNA levels while intra-Acb infusion did not modify any of the mRNAs tested. The long-term increase in basal DA levels in the Acb and TH, and GluR1 mRNA levels in the VTA upon intra-VTA nicotine microinjection indicates that even a single nicotine injection can induce plastic changes of the mesolimbic DA pathway.

Introduction

Nicotine can activate the mesolimbic dopamine (DA) pathway resulting in stimulation of locomotion and reinforcement. Accordingly, lesions of mesolimbic DA neurons attenuate the locomotor stimulant properties of nicotine (Clarke *et al*., 1988; Benwell & Balfour, 1992; Louis & Clarke, 1998; see, however, Vezina *et al*., 1994 for contrasting results), as well as nicotine self-administration in rats (Corrigall *et al*., 1992).

Increases in extracellular DA levels in the nucleus accumbens (Acb) can be elicited by systemic (Imperato *et al*., 1986), as well as local, injections of nicotinic agonists in the ventral tegmental area (VTA) or in the Acb (Nisell *et al*., 1994b; Marshall *et al*., 1997), the site of origin and termination of mesolimbic DA neurons, respectively. However, nicotine differentially affects DA release when injected into the Acb or the VTA (Nisell *et al*., 1994b) and nicotinic antagonists block systemic nicotine effects on extracellular DA levels when microinjected into the VTA, but not the Acb (Nisell *et al*., 1994a). This difference may derive from the presence of nicotinic acetylcholine receptors (nAChRs) with different subunit composition (Le Novère *et al*., 1996; Picciotto *et al*., 1998; Zoli *et al*., 1998; Klink *et al*., 2001) or pharmacology (Reuben *et al*., 2000; Wonnacott, 1997), and/or a different functional role in VTA and Acb.

Correspondence: Dr Michele Zoli, Dipartimento di Scienze Biomediche, sezione di Fisiologia, Università di Modena e Reggio Emilia, via Campi 287, 41100 Modena, Italy.
E-mail: mzoli@unimo.it

Received 17 January 2002, revised 21 March 2002, accepted 8 April 2002

Development of drug dependence is a process that involves drug-induced plastic changes in specific neuronal networks (Berke & Hyman, 2000), including the mesolimbic DA pathway (Nestler & Aghajanian, 1997; Martin-Soelch *et al*., 2001). Several groups have shown that repeated administration of psychostimulants (e.g. cocaine and nicotine) results in sensitization of the mesolimbic DA system to the effect of the drug itself (Pierce & Kalivas, 1997), a phenomenon which may be necessary for development of drug dependence (Robinson & Berridge, 2000). Accordingly, repeated administration of nicotine results in sensitization of both locomotor stimulation (Morrison & Stephenson, 1972; Ksir *et al*., 1985) and DA release in Acb (Benwell & Balfour, 1992; Shim *et al*., 2001) to a nicotine challenge. Yet, a single exposure to nicotine is sufficient to elicit a variety of plastic changes. For instance, a single nicotine injection modifies DA D2 binding and neuropeptide levels in dopaminoceptive striatal neurons (Dhatt *et al*., 1995; Li *et al*., 1995; Houdi *et al*., 1998). A single nicotine application to slices of the ventral mesencephalon induces N-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) in DA neurons (Mansvelder & McGehee, 2000). Finally, a single nicotine injection increases c-fos expression in several brain areas, including input and output regions of the mesolimbic pathway (Lanca *et al*., 2000).

In the present study we have examined several parameters related to the function of the mesolimbic DA pathway in animals treated with nicotine following three modes of drug administration, i.e. systemic intraperitoneal (i.p.) injection, intra-Acb infusion or intra-VTA microinjection. These parameters include (i) acute changes in

TABLE 1. Sequence of the oligonucleotides used for *in situ* hybridization experiments

Target mRNA	Oligonucleotide sequence
Tyrosine hydroxylase	5′-AGGGTGTGCAGCTCATCCTGGACCCCCTCCAAGGAGCGCT-3′
Dopamine transporter	5′-AGGGTGGAGTTGGTCAGCTGCACTCCGTTCTGCTCCTTGACCAGG-3′
Glutamate receptor subunit GluR1 oligoA	5′-TTCTCTGCGGCTGTATCCAAGACTCTCTGCAGGACTGACAGGCCC-3′
Glutamate receptor subunit GluR1 oligoD	5′-CAGTCCACCCTGGTATGGTCTCGGGAGTCACTTGTCCTCCATTGC-3′
Glutamate receptor subunit NR1 oligoA	5′-GTGAAGTGGTCGTTGGGAGTAGGCGGGTGGCTAACTAGGATAGCG-3′
Glutamate receptor subunit NR1 oligoB	5′-CCCCATCCTCATTGAATTCCACACGGCCAGTCACTCCGTCCGCAT-3′

dialysate DA levels from the Acb and locomotor activity in a habituated environment; (ii) long-term changes in dialysate DA levels from the Acb and the levels of mRNAs related to dopaminergic and glutamatergic transmission in the VTA, namely the DA biosynthetic enzyme tyrosine hydroxylase (TH), the dopamine transporter (DAT), the GluR1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) subtype and the NR1 subunit of the NMDA subtype of glutamate receptor. Changes in TH and/or glutamate receptor mRNAs in the VTA have been previously observed following exposure to several drugs of abuse (see e.g. Fitzgerald *et al*., 1996 and Boundy *et al*., 1999 and references therein). Finally, the same neurochemical and behavioural parameters were studied after systemic injection of amphetamine, another drug of abuse which, similar to nicotine, elicits DA release in the Acb and locomotion (see, e.g. Carboni *et al*., 1989; McKinzie *et al*., 2002).

Materials and methods

Animals and experimental groups

Adult male specific pathogen-free Sprague–Dawley rats (250 g body weight) were used for these studies. They were kept under standardized temperature, humidity and lighting conditions (lights on at 08.00 h and off at 20.00 h) and had free access to water and food. All animal experimentation was conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

Animals were placed into the following experimental groups (nicotine doses are expressed as free base).

(i) Animals that received an i.p. injection of nicotine (0.4 mg/kg, $n = 33$) or vehicle ($n = 24$).

(ii) Animals that received nicotine (1 mM, continuously perfused during 60 min, $n = 28$) or vehicle ($n = 24$) administration through the dialysis probe. It has been shown that less than 1% of nicotine crosses the microdialysis probe (Marshall *et al*., 1998; Lecca *et al*., 2000). This dose was chosen in order to obtain an increase of DA perfusate levels comparable with that obtained with intra-VTA injection of nicotine.

(iii) Animals that received nicotine (2 or 12 μg/1 μL infused during 2 min, $n = 6$ and 30, respectively) or vehicle ($n = 22$) administration into the ventral tegmental area through a microinjection cannula. The doses of nicotine microinjected into the VTA were chosen based on previous studies of cytisine microinjection into the VTA (Museo & Wise, 1990a). In a preliminary set of experiments, we tested the effects of the two doses of nicotine on DA perfusate levels in the Acb. The lower dose gave small and inconsistent changes in DA levels. Therefore, we selected the 12 μg/μL dose of nicotine for the following experiments.

(iv) Animals that received an i.p. injection of amphetamine (0.75 mg/kg, $n = 16$).

Each animal was used for a single experimental procedure (intracerebral microdialysis, locomotor activity or *in situ* hybridization).

Intracerebral microdialysis

On day 1 of the experiment, animals were deeply anaesthetized with halothane (Fluothane), and a microdialysis probe guide cannula (Carnegie Medicine, Stockholm, Sweden) was implanted into the right medial Acb (coordinates: bregma +2.0 mm, 0.8 mm or 1.5 mm lateral, and –8.0 mm ventral from dura) and secured to the skull with dental cement. In some animals, a stainless steel guide cannula (26 gauge, 500-μm outer diameter) was implanted into the VTA (coordinates: bregma –5.3 mm, 0.8 mm lateral, and –7.5 mm ventral from dura) and filled with a dummy cannula.

Twenty-four hours after surgery, the awake animal was gently restrained by the experimenter and a microdialysis probe (500-μm outer diameter, with a 2-mm length dialysing membrane, CMA12, Carnegie Medicine) was inserted into the guide cannula. The probe was connected to a microinfusion pump (CMA/102, Carnegie Medicine) and continuously perfused at 2 μL/min with Ringer's solution (KCl, 3.0 mM; NaCl, 147 mM; $CaCl_2$, 1.2 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 0.4 mM; pH 7.2). Starting 1 h after implantation, dialysate samples were collected every 20 min into 0.5-mL Eppendorf tubes containing 10 μL of mobile phase.

Nicotine, amphetamine or vehicle treatments were administered 3 h after probe insertion. In the case of intra-VTA injections, the animal was gently restrained and a stainless steel microinjection cannula (33 gauge) was inserted into the guide cannula. Nicotine or vehicle was injected over 1 min using a microinjection pump (CM100, Carnegie Medicine).

Mean basal dialysate DA levels were determined in the three dialysate samples before drug administration. Three hours after nicotine or vehicle administration, the probe was removed and the animal was returned to its home cage. Twenty-four hours after nicotine, amphetamine or vehicle administration, the microdialysis probe was re-implanted and, after 2 h, three samples were recovered. At the end of the experiment the animals were killed by rapid decapitation and the placement of the microdialysis probe was determined. Animals in which the probe was not correctly located, with large haemorrhages or signs of blood leak into the ventricles were excluded from the study. Using the present procedure, we found stable dialysate DA levels in the three samples preceding drug administration in the second or third experimental day. However, we noticed a slight progressive decrease in dialysate DA levels over the entire sampling period of each day and from day to day, so that basal DA levels on the third day were 70–80% of those of the second day in vehicle-treated animals. In pilot experiments, we found comparable results using animals stably implanted with the microdialysis cannula and tested 24 and 48 h after the implantation. We preferred to use the double insertion rather than the single implantation procedure

because, with the latter procedure, the animals remained connected to the microinfusion pump through the cannula and tubing during two nights, when they have high spontaneous activity.

HPLC determination of dopamine levels

DA levels in the dialysate were determined using high-performance liquid chromatography (HPLC) with electrochemical detection (464B BAS, sensitivity: 20 fmol/sample; Bioanalytical Systems, West Lafayette, IN, USA). A volume of 44 μL from each dialysis sample was injected into a HPLC system with amperometric electrochemical detection for determination of DA (BAS, LC 4B). Separation of DA was performed on a Chrompack Spherisorb 5 ODS column (250 mm × 4.6 mm inner diameter). The mobile phase consisted of 18% methanol in 80 mM NaH_2PO_4, 0.4 mM sodium octane sulphonate and 0.1 mM EDTA (pH 3.0) (Osborne *et al*., 1991). The flow rate was 0.8 mL/min (2150 HPLC pump; LKB, Bromma, Sweden). The column was coupled directly to the amperometric detector cell. The system was equipped with a dual glassy carbon electrode with operating potentials set at 700 mV (vs. an Ag/AgCl reference electrode). The chromatograms were integrated with Gilson 712 HPLC software (Gilson Italia SRL, Milan, Italy) and standard curve ranging from 500 to 30 fmol was used to quantify the concentration of DA.

Locomotor activity

Locomotor activity was measured in motility cages (24 × 45 cm) with 10 photocell beams on the long side connected to a digital counter (Multicounter LE3806; Letica, Barcelona, Spain). The animals were placed in the cages for a 2-h habituation period and then injected with nicotine, amphetamine or vehicle. Habituated locomotion was measured for 90 min following treatment.

In situ *hybridization*

The sequences of the oligonucleotides used for *in situ* hybridization experiments are shown in Table 1. Three oligodeoxynucleotide sequences were chosen in unique regions of the rat TH, DAT, glutamate receptor subunits NR1 and GluR1 mRNAs following analysis for mRNA secondary structure using Genetic Computer Group Wisconsin package (Accelrys Ltd, Cambridge, UK) Sequence Analysis Software 7.1. The oligonucleotides for NR1 were directed to portions of the molecule that are common to all splice variants of this mRNA. Specificity controls included the demonstration that (i) two or more probes for each mRNA showed an identical labelling pattern, (ii) probes with the same base composition but different sequence did not show the specific labelling pattern, (iii) the distribution of the labelling was identical to previously published distribution of the same mRNA. The oligonucleotide probes were labelled at the 3′ end using ^{35}S-dATP (Amersham Pharmacia Biotech Italy, Cologno Monzese, Italy) and terminal deoxynucleotidyl transferase (Boehringer, Mannheim, Germany) following the specifications of the manufacturer to a specific activity of 100–300 kBcq/pmol. The labelled probes were separated from unincorporated ^{35}S-dATP using NucTrap push columns (Stratagene, La Jolla, CA, USA), precipitated in ethanol and resuspended in distilled water containing 50 mM dithiothreitol. After the specificity test, the two oligonucleotides for NR1 or GluR1 were combined to increase the intensity of the hybridization signal.

Animals were killed by decapitation, frozen brains were cut on a cryostat (14-μm-thick sections) at a level –5.5 mm from bregma, thaw-mounted on poly L-lysine coated slides and stored at –80 °C for 1–3 days. The procedure was carried out according to Zoli *et al*. (1995). Probes were applied at a concentration of 2000–3000 Bcq/30 μL per section (corresponding to around 15 fmol per section). The slides were exposed for 7 days (TH and DAT) or 21 days (NR1 or GluR1) to Kodak BioMax MR (Amersham Pharmacia Biotech Italy).

Statistical analysis

Statistical analysis was performed using the SPSS version 10 statistical package (SPSS, Chicago, IL, USA). In microdialysis and *in situ* hybridization experiments, statistical comparisons between treatments were carried out by means of the nonparametric Mann–Whitney *U*-test. In locomotor activity experiments, statistical comparisons were carried out by means of repeated-measures analysis of variance using treatment as between-subject factor and number of beam breaks per 5 min as within-subject factor. $P < 0.05$ was defined as a threshold for significant difference.

Results

Changes in dialysate dopamine levels from the nucleus accumbens after systemic or local nicotine administration

Systemic nicotine injections (0.4 mg/kg) elicited a significant increase in DA levels in the dialysate recovered from the Acb. The peak increase was detected 40 min after nicotine administration (Fig. 1A). The placement of the probe in different parts of the Acb markedly influenced the magnitude of the response. Nicotine elicited more than a 200% peak increase in DA levels when the probe was placed medial to the anterior commissure (corresponding to the shell of the Acb), but around a 40% peak increase when the probe was placed lateral to the anterior commissure (corresponding to the core of the Acb) (Fig. 1A). We have chosen the medial location for all the following experiments. Saline i.p. treatment did not elicit any significant increase in DA perfusate levels (Fig. 1A).

In another group of animals, we administered nicotine directly into the VTA (DA cell bodies) or the Acb (DA nerve terminals). In both cases, nicotine elicited a significant increase in DA perfusate levels, with peaks of ≈300% for intra-Acb or intra-VTA administration (Fig. 1B and C). In the case of intra-Acb administration, DA levels remained elevated throughout the entire period of nicotine administration, but rapidly decreased when nicotine was withdrawn. After intra-VTA nicotine administration, the increase in DA levels was sustained and was still higher (30%) than basal DA level 3 h after nicotine administration. Intra-VTA administration of saline did not significantly change DA dialysate levels (Fig. 1C).

Changes in dopamine levels in the nucleus accumbens 24 h after systemic or local nicotine administration

When the same animals were tested on the day after the nicotine or vehicle challenge, basal DA levels differed significantly across treatment groups. Basal DA levels were decreased by 30–40% in both animal groups treated on the previous day with systemic saline or nicotine (Fig. 2). The animals previously treated with intra-Acb nicotine showed a decrease in basal DA levels (40%) similar to that observed after i.p. nicotine administration (Fig. 2). Instead, the animals previously treated with intra-VTA nicotine showed a more than twofold increase in extracellular DA levels as compared with basal levels of the previous day, which was significantly different from that induced by intra-VTA vehicle administration (Fig. 2). When the animals treated with intra-VTA nicotine on the previous day were challenged with a further intra-VTA injection of nicotine, the magnitude of the percentage increase in dialysate DA levels from the Acb was similar to that elicited by nicotine the previous day,

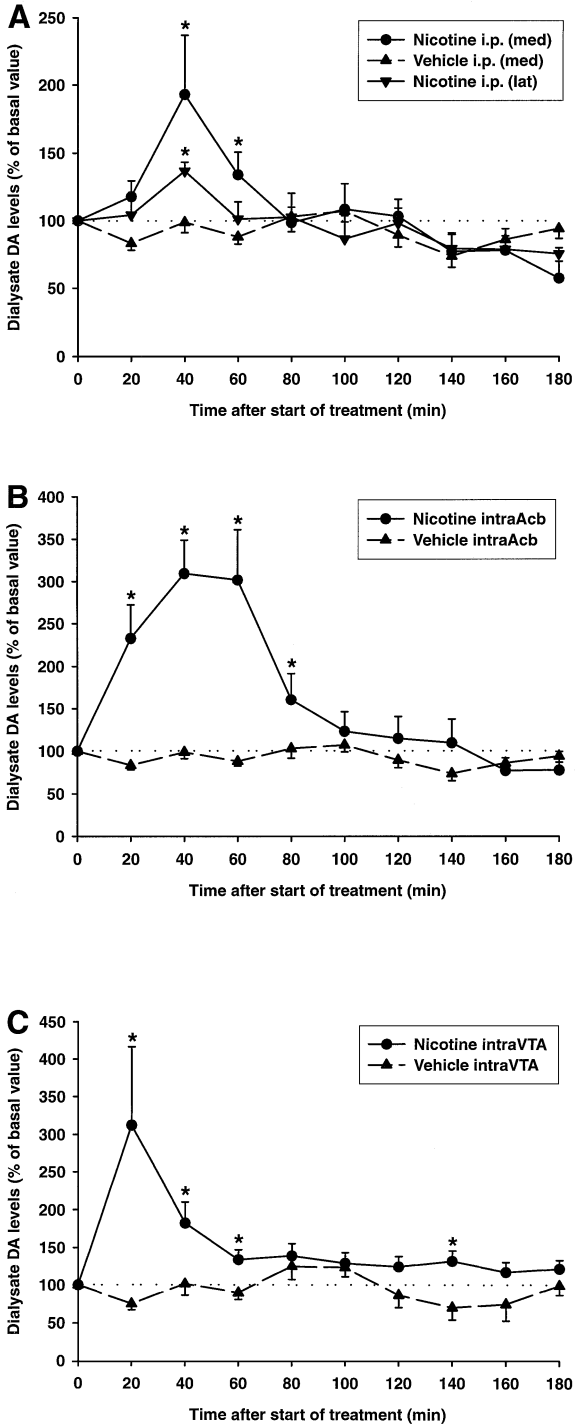

FIG. 1. Changes in dialysate dopamine (DA) levels from the nucleus accumbens (Acb) of rats after systemic (i.p.) (A), intra-Acb (B) or intraventral tegmental area (intra-VTA) (C) nicotine administration. In the case of nicotine i.p. treatment (A) the microdialysis probe was placed in the medial (med) or lateral (lat) Acb, whereas in the case of intra-Acb (B) or intra-VTA (C) administration the probe was always located in the medial Acb. Dialysate DA levels are expressed as a percentage of basal DA levels, calculated as the mean of the three dialysate samples obtained before drug injection. Statistical analysis according to Mann–Whitney *U*-test, $*P < 0.05$, nicotine vs. respective vehicle treatment. Animals per group: i.p. treatment: nicotine med (8), nicotine lat (8), vehicle (8); intra-Acb: nicotine (11), vehicle (8); intra-VTA: nicotine (13), vehicle (6).

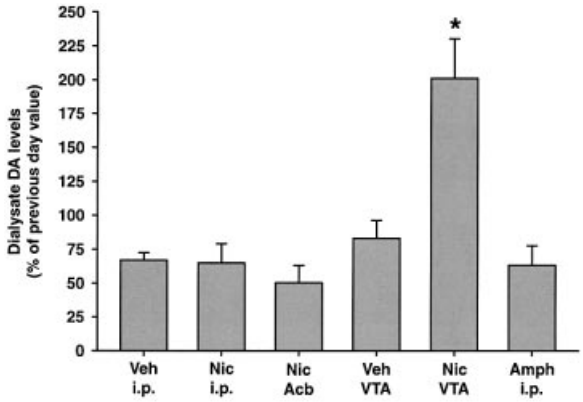

FIG. 2. Basal dialysate dopamine (DA) levels from the medial nucleus accumbens 24 h after systemic (i.p.), intranucleus accumbens (intra-Acb) or intraventral tegmental area (intra-VTA) nicotine administration. Statistical analysis according to Mann–Whitney *U*-test, $*P < 0.05$, nicotine or amphetamine vs. respective vehicle treatment.

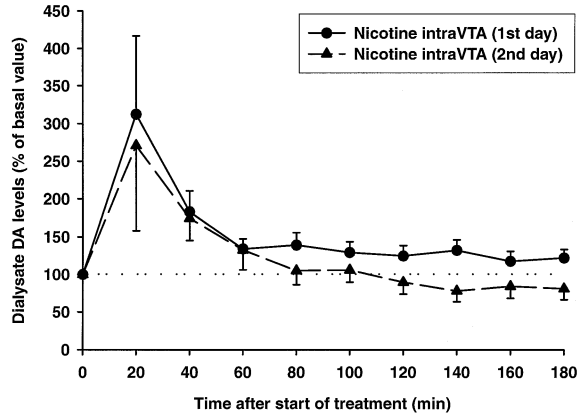

FIG. 3. Changes in dialysate dopamine (DA) levels from the medial nucleus accumbens of rats after intraventral tegmental area (intra-VTA) nicotine administration injected in naive rats (day 1) or rats which received a nicotine intra-VTA injection 24 h before (day 2). Dialysate DA levels are expressed as a percentage of basal DA levels, calculated as the mean of the three dialysate samples obtained before drug injection.

showing no sign of sensitization (Fig. 3). However, no further post-treatment augmentation in basal DA levels could be observed.

Locomotion elicited by i.p., intranucleus accumbens or intraventral tegmental area nicotine administration

Nicotine-stimulated locomotion in a familiar environment is thought to be dependent on activation of the mesolimbic DA system (see Introduction). We have therefore tested the rats treated with nicotine i.p., intra-VTA or intra-Acb, in a paradigm of habituated locomotion (Fig. 4). The animals were treated with nicotine or saline 2 h after their placement in a locomotor activity cage. While both i.p. and intra-VTA nicotine elicited a significant increase in locomotion (Fig. 4A and C), locomotion elicited by nicotine administered intra-Acb was not significantly different from that elicited by vehicle injection (Fig. 4B).

Nicotine-administered i.p. reaches cell bodies and terminals of the mesolimbic DA pathway of both sides of the brain. It is also likely that nicotine injected into the VTA can diffuse and at least partially

activate the contralateral side of the VTA. Diffusion of nicotine to the contralateral side at an effective concentration is less likely in the case of intra-Acb administration. In order to exclude that the differences observed in nicotine-elicited locomotor activation are simply due to unilateral (intra-Acb) vs. bilateral (i.p. or intra-VTA) activation of the mesolimbic pathway, we placed a probe in each medial Acb and administered nicotine simultaneously through both probes. However, no significant increase in locomotion was observed after bilateral nicotine intra-Acb administration (Fig. 4B).

Dialysate dopamine levels from the nucleus accumbens and locomotion elicited by systemic amphetamine

Amphetamine can potently stimulate the release of DA as well as effectively increase locomotor activity. We have therefore compared the effects of i.p. amphetamine on dialysate DA levels from the Acb as well as habituated locomotion to those elicited by i.p. nicotine. As expected, i.p. injection of amphetamine (0.75 mg/kg) elicited a large increase in dialysate DA levels with a peak value of ≈ 500% (Fig. 5). The same animals were tested the day after the amphetamine challenge and basal dialysate DA levels did not differ significantly from those of the animals injected with saline (Fig. 2). Locomotor activity after habituation to the cage was markedly increased by amphetamine (0.75 mg/kg) (not shown).

Analysis of dopamine transporter, tyrosine hydroxylase, NR1 and GluR1 mRNA levels in mesencephalic dopaminergic nuclei 24 h after nicotine intraventral tegmental area injection

We determined whether the increase in DA levels in the Acb observed 24 h after an intra-VTA injection of nicotine was accompanied by alterations in the expression of dopaminergic or glutamatergic markers in the VTA. Glutamate transmission in the VTA is considered to be critical for nicotine-elicited activation of the mesolimbic pathway (Fu *et al.*, 2000; Schilstrom *et al.*, 1998). Both AMPA and NMDA subtypes of glutamate receptors are expressed in the VTA. The present analysis was carried out using two dopaminergic markers, TH and DAT, and two glutamatergic markers, the AMPA subunit GluR1 and the NMDA subunit NR1. TH mRNA levels were markedly increased in the VTA of rats treated with nicotine in the VTA (≈ 40%, $P < 0.01$, Mann–Whitney *U*-test) and to a lesser extent in rats treated with nicotine i.p. (≈ 25%, $P < 0.05$, Mann–Whitney *U*-test), but not in rats treated with nicotine in the Acb (Figs 6A and B, and 7A). No significant change was observed in the substantia nigra pars compacta (SNc) of any treatment group. A marked and significant (≈ 55%, $P < 0.001$, Mann–Whitney *U*-test) increase of GluR1 mRNA levels in the ventromedial mesencephalon, including the dopaminergic portion of the VTA, but not in the SNc, superior colliculus or frontoparietal cortex (not shown), was detected in rats that had received intra-VTA nicotine (Figs 6C and D, and 7B). No significant change was observed 24 h after i.p. or intra-Acb nicotine treatments. NR1 and DAT mRNA levels were unchanged in all analysed regions, namely SNc, VTA, periaqueductal grey and frontoparietal cortex for NR1, and VTA and SNc for DAT, 24 h after i.p., intra-Acb or intra-VTA nicotine treatment (not shown).

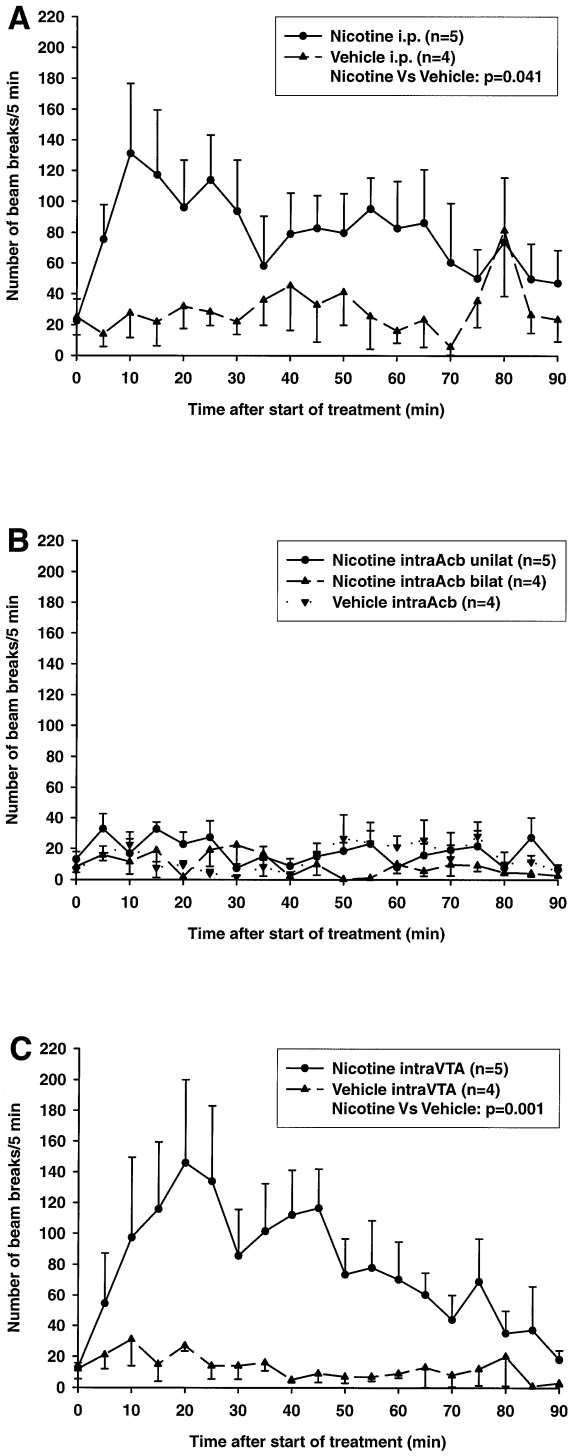

FIG. 4. Locomotor activity in a habituated environment of rats after systemic (i.p.) (A), intranucleus accumbens (intra-Acb) (B) or intraventral tegmental area (intra-VTA) (C) nicotine administration. In the case of nicotine intra-Acb administration, both unilateral (unilat) and bilateral (bilat) administrations were studied (B). Locomotor activity was evaluated as the number of photocell beam breaks per 5 min. Nicotine or vehicle was administered after 90 min habituation time to the cage. Statistical analysis according to repeated-measures analysis of variance, $^*P < 0.05$, nicotine vs. respective vehicle treatment. Animals per group: i.p. treatment: nicotine (5), vehicle (4); intra-Acb: unilateral nicotine (5), bilateral nicotine (4), vehicle (4); intra-VTA: nicotine (5), vehicle (4).

Discussion

Systemic, intranucleus accumbens and intraventral tegmental area administrations of nicotine differ in their neurochemical and behavioural effects

The present results show that a single peripheral dose of nicotine results in both an increase in dialysate DA levels from Acb, and an

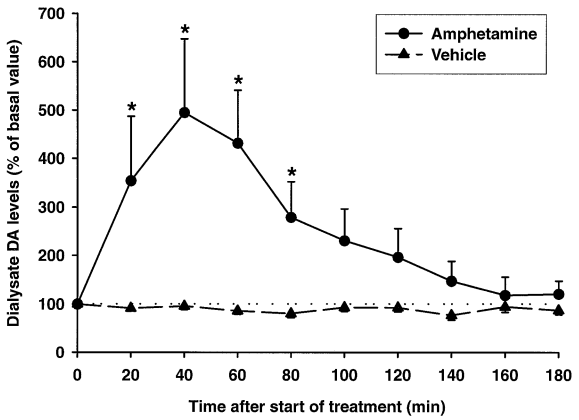

FIG. 5. Changes in dialysate dopamine (DA) levels from the medial nucleus accumbens of rats after systemic (i.p.) amphetamine administration. Dialysate DA levels are expressed as a percentage of basal DA levels, calculated as the mean of the three dialysate samples obtained before drug injection. Statistical analysis according to Mann–Whitney *U*-test, $*P < 0.05$, amphetamine vs. vehicle treatment. Animals per group: amphetamine (11), vehicle (8).

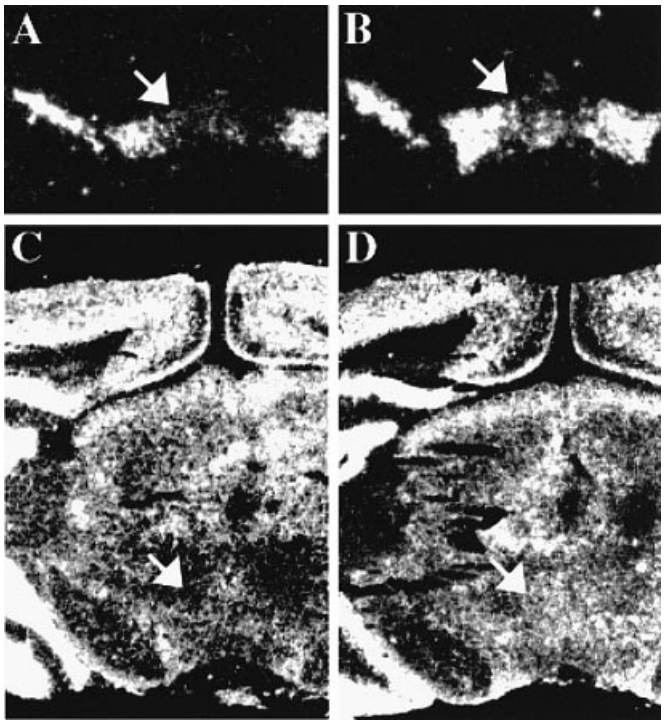

FIG. 6. Dark-field microphotographs of film autoradiograms showing tyrosine hydroxylase mRNA (A and B) or GluR1 mRNA (C and D) signal in the mesencephalon of rats 24 h after an intraventral tegmental area (intra-VTA) injection of vehicle (A and C) or nicotine (B and D).

increase in habituated locomotion, but no significant changes in dialysate DA levels from the Acb 24 h postinjection. A dissociation between these effects occurs when local injection of nicotine is performed in different sites of the mesoaccumbens pathway. Systemic, intra-VTA and intra-Acb nicotine administration are all able to increase dialysate DA levels from the Acb acutely to a roughly comparable extent. In contrast, while both systemic and intra-Acb nicotine administrations result in no long-term changes in dialysate DA levels, intra-VTA nicotine administration causes an increase in basal dialysate DA levels from Acb 24 h following injection. A further dissociation is seen in nicotine-elicited locomotor activation depending on the mode of administration. Whereas either i.p. or intra-VTA injection of nicotine results in an increase in locomotion, intra-Acb injection is ineffective in altering this behaviour.

Effects on dialysate dopamine levels from the nucleus accumbens

As previously shown by several groups (Imperato *et al*., 1986; Nisell *et al*., 1994b; Marshall *et al*., 1997), nicotine administered systemically, either into the Acb or into the VTA, elicits a marked increase in dialysate DA levels from the medial Acb (mostly corresponding to the shell subdivision). Nicotine administration into the Acb was at least as effective as systemic or intra-VTA nicotine administration, although DA levels rapidly dropped when nicotine administration through the dialysis cannula was withdrawn. This is consistent with what has been shown by Marshall *et al*. (1997), but differs from what has been reported by Nisell *et al*., (1994b). Overall, the present data support the notion that nAChRs expressed in the DA cell body and terminal areas are capable of eliciting DA release in the Acb of the awake, freely moving rat. However, the outcome of the treatment seems to be dependent on both the site of injection as well as the mode (e.g. cannula vs. microdialysis probe, time of infusion) of nicotine administration and its dose.

Effects on locomotor activity

Systemic, intra-VTA and intra-Acb administrations of nicotine had different effects on locomotor activation. Unilateral injection of nicotine into the VTA resulted in locomotor activation comparable with that recorded upon systemic administration. In contrast, unilateral or bilateral injections of nicotine in the Acb were ineffective. These data are in line with previous studies on intra-VTA (Museo & Wise, 1990a; Reavill & Stolerman, 1990; Panagis *et al*., 1996) and intra-Acb injection of nicotine or cytisine (Museo & Wise, 1990b; Reavill & Stolerman, 1990; Leikola-Pelho & Jackson, 1992). However, we show here that differences in locomotor activation with the two treatments are paralleled by comparable increases in dialysate DA levels from the Acb. Thus, increase in accumbal dialysate DA levels alone is not sufficient to elicit locomotion. A similar dissociation has previously been reported by Balfour and coworkers (1998) who showed that pretreatment with the NMDA antagonist D-CPPene prevents the increase in dialysate DA levels from Acb, but not the sensitized locomotor response elicited by repeated nicotine administration. The link between nicotine-elicited DA increase observed in microdialysis experiments and locomotion is probably more complex than previously thought (see also discussion in Balfour *et al*., 1998). The intracerebral microdialysis technique samples extracellular fluid in the minute time-scale and cannot distinguish between different spatiotemporal patterns of DA release, leading to, for example, the activation of synaptic vs. nonsynaptic DA receptors (Agnati *et al*., 1995; Zoli & Agnati, 1996; Balfour *et al*., 1998). Another possibility is that, besides DA transmission, intra-Acb nicotine activates other neurotransmissions with opposite action on locomotion (see, e.g. the evidence for DA glutamate antagonism on reward mechanisms in the Acb, Carlezon & Wise, 1996). Alternatively, coactivation of other DA terminal fields besides Acb may be necessary for nicotine-elicited locomotion. In fact, while diffusion of nicotine from Acb should not influence DA terminals in caudate putamen or prefrontal cortex, intra-VTA nicotine injection elicits DA release in the prefrontal cortex and may also influence DA

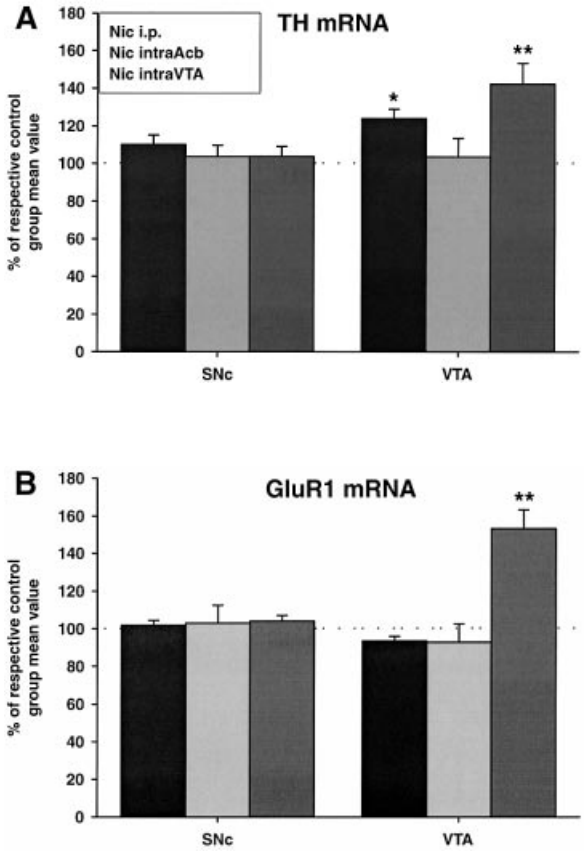

FIG. 7. Bar histogram showing the semiquantitative analysis of tyrosine hydroxylase (TH) (A) and GluR1 (B) mRNA levels in the ventral mesencephalon of rats 24 h after i.p. intranucleus accumbens (intra-Acb) or intraventral tegmental area (intra-VTA) injection of nicotine. For each mRNA, two experiments with six animals per treatment were performed, obtaining very similar results. Therefore, the data were pooled for the statistical analysis. Results are shown as mean percentage value ± SEM of the respective vehicle-treated mean value. Statistical analysis according to Mann–Whitney *U*-test, $^{**}P < 0.01$, $^{*}P < 0.05$, nicotine vs. respective vehicle treatment.

cells bodies in the SNc and, thus, DA release in the caudate putamen. Although nicotine activation of the nigrostriatal or mesocortical pathway alone does not elicit locomotion (Museo & Wise, 1990b, 1995), their coactivation together with the mesolimbic pathway may be necessary for full expression of nicotine-elicited locomotion.

A single nicotine injection into the ventral tegmental area results in long-term increases in dialysate dopamine levels from the nucleus accumbens, as well as in tyrosine hydroxylase and GluR1 mRNA levels in the VTA

Three hours and, more markedly, 24 h after a single nicotine injection in the VTA, dialysate DA levels from the Acb were significantly increased with respect to vehicle-treated animals. This change was dependent on the site of nicotine administration (see the lack of effect after i.p. and intra-Acb nicotine administration) and unrelated to the extent of the acute increase in accumbal dialysate DA level (see the lack of effect after amphetamine administration). The increase in baseline dialysate DA levels was accompanied by a significant increase in the expression of TH and GluR1 mRNA levels in the VTA. Notably, increase in GluR1 expression in the VTA parallels development of sensitization to several drugs of abuse, including cocaine and morphine, as well as stress (Fitzgerald *et al*., 1996; Carlezon *et al*., 1997). By increasing the rate of DA synthesis (TH) and the strength of the excitatory glutamatergic input (GluR1), the alterations in TH and GluR1 expression may therefore contribute to the basal hyperfunctioning of the mesolimbic DA pathway.

While the absence of nicotine-induced potentiation of the DA mesolimbic pathway after intra-Acb administration can be explained on the basis of different nAChR composition or circuitry in the Acb and VTA, respectively (see later discussion on LTP and long-term depression, LTD), i.p. nicotine administration can activate nAChRs in the VTA and therefore should, in principle, mimic the effects of intra-VTA nicotine injection. Among the factors that may account for the difference between i.p. and intra-VTA nicotine administration, nicotine concentration could be the most critical. Intra-VTA administration very likely results in a higher local concentration of nicotine than i.p. administration. This may lead to greater activation (or desensitization) of nAChRs in the VTA than what can be achieved by systemic nicotine administration. On the other hand, the high local concentration of nicotine may prolong its effects on nAChRs. It is therefore possible that, in this way, local nicotine can mimic the effects of repeated systemic nicotine administration causing a sensitization of DA mesolimbic pathway.

Increased DA accumbal levels upon single nicotine intra-VTA injection resemble the sensitization to the locomotor stimulant and DA-releasing effects of nicotine that are obtained upon repeated administration of systemic nicotine (Balfour *et al*., 1998; Shim *et al*., 2001). Nicotine sensitization is dependent on activation of nAChRs in the VTA, as it can be produced by repeated nicotine injections in this area (Balfour *et al*., 1998). However, the phenomenon described here is different from, although possibly related to, sensitization (discussed earlier). Sensitization is defined as an increased effect elicited by subsequent administrations of the same dose of a drug. In contrast, what is shown here is the effect of a single dose of nicotine leading to a long-term (at least 24 h) increase in baseline dialysate DA levels from the Acb.

Possible mechanisms for intraventral tegmental area nicotine-elicited potentiation of mesolimbic dopamine pathway

In the VTA, nicotine excites dopaminergic neurons via activation of somatic and/or dendritic nAChRs (Pidoplichko *et al*., 1997; Klink *et al*., 2001) and by stimulating glutamate release via nAChRs located on nerve terminals possibly projecting from the hippocampus or neocortex (Fu *et al*., 2000; Mansvelder & McGehee, 2000; Schilstrom *et al*., 1998). A recent paper reported that NMDA receptor-dependent LTP can be induced in VTA DA neurons by pairing postsynaptic depolarization with nicotine application (Mansvelder & McGehee, 2000). Indeed, late-phase LTP in the CA1 field of the hippocampus is paralleled by increased GluR subunit expression (Nayak *et al*., 1998), similar to what we observed 24 h after the intra-VTA nicotine challenge. Interestingly, it has recently been shown that a single cocaine exposure induces an AMPA receptor-dependent LTP of excitatory synapses onto DA neurons in the VTA (Ungless *et al*., 2001). Therefore, a possible explanation for nicotine-induced potentiation of the mesolimbic DA pathway is that intra-VTA nicotine treatment results in LTP of the glutamatergic input onto DA neurons in the VTA (a similar mechanistic hypothesis has been proposed for nicotine sensitization by Balfour *et al*., 1998).

Interestingly, activation of both glutamatergic and dopaminergic transmission in the striatum leads to LTD (Lovinger & Tyler, 1996; Calabresi *et al*., 1997) rather than LTP. This difference may explain

the different outcome of intra-Acb and intra-VTA nicotine injection on accumbal DA levels detected 24 h after the nicotine challenge. A balance between LTP in the VTA and LTD in the Acb may indeed explain why systemic nicotine treatment does not reproduce the effects of intra-VTA nicotine injection.

It has previously been shown that long-term exposure to nicotine, via activation of β2* or α7* nAChRs, causes a long-lasting rise of intracellular Ca^{2+} level (Tsuneki *et al*., 2000). Therefore, both nAChR-elicited potentiation of NMDA transmission (discussed earlier) and direct activation of postsynaptic nAChRs lead to increase in Ca^{2+} levels in DA neurons. Similar to what has been observed in other neuronal cells (Cammarota *et al*., 1998; Gueorguiev *et al*., 2000), activation of Ca^{2+}/calmodulin-dependent kinases may, in turn, increase GluR1 and TH expression, thus leading to the long-term increase in basal dialysate DA levels from the Acb.

Prolonged nicotine exposure is known to result in desensitization of several types of neuronal nAChRs, a phenomenon that may contribute to development of nicotine dependence (Katz & Thesleff, 1957; Changeux, 1990; Dani & Heinemann, 1996; Changeux & Edelstein, 1998). Long-term desensitization of nAChRs could cause the observed changes in DA transmission if DA neurons were under tonic inhibitory nicotinic tone. Recent papers have shown that specific nAChR subtypes are expressed in various γ-aminobutyric acid (GABA) subpopulations in the VTA/SN (Klink *et al*., 2001), and GABA transmission tonically inhibits mesencephalic DA neurons (Ikemoto *et al*., 1997; Paladini *et al*., 1999). Although available evidence suggests that intra-VTA or intra-Acb infusion of nicotinic antagonists (Fu *et al*., 2000) does not modify DA perfusate levels in the Acb, it remains possible that desensitization of nAChR subtypes expressed in specific GABA subpopulations may relieve an inhibitory nicotinic tone on the mesolimbic DA pathway.

Conclusions

In conclusion, we have shown that systemic, intra-Acb and intra-VTA nicotine administration elicit comparable increases in dialysate DA levels from the Acb. This increase in extracellular DA levels is not by itself sufficient to enhance locomotion in a habituated environment, as intra-Acb nicotine administration does increase DA levels markedly but does not alter locomotor activity significantly. Finally, in the case of intra-VTA nicotine administration, in addition to acute increases in dialysate DA levels from Acb and locomotor activity, a long-term (at least 24 h) alteration occurs in basal dialysate DA levels from the Acb as well as in TH and GluR1 mRNA levels in the VTA, demonstrating a new form of plasticity of the mesolimbic DA pathway elicited by a single injection of nicotine.

Acknowledgements

This work has been supported by an Italian MURST grant ('Neuronal nicotinic receptors: from structure to function', MM05152538).

Abbreviations

Acb, nucleus accumbens; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; DA, dopamine; DAT, dopamine transporter; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; i.p., intraperitoneal; LTD, long-term depression; LTP, long-term potentiation; nAChR, nicotinic acetylcholine receptor; NMDA, N-methyl-D-aspartate; SNc, substantia nigra, pars compacta; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

References

Agnati, L.F., Zoli, M., Strömberg, I. & Fuxe, K. (1995) Intercellular communication in the brain: Wiring versus Volume transmission. *Neuroscience*, **69**, 711–726.

Balfour, D.J., Benwell, M.E., Birrell, C.E., Kelly, R.J. & Al Aloul, M. (1998) Sensitization of the mesoaccumbens dopamine response to nicotine. *Pharmacol. Biochem. Behav.*, **59**, 1021–1030.

Benwell, M.E.M. & Balfour, D.J.K. (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *Br. J. Pharmacol.*, **105**, 849–856.

Berke, J.D. & Hyman, S.E. (2000) Addiction, dopamine, and the molecular mechanisms of memory. *Neuron*, **25**, 515–532.

Boundy, V.A., Gold, S.J., Messer, C.J., Chen, J., Son, J.H., Joh, T.H. & Nestler, E.J. (1999) Regulation of tyrosine hydroxylase promoter activity by chronic morphine in TH9.0-LacZ transgenic mice. *J. Neurosci.*, **18**, 9989–9995.

Calabresi, P., Pisani, A., Centonze, D. & Bernardi, G. (1997) Synaptic plasticity and physiological interactions between dopamine and glutamate in the striatum. *Neurosci. Biobehav. Rev.*, **21**, 519–523.

Cammarota, M., Bernabeu, R., Levi De Stein, M., Izquierdo, I. & Medina, J.-H. (1998) Learning-specific, time-dependent increases in hippocampal Ca2+/calmodulin-dependent protein kinase II activity and AMPA GluR1 subunit immunoreactivity. *Eur. J. Neurosci.*, **10**, 2669–2676.

Carboni, E., Imperato, A., Perezzani, L. & Di Chiara, G. (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. *Neuroscience*, **28**, 653–661.

Carlezon, W.A. Jr, Boundy, V.A., Haile, C.N., Lane, S.B., Kalb, R.G., Neve, R.L. & Nestler, E.J. (1997) GluR1 increases after morphine administration Sensitization to morphine induced by viral-mediated gene transfer. *Science*, **277**, 812–814.

Carlezon, W.A. Jr & Wise, R.A. (1996) Microinjections of phencyclidine (PCP) and related drugs into nucleus accumbens shell potentiate medial forebrain bundle brain stimulation reward. *Psychopharmacology (Berlin)*, **128**, 413–420.

Changeux, J.-P. (1990) Functional architecture and dynamics of the nicotinic acetylcholine receptor: an allosteric ligand-gated ion channel. In Changeux, J.-P., Llinas, R.R., Bloom, F.E. (eds), *Fidia Research Foundation Neuroscience Award Lectures*, **Vol. 4**. Raven Press Ltd, New York, pp. 21–168.

Changeux, J.-P. & Edelstein, S.J. (1998) Allosteric receptors after 30 years. *Neuron*, **21**, 959–980.

Clarke, P.B.S., Fu, D.S., Jakubovic, A. & Fibiger, H.C. (1988) Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. *J. Pharmacol. Exp. Ther.*, **246**, 701–708.

Corrigall, W.A., Franklin, K.B., Coen, K.M. & Clarke, P.B. (1992) The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berlin)*, **107**, 285–289.

Dani, J.A. & Heinemann. S. (1996) Molecular and cellular aspects of nicotine abuse. *Neuron*, **16**, 905–908.

Dhatt, R.K., Gudehithlu, K.P., Wemlinger, T.A., Tejwani, G.A., Neff, N.H. & Hadjiconstantinou, M. (1995) Preproenkephalin mRNA and methionine-enkephalin content are increased in mouse striatum after treatment with nicotine. *J. Neurochem.*, **64**, 1878–1883.

Fitzgerald, L.W., Ortiz, J., Hamedani, A.G. & Nestler, E.J. (1996) Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J. Neurosci.*, **16**, 274–282.

Fu, Y., Matta, S.G., Gao, W. & Sharp, B.M. (2000) Local alpha-bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion *in vivo*. *Neuroscience*, **101**, 369–375.

Gueorguiev, V.D., Zeman, R.J., Meyer, E.M. & Sabban, E.L. (2000) Involvement of alpha7 nicotinic acetylcholine receptors in activation of tyrosine hydroxylase and dopamine beta-hydroxylase gene expression in PC12 cells. *J. Neurochem.*, **75**, 1997–2005.

Houdi, A.A., Dasgupta, R. & Kindy, M.S. (1998) Effect of nicotine use and withdrawal on brain preproenkephalin A mRNA. *Brain Res.*, **799**, 257–263.

Ikemoto, S., Kohl, R.R. & McBride, W.J. (1997) GABA (A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. *J. Neurochem.*, **69**, 137–143.

Imperato, A., Mulas, A. & Di Chiara, G. (1986) Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur. J. Pharmacol.*, **132**, 337–338.

Katz, B. & Thesleff, S. (1957) A study of "desensitization" produced by acetylcholine at the motor end-plate. *J. Physiol. (London)*, **138**, 63–80.

Klink, R., de Kerkhove d'Exaerde, A., Zoli, M. & Changeux, J.-P. (2001) Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J. Neurosci.*, **21**, 1452–1463.

Ksir, C., Hakan, R., Hall, D.P. Jr & Kellar, K.J. (1985) Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [3H]acetyl choline to nicotinic receptors. *Neuropharmacology*, **24**, 527–531.

Lanca, A.J., Sanelli, T.R. & Corrigall, W.A. (2000) Nicotine-induced fos expression in the pedunculopontine mesencephalic tegmentum in the rat. *Neuropharmacology*, **39**, 2808–2817.

Le Novère, N., Zoli, M. & Changeux, J.P. (1996) Neuronal nicotinic receptor alpha6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur. J. Neurosci.*, **8**, 2428–2439.

Lecca, D., Shim, I., Costa, E. & Javaid, J.I. (2000) Striatal application of nicotine, but not of lobeline, attenuates dopamine release in freely moving rats. *Neuropharmacology*, **39**, 88–98.

Leikola-Pelho, T. & Jackson, D.M. (1992) Preferential stimulation of locomotor activity by ventral tegmental micro-injections of (–)-nicotine. *Pharmacol. Toxicol.*, **70**, 50–52.

Li, X.M., Zoli, M., Finnman, U.B., Le Novère, N., Changeux, J.P. & Fuxe, K. (1995) A single (–)nicotine injection causes change with a time delay in the affinity of striatal D2 receptors for antagonist but not for agonist, nor in the D2 receptor levels in the rat substantia nigra. *Brain Res.*, **679**, 157–167.

Louis, M. & Clarke, P.B. (1998) Effect of ventral tegmental 6-hydroxydopamine lesions on the locomotor stimulant action of nicotine in rats. *Neuropharmacology*, **37**, 1503–1513.

Lovinger, D.M. & Tyler, E. (1996) Synaptic transmission and modulation in the neostriatum. *Int. Rev. Neurobiol.*, **39**, 3977–4111.

Mansvelder, H.D. & McGehee, D.S. (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron*, **27**, 349–357.

Marshall, D.L., Redfern, P.H. & Wonnacott, S. (1997) Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by *in vivo* microdialysis: comparison of naive and chronic nicotine-treated rats. *J. Neurochem.*, **68**, 1511–1519.

Martin-Soelch, C., Leenders, K.L., Chevalley, A.F., Missimer, J., Kunig, G., Magyar, S., Mino, A. & Schultz, W. (2001) Reward mechanisms in the brain and their role in dependence: evidence from neurophysiological and neuroimaging studies. *Brain Res. Rev.*, **36**, 139–149.

McKinzie, D.L., McBride, W.J., Murphy, J.M., Lumeng, L. & Li, T.K. (2002) Effects of amphetamine on locomotor activity in adult and juvenile alcohol-preferring and -nonpreferring rats. *Pharmacol. Biochem. Behav.*, **71**, 29–36.

Morrisson, C.F. & Stephenson, J.A. (1972) The occurrence of tolerance to a central depressant effect of nicotine. *Br. J. Pharmacol.*, **45**, 151–156.

Museo, E. & Wise, R.A. (1990a) Locomotion induced by ventral tegmental microinjections of a nicotinic agonist. *Pharmacol. Bioch. Behav.*, **35**, 735–737.

Museo, E. & Wise, R.A. (1990b) Microinjections of a nicotinic agonist into dopamine terminal fields: effects on locomotion. *Pharmacol. Bioch. Behav.*, **37**, 113–116.

Museo, E. & Wise, R.A. (1995) Cytisine-induced behavioral activation: delineation of neuroanatomical locus of action. *Brain Res.*, **670**, 257–263.

Nayak, A., Zastrow, D.-J., Lickteig, R., Zahniser, N.-R. & Browning, M.-D. (1998) Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. *Nature*, **394**, 680–683.

Nestler, E.J. & Aghajanian, G.K. (1997) Molecular and cellular basis of addiction. *Science*, **278**, 58–63.

Nisell, M., Nomikos, G.G. & Svensson, T.H. (1994a) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse*, **16**, 36–44.

Nisell, M., Nomikos, G.G. & Svensson, T.H. (1994b) Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacol. Toxicol.*, **75**, 348–352.

Osborne, P.G., O'Connor, W.T. & Ungerstedt, U. (1991) Effect of varying the ionic concentration of a microdialysis perfusate on basal striatal dopamine levels in awake rats. *J. Neurochem.*, **56**, 452–456.

Paladini, C.-A., Celada, P. & Tepper, J.-M. (1999) Striatal, pallidal, and pars reticulata evoked inhibition of nigrostriatal dopaminergic neurons is mediated by GABA (A) receptors *in vivo*. *Neuroscience*, **89**, 799–812.

Panagis, G., Nisell, M., Nomikos, G.-G., Chergui, K. & Svensson, T.-H. (1996) Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. *Brain Res.*, **730**, 133–142.

Picciotto, M.R., Zoli, M., Rimondini, R., Léna, C., Marubio, L., Merlo Pich, E., Fuxe, K. & Changeux, J.-P. (1998) Acetylcholine receptors containing β2 subunits are involved in the reinforcing properties of nicotine. *Nature*, **391**, 173–177.

Pidoplichko, V.-I., DeBiasi, M., Williams, J.-T. & Dani, J.-A. (1997) Nicotine activates and desensitizes midbrain dopamine neurons. *Nature*, **390**, 401–404.

Pierce, R.C. & Kalivas, P.W. (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res. Rev.*, **25**, 192–216.

Reavill, C. & Stolerman, I.-P. (1990) Locomotor activity in rats after administration of nicotinic agonists intracerebrally. *Br. J. Pharmacol.*, **99**, 273–278.

Reuben, M., Boye, S. & Clarke, P.B. (2000) Nicotinic receptors modulating somatodendritic and terminal dopamine release differ pharmacologically. *Eur. J. Pharmacol.*, **393**, 39–49.

Robinson, T.E. & Berridge, K.C. (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction*, **95** (Suppl. 2), S91–S117.

Schilstrom, B., Svensson, H.M., Svensson, T.H. & Nomikos, G.G. (1998) Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of alpha7 nicotinic receptors in the ventral tegmental area. *Neuroscience*, **85**, 1005–1009.

Shim, I., Javaid, J.I., Wirtshafter, D., Jang, S.Y., Shin, K.H., Lee, H.J., Chung, Y.C. & Chun, B.G. (2001) Nicotine-induced behavioral sensitization is associated with extracellular dopamine release and expression of c-Fos in the striatum and nucleus accumbens of the rat. *Behav. Brain Res.*, **121**, 137–147.

Tsuneki, H., Klink, R., Lena, C., Korn, H. & Changeux, J.-P. (2000) Calcium mobilization elicited by two types of nicotinic acetylcholine receptors in mouse substantia nigra pars compacta. *Eur. J. Neurosci.*, **12**, 2475–2485.

Ungless, M.A., Whistler, J.L., Malenka, R.C. & Bonci, A. (2001) Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. *Nature*, **411**, 583–587.

Vezina, P., Herve, D., Glowinski, J. & Tassin, J.P. (1994) Injections of 6-hydroxydopamine into the ventral tegmental area destroy mesolimbic dopamine neurons but spare the locomotor activating effects of nicotine in the rat. *Neurosci. Lett.*, **168**, 111–114.

Wonnacott, S. (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci.*, **20**, 92–98.

Zoli, M. & Agnati, L.F. (1996) Wiring and Volume transmission in the central nervous system: the concept of closed and open synapses. *Prog. Neurobiol.*, **49**, 363–380.

Zoli, M., Le Novère, N., Hill, J.A. & jr. & Changeux, J.-P. (1995) Developmental regulation of nicotinic receptor subunit mRNAs in the rat central and peripheral nervous systems. *J. Neurosci.*, **15**, 1912–1939.

Zoli, M., Léna, C., Picciotto, M.R. & Changeux, J.-P. (1998) Identification of four classes of brain nicotinic receptors using β2-mutant mice. *J. Neurosci.*, **18**, 4461–4472.