

Systemic Nicotine Stimulates Dopamine Release in Nucleus Accumbens: Re-evaluation of the Role of *N*-Methyl-D-aspartate Receptors in the Ventral Tegmental Area¹

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ABSTRACT

Systemic nicotine stimulates dopamine (DA) release in the nucleus accumbens (NAcc), and *N*-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area (VTA) appear to be involved. However, it is not known whether the secretion of DA elicited by nicotine depends on the tonic and/or phasic activation of NMDA receptors by glutamate (Glu). To clarify this, *in vivo* microdialysis was conducted in freely moving, alert rats to measure DA and Glu overflows in the NAcc and Glu in the VTA. Nicotine (0.065, 0.09, or 0.135 mg/kg delivered *i.v.* at 0.09 mg/kg/60 s via a jugular cannula) dose dependently stimulated NAcc DA secretion ($P < .05$). However, 0.065 mg/kg nicotine failed to stimulate Glu release in the VTA, whereas higher doses of nicotine (≥ 0.09 mg/kg) were effective ($P < .05$). Administering the competitive NMDA receptor antagonists, 2-amino-5-phosphonopentanoic acid (AP-5; 1 mM) or 0.2 mM *cis*-4-phosphonomethyl-2-piperidine carboxylic acid

(CGS 19755) through the VTA probe, abolished NAcc DA release after 0.065 mg/kg nicotine ($P < .01$) and reduced the response to 0.09 mg/kg nicotine. Therefore, the NAcc DA response to a relatively low dose of nicotine depends on the tonic activation of NMDA receptors in the VTA. In contrast, infusing 1 mM 2-amino-5-phosphonopentanoic acid or 1 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an α -amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor antagonist, into the NAcc through the microdialysis probe had no effect on NAcc DA secretion in response to 0.09 mg/kg nicotine. These findings, coupled with data showing that Glu secretion in the VTA was stimulated only by higher doses of nicotine, indicate that the phasic release of VTA Glu is involved in the NAcc DA response to higher doses of nicotine (≥ 0.09 mg/kg).

Recent studies suggest that the reinforcing effects of nicotine, the major psychoactive agent in cigarette smoke, depend on dopamine (DA) secretion in the mesolimbic system of the brain. (Corrigall and Coen, 1989; Corrigall et al., 1992) This holds, as well, for other addictive substances, such as cocaine, opiates, and alcohol (Nisell et al., 1995; Pontieri et al., 1996; Rose and Corrigall, 1997). The mesolimbic dopaminergic system originates in the ventral tegmental area (VTA) and projects, in large part, to the nucleus accumbens (NAcc). Nicotine increases the firing rate and burst firing of DA neurons in VTA (Nisell et al., 1996; Fisher et al., 1998) and, when administered systemically or infused locally into VTA, stimulates NAcc DA release (Nisell et al., 1994; Schilstrom et al., 1998a,b). In addition, nicotinic antagonists infused into VTA reduce nicotine self-administration in rats (Corrigall et al., 1992, 1994; Fisher et al., 1998).

The increase in extracellular DA in the NAcc is a major factor mediating the self-administration of nicotine and depends, in part, on nicotinic receptors (nAChRs) within the VTA (Corrigall et al., 1994; Nisell et al., 1994). Recent studies, showing that the action of glutamate (Glu) at *N*-methyl-D-aspartate (NMDA)-sensitive receptors within the VTA is required for nicotine to stimulate DA release in the NAcc, were based on the observation that DA secretion was reduced after a VTA infusion of the NMDA antagonist 2-amino-5-phosphopentanoic acid (AP-5; Schilstrom et al., 1998a). Although Glu release was not measured, Schilstrom et al. (1998a) hypothesized that nicotine may stimulate presynaptic nAChRs on glutamatergic afferents, increasing the release of Glu within the VTA which, in turn, activated DA neurons (Schilstrom et al., 1998a,b).

The VTA contains the soma of two major neuronal phenotypes: dopaminergic neurons and γ -aminobutyric acid (GABA) interneurons. Both receive excitatory glutamatergic projections from the medial prefrontal cortex (Christie et al.,

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ABBREVIATIONS: DA, dopamine; VTA, ventral tegmental area; NAcc, nucleus accumbens; nAChR, nicotinic cholinergic receptor; Glu, glutamate; NMDA, *N*-methyl-D-aspartate; AP-5, 2-amino-5-phosphonopentanoic acid; GABA, γ -aminobutyric acid; CGS 19755, *cis*-4-phosphonomethyl-2-piperidine carboxylic acid; AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; OPA, *ortho*-(1,2)-phthalic dicarboxaldehyde; β ME, 2- β -mercaptoethanol; KRB, Krebs-Ringer buffer.

1985; Sesack and Pickel, 1992; Taber et al., 1995) that may tonically regulate VTA DA (Karreman et al., 1996; Takahata and Moghaddam, 1998) and GABA neurons (Overton and Clark, 1997; Steffensen et al., 1998). Indeed, studies have shown that synaptic currents recorded from both DA and GABA cells in the VTA were blocked by the NMDA antagonist AP-5 (Bonci and Malenka, 1999). In addition, an intra-VTA infusion of AP-5 dose dependently decreased DA release within the NAcc, and, conversely, NMDA increased DA efflux (Karreman et al., 1996).

To clarify the role of Glu in nicotine-induced DA secretion within the NAcc, initial experiments were carried out in alert, freely moving rats receiving an acute i.v. injection of nicotine. These studies were designed to determine whether nicotine-stimulated DA release in the NAcc depends on the tonic activity of NMDA receptors in VTA and/or on the phasic stimulation of these receptors resulting from the enhanced release of Glu. To permit concurrent neurotransmitter measurements from the NAcc and VTA, alert, freely mobile rats were equipped with guide cannulas for simultaneous microdialysis in both sites: one probe measured Glu in the VTA and a second probe detected DA and Glu secretion in the NAcc. By infusing specific antagonists into the VTA, we obtained site-directed blockade of NMDA receptors [*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) or AP-5] or α -amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kainate receptors [6-cyano-7-nitroquinoline-2,3-dione (CNQX)]. These experiments showed that 1) tonic Glu activation of NMDA receptors within the VTA facilitated the NAcc DA response to a low dose of nicotine (0.065 mg/kg), whereas 2) the phasic release of Glu in the VTA was primarily involved in DA responses to higher doses of nicotine (≥ 0.09 mg/kg).

Experimental Procedures

Materials. (–)-Nicotine hydrogen tartrate (all doses were based on the free base), glutamate, and triethylamine were purchased from Sigma (St. Louis, MO). Dopamine hydrochloride, CGS 19755, (\pm)-2-amino-5-phosphopentanoic acid, and CNQX were purchased from Research Biochemicals (Natick, MA). Sodium dihydrogen phosphate monohydrate, methanol, acetonitrile, EDTA, *ortho*-(1,2)-phthalic dicarboxaldehyde (OPA), sodium tetraborate, and phosphoric acid were obtained from Fisher Scientific (Fair Lawn, NJ). Octanesulfonic acid sodium salt was purchased from J.T. Baker (Phillipsburg, NJ) and 2- β -mercaptoethanol (β ME) was from Bio-Rad (Hercules, CA). The alert-rat microdialysis systems and CMA 110 liquid switches were obtained from CMA/Microdialysis (Acton, MA). For constructing dialysis probes, cellulose fiber tubing was obtained from Spectrum (Laguna Hills, CA) and silica tubing (148 μ m o.d.; 73 μ m i.d.) was from Polymicron Technologies Inc. (Phoenix, AZ).

Animals. Adult male Sprague-Dawley rats (250–350 g; HSD, Inc., Indianapolis, IN) were given access to standard rat chow and water ad libitum. They were individually housed on a 12-h reversed light cycle (lights off at 9:00 AM and on at 9:00 PM) for 14 days before the microdialysis experiments. This reversed light cycle was designed so that experiments could be conducted during the rats' active (dark) phase. After such housing for 7 days, rats were anesthetized with xylazine-ketamine (5.35 mg/kg b.wt. i.m.; Parke-Davis, Morris Plains, NJ), and chronic guide cannulas (20 gauge) were stereotaxically implanted into the VTA and NAcc, according to the atlas coordinates of Paxinos and Watson (1986). The coordinates for VTA were AP, –5.2 mm; DV, –8.0 mm; and ML, 0.2 mm, from bregma with a flat skull. For NAcc, coordinates were AP, +1.4 mm; DV, –6.0 mm;

and ML, 0.5 mm, from bregma with a flat skull. Five days later, rats received jugular cannulas under Innovar Vet anesthesia (3.75 mg/kg droperidol plus 0.08 mg/kg i.m. fentanyl; Far-Vet, St Paul, MN) and were allowed to recover for another 2 days. All procedures were conducted in accordance with the National Institutes of Health Guidelines concerning the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of University of Tennessee College of Medicine.

In Vivo Microdialysis. The microdialysis method and probes have been described previously (Fu et al., 1997). Briefly, concentric microdialysis probes (molecular mass cutoff 13,000 Da; 235 μ m o.d.; 1 mm for VTA or 2 mm for NAcc) were constructed in our laboratory. The recovery efficiency for 1-mm probes was $3.7 \pm 0.5\%$ ($n = 15$) for DA and $4.0 \pm 0.5\%$ ($n = 10$) for Glu. For 2-mm probes, they were $6.5 \pm 0.7\%$ ($n = 15$) for DA and $7.1 \pm 0.3\%$ ($n = 10$) for Glu.

On the day of microdialysis, rats were transferred to alert-rat microdialysis chambers in an isolated darkroom illuminated by a red safe-light; all connections were made quickly to minimize stress to the animal. Microdialysis probes were perfused at 1 μ l/min with a solution of Krebs-Ringer buffer (KRB; 147 mM NaCl, 4.0 mM KCl, and 3.4 mM CaCl₂ in polished water; 0.2- μ m filter sterilized and degassed). Two hours after insertion of the probes through the guide cannulas, five consecutive samples were collected to measure basal levels of DA and Glu before nicotine administration. Each sample was collected over 15 min into vials containing 1 μ l of 5% perchloric acid to prevent the degradation of DA. At the end of each experiment, probe position was verified by histological examination; only data obtained from animals with probes correctly placed within both the NAcc and VTA were analyzed.

HPLC-Electrochemical Analysis. The procedure for DA detection was as previously described for norepinephrine (Fu et al., 1997). An isocratic chromatographic method with precolumn derivatization of amino acids with OPA/ β ME was used to separate and detect Glu in the microdialysates. The mobile phase contained 0.1 M disodium hydrogen phosphate in 25% methanol and 8% acetonitrile, pH 6.8 (adjusted by phosphoric acid). An ESA 580 pump was used to perfuse mobile phase through a 15 cm \times 4.6 mm ODS C18 column (ESA, Chelmsford, MA) at a flow rate of 2.2 ml/min. OPA/ β ME stock solution was made by dissolving 27 mg of OPA in 1 ml of methanol, and then adding 5 μ l of β ME and 9 ml of 0.1 M sodium tetraborate, pH 9.3. The working OPA/ β ME solution was prepared daily by diluting 1 ml of the stock OPA/ β ME solution with 3 ml of 0.1 M sodium tetraborate. For automated derivatization of a sample (with the CMA 200), 15 μ l from the VTA dialysate or 5 μ l from the NAcc dialysate was mixed with 15 μ l of working OPA/ β ME solution. After exactly 2 min, 16 μ l of the mixture was injected onto the HPLC column and analyzed with an ESA Coulochem II 5200A electrochemical detector, with ESA 5011 analytical and 5020 guard cells, respectively. Electrochemical detection was performed at –400 mV (first electrode) and +600 mV (second electrode) with the gain at 200 nA and guard cell at 350 mV. The limit of detection for Glu was 100 pg/injection.

Two neurotransmitters were quantified simultaneously from each dialysis sample. NAcc samples were divided into two aliquots for measurements of Glu and DA; 8 μ l was injected onto the HPLC to determine DA levels and 5 μ l was used to analyze Glu, with a separate detection system. Samples collected from the VTA were analyzed for only Glu and, therefore, were not split.

Experimental Protocols. Rats were not restrained by hand or device during these experiments; they were freely moving in the alert-rat apparatus bowls during the time antagonists and/or nicotine were administered, as described previously. In all experiments, microdialysis probes were inserted into the VTA and NAcc guide cannulas for 10 min on day 1 and then removed without further microdialysis. On days 3 and 5, probes were reinserted and the rats received randomized experimental treatments. This was done because previous studies showed no significant “within-rat” change in basal norepinephrine levels nor in norepinephrine responses to nic-

otine when using this protocol of testing on days 3 and 5 in the same rat (Fu et al., 1997, 1998). The efficacy of this protocol was borne out in this study. For example, no differences in the basal levels of any neurotransmitter were observed between days 3 and 5: basal DA levels in NAcc dialysates were 0.83 ± 0.14 pg/8 μ l (mean \pm S.E.) on day 3 and 0.74 ± 0.17 pg/8 μ l on day 5 ($n = 20$); basal Glu levels in the VTA dialysates were 732 ± 46 pg/12 μ l on day 3 and 643 ± 57 pg/12 μ l on day 5 ($n = 20$). With this approach, each rat was tested twice, and only within the same series of experiments, to reduce animal use.

Preliminary experiments were conducted with the sodium channel blocker tetrodotoxin to validate that the secretion of DA was neuron-dependent. When 1 μ M tetrodotoxin was dialyzed into the VTA, DA levels in the NAcc were less than 40% of control. The first series of experiments was conducted to determine the dose-response relationship for i.v. nicotine on DA release in the NAcc, as well as Glu secretion in the VTA. After five basal samples were collected, rats randomly received i.v. infusions of saline or one of three doses of nicotine (each delivered at a constant rate of 0.09 mg/kg/0.1 ml/60 s; Fu et al., 1997): 0.065 mg/kg over 44 s, 0.09 mg/kg over 60 s, or 0.135 mg/kg over 90 s; saline was delivered over 60 s. Then, consecutive samples were collected for another 60 min.

In a second series of experiments designed to evaluate the role of the VTA NMDA receptors in nicotine-stimulated DA release in the NAcc, the NMDA receptor antagonist AP-5 or CGS 19755 was perfused directly through the microdialysis probe into the VTA and extracellular DA was measured concurrently via a probe in the NAcc. Briefly, after three 15-min basal samples were collected from both sites, the VTA was perfused with either 1 mM AP-5 or KRB (vehicle control) for 60 min; thereafter, only KRB was perfused. Thirty minutes after the onset of AP-5 (or KRB) perfusion, nicotine (0.065 or 0.09 mg/kg) was administered i.v. over 44 or 60 s, respectively, and consecutive 15-min samples were collected for the next 60 min. The effect of AP-5 alone was assessed in one group of rats; after three 15-min basal samples were collected, the VTA was perfused with 1 mM AP-5 for 60 min. Thereafter, AP-5 was replaced with KRB. These rats did not receive nicotine injections. With a similar protocol, CGS 19755 (0.1 and 0.2 mM) was delivered followed by 0.065 mg/kg nicotine i.v.

The third series of experiments evaluated the potential role of the NAcc glutamatergic receptors in nicotine-stimulated DA secretion within the NAcc. To accomplish this, KRB or 1 mM AP-5 or 1 mM CNQX was perfused directly through the microdialysis probe into the NAcc for 60 min. After AP-5 or CNQX had been perfused for 30 min, 0.09 mg/kg nicotine was administered i.v. DA levels were measured in NAcc microdialysates obtained through the same probe;

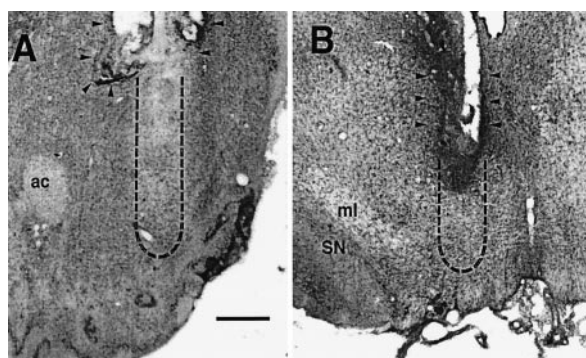


Fig. 1. Photomicrographs of dialysis probe placement in the NAcc (ac-cumbens shell; A) and VTA (B). Rats were intracardially perfused with 4% paraformaldehyde in 0.05 M PBS, pH 6.8, at 4°C. Brains were removed, sucrose infused, cryosectioned at 20 μ m, and stained with cresyl violet. The track of the probe in the tissue is identified by the dotted line. Arrowheads indicate the track of the guide cannula. ac, anterior commissure; ml, medial lemniscus; SN, substantia nigra. Magnification bar, 500 μ m.

basal samples were obtained before the administration of antagonists and sample collection continued.

Data Analysis and Statistics. Chromatographic data were collected and analyzed with the PowerChrom system (AD Instruments, Castle Hill, New South Wales, Australia). Data (mean \pm S.E.) were expressed as picograms of DA or Glu per HPLC injection volume or as a percentage of basal levels. Basal values were defined as the mean level detected in the three or five samples obtained before administering nicotine, AP-5, CGS 19755, CNQX, or vehicle. Peak levels were the samples collected 15 min after i.v. nicotine or saline. As specified in the text and figure legends, data were analyzed by one-way or two-way ANOVA with repeated measures, followed by post hoc Dunnett's test, with StatView version 5.0.1 (SAS Institute Inc., Cary, NC). Some data also were analyzed by linear regression with StatView.

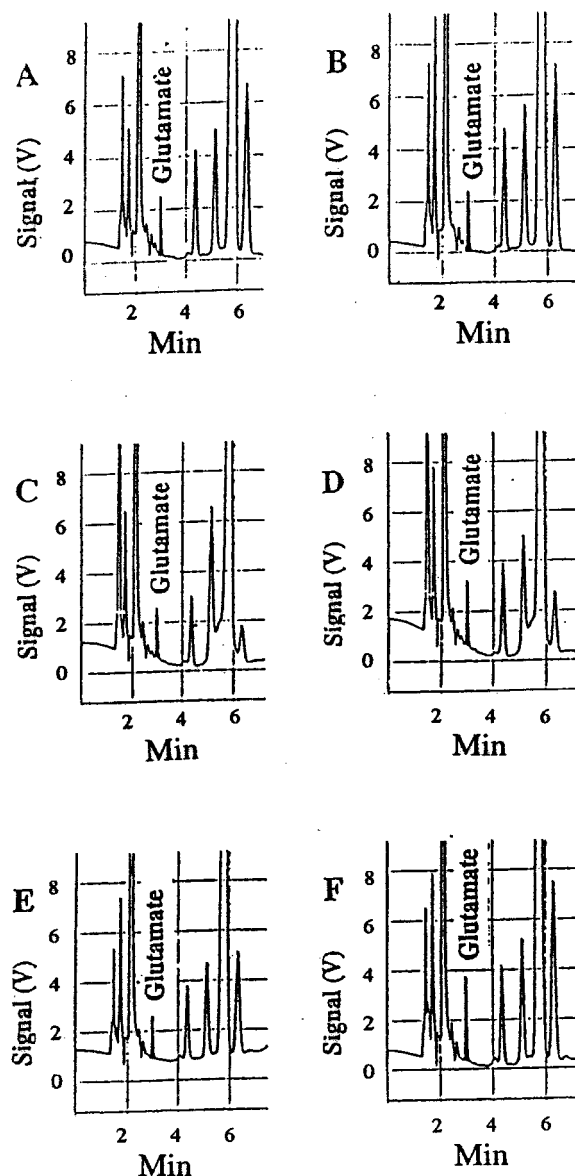


Fig. 2. Chromatograms of Glu in the dialysate samples from the VTA of three separate rats. A and B show the basal levels and responses to 0.065 mg/kg nicotine, respectively, in the same rat. C and D are the respective basal levels and responses to 0.09 mg/kg in a different animal, whereas E and F are the respective basal and stimulated responses to nicotine 0.135 mg/kg in a third. The levels of Glu were increased by nicotine 0.09 mg/kg i.v. (D) and 0.135 mg/kg (F) but not 0.065 mg/kg nicotine (B).

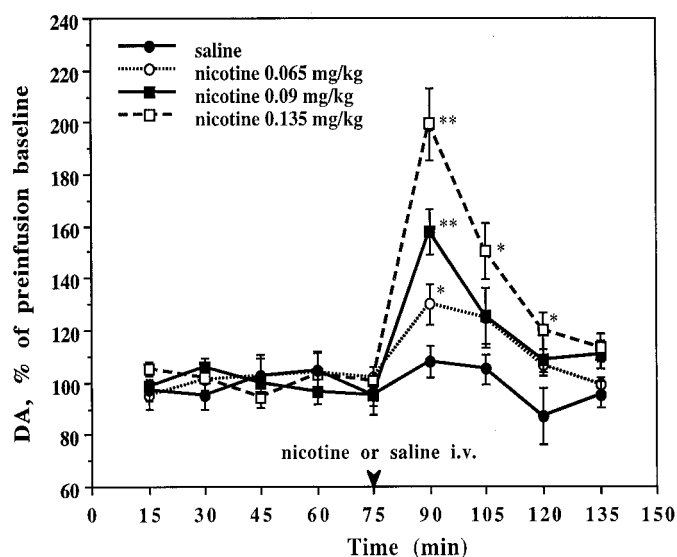


Fig. 3. Nicotine stimulates DA release in the NAcc in a dose-dependent manner. Nicotine or saline was delivered at +75 min, after five basal microdialysates had been collected. DA levels are expressed as percentage of preinfusion basal levels; peak levels are the samples collected 15 min after i.v. nicotine (see *Experimental Procedures*). Arrowhead indicates the time of saline or nicotine infusion. Data were analyzed with two-way ANOVA with repeated measures. $F(\text{treatment}) = 5.02, P = .013$; $F(\text{time}) = 3.475, P = .004$; $F(\text{time} \times \text{treatment}) = 2.185, P = .006$. * $P < .05$ or ** $P < .01$ compared with the samples collected at the same time point from saline controls ($n = 7-9$ rats per group).

Results

Figure 1 shows representative histological sections of probe placement in the NAcc and VTA. Figure 2 demonstrates representative HPLC chromatograms of the Glu detected in microdialysate samples from the VTA of three separate rats treated with different doses of nicotine. Basal chromatograms are shown in Fig. 1A, C, and E; responses to 0.065, 0.09, or 0.135 mg/kg nicotine are shown in Fig. 1B, D, and F, respectively. In saline-treated animals ($n = 7$), the mean baseline level from the VTA dialysates was 784 ± 53 pg/12 μ l. Baseline NAcc dialysates from the same saline-treated rats contained 438 ± 41 pg/4 μ l of Glu and 1.06 ± 0.3 pg/8 μ l of DA. Figure 3 demonstrates the time course for DA release in the NAcc in response to systemic nicotine that was delivered at +75 min. The DA response was dose-dependent, resulting in maximal DA concentrations within the first 15 min. DA levels were significantly elevated for another 15 to 30 min and returned to basal levels. Nicotine at 0.135 mg/kg routinely induced overt behavioral responses such as hyper-ventilation, prolonged locomotion, and, occasionally, brief seizures, whereas 0.065 or 0.09 mg/kg occasionally produced slight behavioral activation (e.g., increased respiratory rate, a brief increase in locomotor activity, and head nodding).

The results presented in Fig. 4, A and B show that nicotine stimulated Glu release in the VTA and NAcc, respectively. Nicotine, at doses of 0.09 mg/kg or higher, significantly increased the extracellular concentration of Glu in both regions. Glu levels were elevated for 30 to 45 min after 0.135 mg/kg nicotine and for 15 to 30 min after 0.09 mg/kg nicotine. However, in neither region was Glu release observed after 0.065 mg/kg nicotine.

Figure 5 shows that nicotine-induced DA release in the NAcc was significantly inhibited by infusing AP-5 into the

VTA. Compared with rats receiving only 0.065 mg/kg nicotine, the peak DA response was reduced by 83% after pre-treatment with 1 mM AP-5 (Fig. 5A); this concentration of AP-5 also inhibited 55% of the DA response to 0.09 mg/kg nicotine (Fig. 5B). Thus, AP-5 significantly inhibited nicotine-induced DA secretion at a dose of nicotine (0.065 mg/kg) that was not sufficient to elevate VTA Glu levels. In rats treated with AP-5 1 mM followed by 0.065 mg/kg nicotine (Fig. 5A), the basal level of DA in the NAcc was significantly reduced (by 17%) during the initial 15 min that AP-5 perfused the VTA. However, DA returned to basal levels within the next 15 min. A similar trend was observed in rats treated with AP-5 alone.

Figure 6 shows that a second competitive NMDA receptor

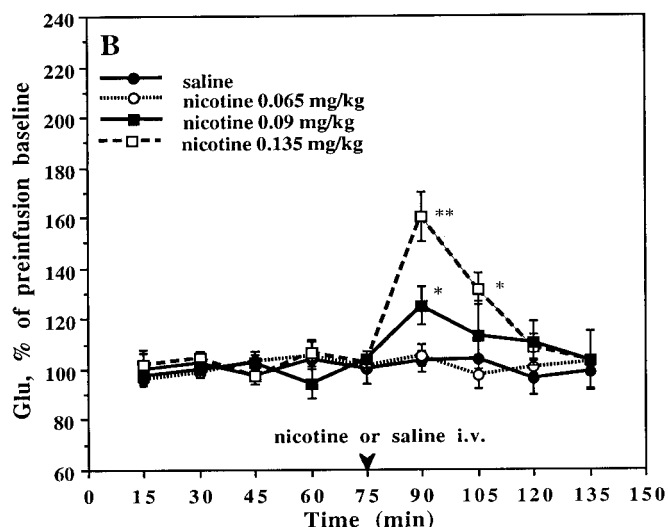
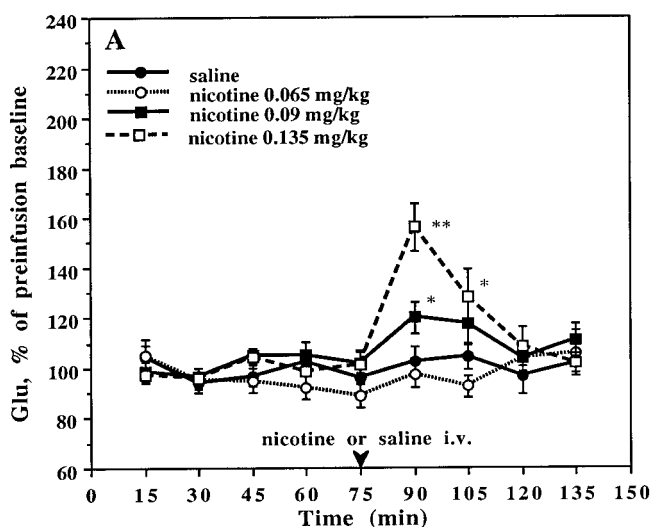


Fig. 4. Nicotine dose dependently stimulates Glu release in the VTA (A) and the NAcc (B). Nicotine or saline was infused at +75 min, after five basal microdialysate samples were collected. Glu levels are expressed as percentage of preinfusion basal levels. Data were analyzed with two-way ANOVA with repeated measures. $F(\text{treatment}) = 4.733, P = .018$; $F(\text{time}) = 3.291, P = .006$; $F(\text{time} \times \text{treatment}) = 2.103, P = .006$. * $P < .05$ or ** $P < .01$ compared with the samples collected at the same time point from saline controls ($n = 7-9$ rats per group).

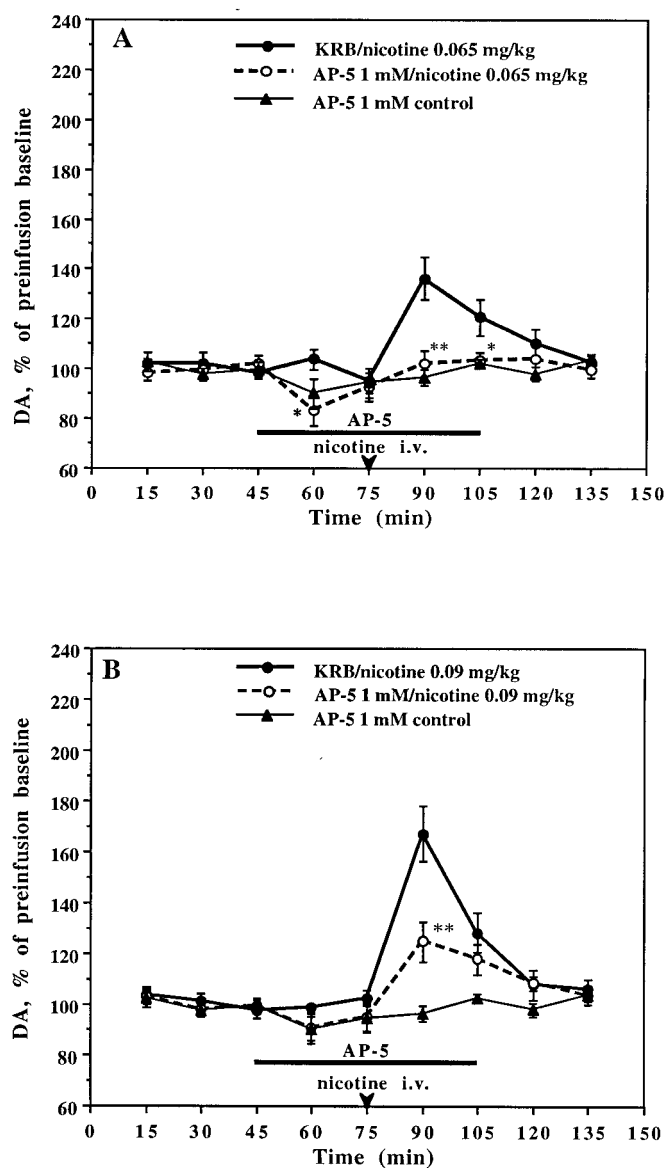


Fig. 5. AP-5 in the VTA inhibited DA release in the NAcc in response to 0.065 mg/kg (A) or 0.09 mg/kg (B) nicotine. After three basal microdialysate samples were collected, AP-5 was infused from +45 to +105 min. Nicotine was delivered at +75 min. DA levels are expressed as percentage of preinfusion basal levels. Arrowheads show the start of the nicotine infusion. Solid bars indicate that AP-5 was perfused for 30 min before the nicotine infusion and continued for 30 min thereafter. Data were analyzed with two-way ANOVA with repeated measures. For A, $F(\text{treatment}) = 3.482, P = .039$; $F(\text{time}) = 2.389, P = .010$; $F(\text{time} \times \text{treatment}) = 1.531, P = .018$. For B, $F(\text{treatment}) = 3.201, P = .048$; $F(\text{time}) = 2.144, P = .022$; $F(\text{time} \times \text{treatment}) = 1.653, P = .019$. * $P < .05$ or ** $P < .01$ compared with the samples collected at the same time point from the KRB/nicotine group ($n = 9$ rats per group).

antagonist, CGS19755, dose dependently reduced DA secretion within the NAcc in response to 0.065 mg/kg nicotine. Although a VTA infusion of CGS 19755 alone tended to reduce DA levels, no significant differences were identified ($F = 1.223; P = .32$). At 0.1 mM, CGS 19755 inhibited 38% of the DA response to nicotine, and the DA response was abolished by 0.2 mM CGS 19755. Thus, this study confirms the involvement of VTA NMDA receptors in the DA response to nicotine, at a dose that was not sufficient to enhance VTA Glu levels.

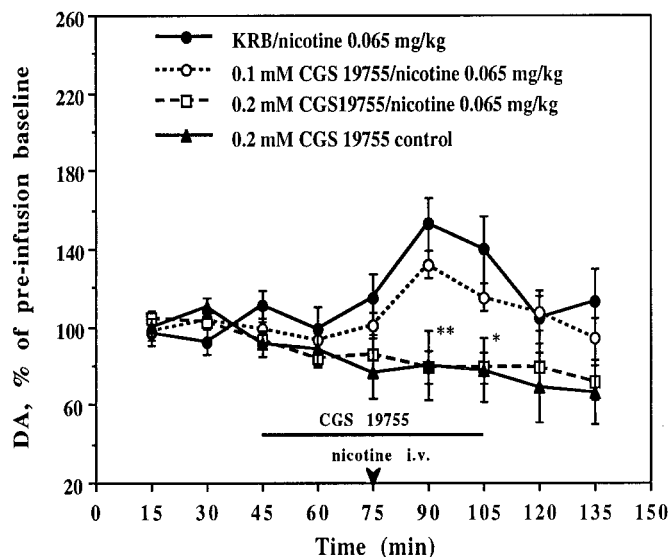


Fig. 6. CGS 19755 infused into the VTA dose dependently inhibited DA release in the NAcc in response to 0.065 mg/kg. After three basal microdialysate samples were collected, CGS 19755 was infused from +45 to +105 min. Nicotine was delivered at +75 min. DA levels are expressed as percentage of preinfusion basal levels. Data were analyzed with two-way ANOVA with repeated measures. $F(\text{treatment}) = 3.911, P = .018$; $F(\text{time}) = 2.774, P = .008$; $F(\text{time} \times \text{treatment}) = 1.820, P = .007$. * $P < .05$ or ** $P < .01$ compared with the samples collected at the same time point from KRB/nicotine group ($n = 4-6$ rats per group).

Figure 7 is a correlative analysis of peak levels (collected from samples 15 min after i.v. nicotine or saline, and expressed as a percentage of baseline) of VTA Glu versus those of NAcc DA, shown as a function of nicotine dose. As expected, no relationship existed between these two neurotransmitters in rats receiving saline (Fig. 7A) or a low dose of nicotine (0.065 mg/kg; Fig. 7B). In contrast, 0.09 mg/kg nicotine (Fig. 7C) resulted in a significant correlation between VTA Glu and NAcc DA ($P < .05$), suggesting that Glu may, in part, mediate the DA response to this dose of nicotine. This correlation was absent at the highest dose of nicotine where the behavioral responses to nicotine were most notable (e.g., hyperventilation, prolonged locomotion, and, occasionally, brief seizures). Therefore, the neurotransmitter responses to 0.135 mg/kg nicotine may result, in part, from nonspecific behavioral responses characteristic of this high dose. It is unlikely that these changes reflect the effects of increased accumbal DA secretion.

Figure 8 shows the results of experiments that evaluated the effects of dialyzing the selective glutamatergic receptor antagonists AP-5 or CNQX into the NAcc before administering nicotine i.v. The results demonstrate that neither antagonist reduced basal DA levels nor affected the NAcc DA response to nicotine; higher concentrations (1.8 mM or higher) of either antagonist actually increased basal DA levels (data not shown). This contrasts with the marked effects in the VTA, where the same concentration of AP-5 significantly reduced the NAcc DA response to i.v. nicotine (Fig. 5).

Discussion

This study demonstrates that nicotine dose dependently stimulates DA release in the NAcc and Glu secretion in both the VTA and NAcc. In addition, when dialyzed into the VTA, AP-5 or CGS 19755 significantly reduced the NAcc DA re-

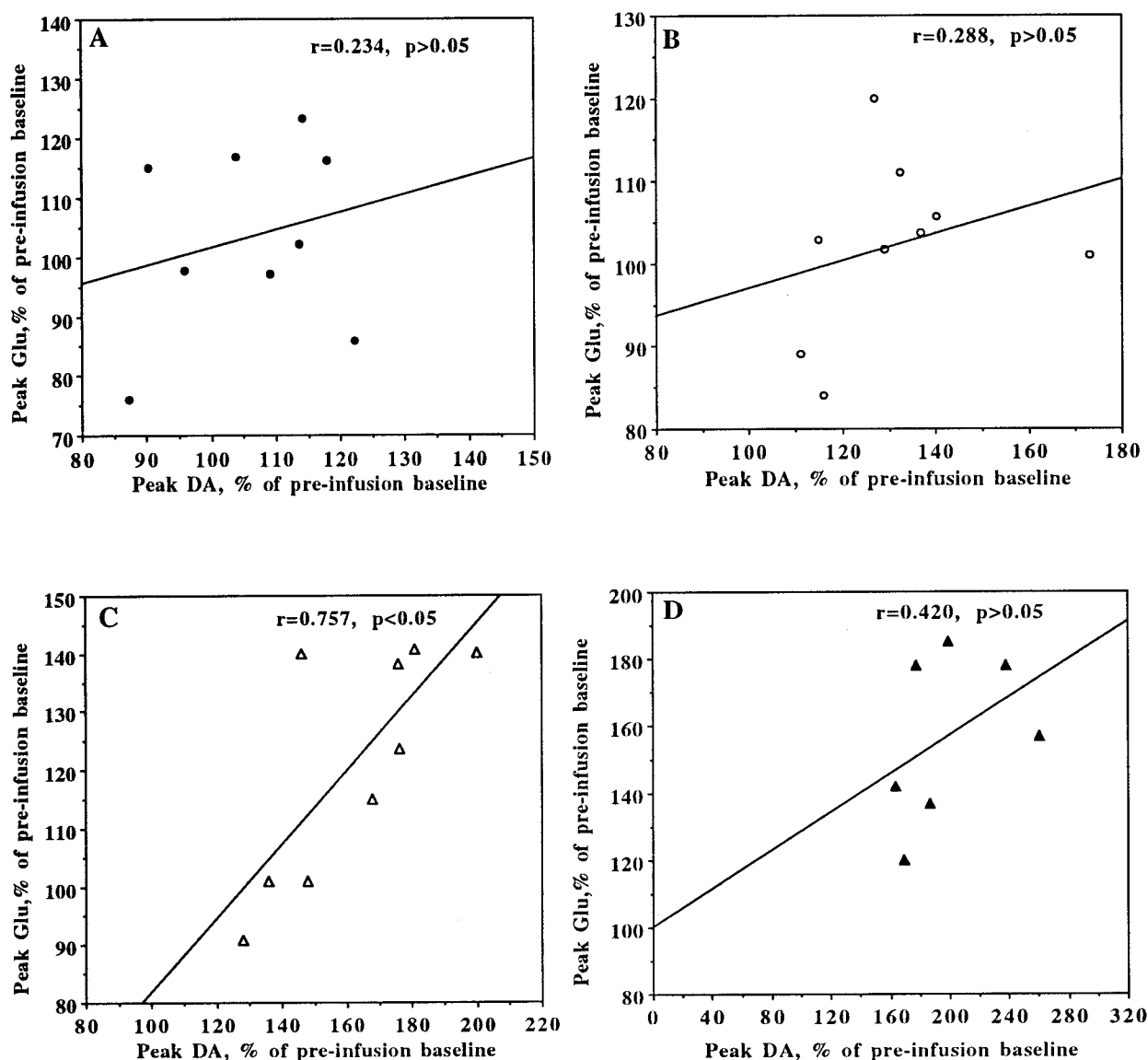


Fig. 7. Dose-specific correlation between nicotine-induced Glu release in the VTA and DA secretion in the NAcc. Peak DA or Glu values (expressed as a percentage of the preinfusion basal levels) were measured in samples collected 15 min after the injection of nicotine. A, data from the saline controls; B, C, and D, 0.065, 0.09, and 0.135 mg/kg nicotine cohorts, respectively. A significant correlation between VTA Glu release and NAcc DA secretion was found only in rats treated with 0.09 mg/kg nicotine ($n = 7-9$ rats per group).

sponses to 0.065 or 0.09 mg/kg nicotine. In contrast, when dialyzed into NAcc, neither the NMDA receptor antagonist AP-5 nor the AMPA receptor antagonist CNQX had an effect on NAcc DA release by 0.09 mg/kg nicotine. These results indicate that NMDA receptors in the VTA, but not in the NAcc, are involved in nicotine-induced DA secretion in the NAcc.

We hypothesized that VTA NMDA receptors may be involved in nicotine-stimulated DA secretion through two mechanisms. First, glutamatergic afferents to VTA appear to exert a tonic effect on the activity of dopaminergic neurons through NMDA receptors (Karreman et al., 1996). In this study, this effect was reflected in the trend toward a reduction of basal DA secretion in the NAcc induced by administering AP-5 or CGS 19755 into the VTA (Figs. 5 and 6). This is consistent with the observations previously made in other studies with AP-5 (Karreman et al., 1996; Taber and Fibiger, 1997). The experiments in this study also demonstrated that

blocking the tonic activity of VTA NMDA receptors, with AP-5 or CGS 19755, reduced nicotine-stimulated DA secretion in the NAcc when a low dose of nicotine was administered. Although nicotine-stimulated Glu release in both the VTA and NAcc was dose-dependent, at a low dose of nicotine (0.065 mg/kg) there was no evidence of enhanced Glu release. Therefore, a low dose of nicotine enhanced the secretion of NAcc DA without a concomitant increase in Glu levels. Because the infusion of AP-5 or CGS 19755 diminished the enhanced release of DA by this dose of nicotine, the experiments demonstrate that tonic glutamatergic input to VTA DA neurons is required to maintain their nicotine responsiveness. In the presence of NMDA receptor antagonists, the lack of nicotine responsiveness may be related to the marked decline in the firing rate and burst firing pattern of midbrain DA neurons that has been reported to occur in the absence of glutamatergic input (Overton and Clark, 1992; Chergui et al., 1993). Thus, the tonic glutamate-dependent activation of

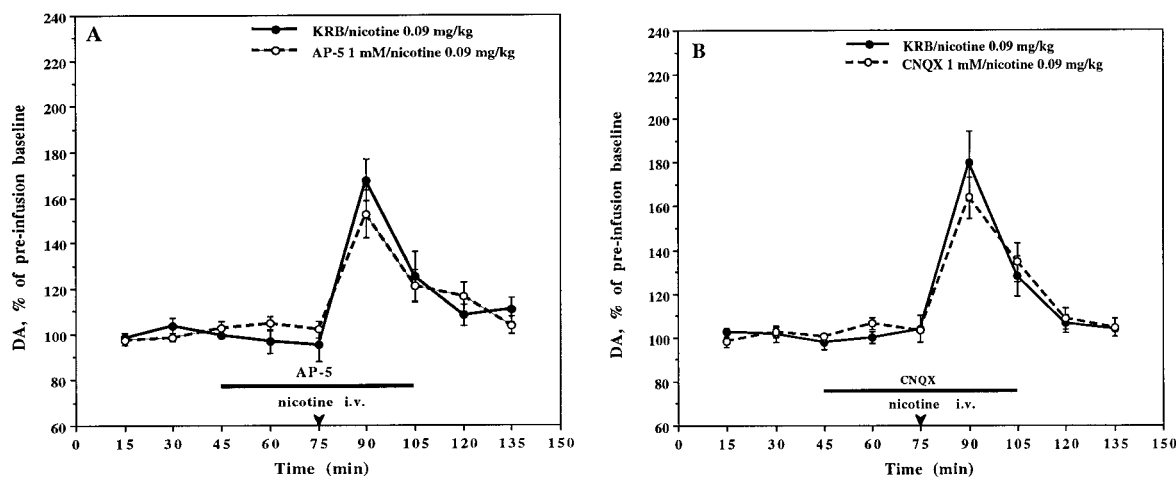


Fig. 8. Lack of an effect on NAcc DA release by the glutaminergic antagonists AP-5 (A) or CNQX (B) dialyzed directly into the NAcc. Arrowhead indicates the start of nicotine administration; the line indicates the perfusion of AP-5 or CNQX for 30 min before and after the nicotine infusion. Data were analyzed with two-way ANOVA with repeated measures. For A, $F(\text{treatment}) = 1.205$, $P = .632$; $F(\text{time}) = 2.189$, $P = .026$; $F(\text{time} \times \text{treatment}) = .633$, $P = .227$. For B, $F(\text{treatment}) = 1.047$, $P = .466$; $F(\text{time}) = 2.308$, $P = .017$; $F(\text{time} \times \text{treatment}) = .832$, $P = .138$ ($n = 6$ rats per group).

VTA DA neurons appears to be required for these neurons to respond to nicotine with enhanced DA secretion in the NAcc.

The second mechanism whereby VTA NMDA receptors mediate nicotine-stimulated DA release in the NAcc may depend on the enhanced (phasic) release of Glu in the VTA. It has been reported that an infusion of NMDA into the VTA stimulated DA release in the NAcc (Karreman et al., 1996; Schilstrom et al., 1998a). Moreover, electrical stimulation of the prefrontal cortex, the origin of glutamatergic afferents to the VTA, increased DA release in the NAcc (Taber et al., 1995). Glutamatergic regulation of DA release also was seen in other brain regions receiving dopaminergic projections from VTA. For example, infusion of NMDA into VTA increased extracellular DA levels in the prefrontal cortex (Westerink et al., 1998). In this study, nicotine (at a dose of 0.09 mg/kg or higher) stimulated Glu release in the VTA. Linear regression analysis showed that this release of Glu was significantly correlated with the enhanced secretion of DA in the NAcc. Therefore, the enhanced release of Glu by higher doses of nicotine appears to play a role in increasing the activity of dopaminergic neurons within the VTA.

Schilstrom et al. (1998a) previously reported that administering AP-5 into the VTA blocked nicotine-stimulated DA secretion in the rat NAcc. In that study, 0.5 mg/kg nicotine s.c. stimulated the release of DA that peaked at 77% above basal levels. This is slightly greater than the 62% elevation observed after 0.09 mg/kg i.v. nicotine in this study. Based on this comparison, it is conceivable that enhanced Glu release within the VTA by s.c. nicotine may have contributed to nicotine-stimulated DA secretion, as postulated by those authors (although excitatory amino acids were not actually measured). However, the tonic involvement of Glu in mediating the responsiveness of VTA DA neurons to nicotine could not be appreciated in that study because low doses of nicotine were not used, nor was Glu measured. Because a dose of AP-5 greater than 1 mM was not tolerated by the animals in the current study, we could not definitively show, with pharmacological methods, that the unblocked component of the enhanced DA response to higher doses of nicotine was mediated through NMDA receptors. The evidence we

have demonstrating that this unblocked portion was dependent on Glu secretion rests on the correlation that exists between Glu release and DA secretion (Fig. 4C).

In addition to the NMDA receptor, other VTA glutamatergic receptors (i.e., AMPA or metabotropic subtypes) may be involved. However, AMPA receptors are probably not involved because the administration of CNQX, an AMPA receptor antagonist, into the VTA had no effect on DA secretion in response to s.c. nicotine (Schilstrom et al., 1998a). In addition, we found that CGS 19755 or AP-5 abolished DA release after 0.065 mg/kg i.v. nicotine, indicating that the NMDA receptor is predominantly involved. These results are also consistent with the reports by Wang and French, who found that low extracellular levels of Glu (less than or equal to 30 μM , physiologically relevant concentrations) excited VTA DA neurons through the preferential activation of NMDA receptors. Non-NMDA receptors were involved only at higher concentrations (greater than or equal to 300 μM) of Glu (Wang and French, 1993). The potential involvement of VTA metabotropic receptors in nicotine-stimulated NAcc DA secretion remains to be clarified.

The precise mechanism(s) whereby systemic nicotine stimulates VTA Glu secretion remains to be determined. It has been reported that nicotine stimulates Glu release in cultured neonatal hippocampal neurons through presynaptic $\alpha 7$ nAChRs (Gray et al., 1996). Administration of the $\alpha 7$ -selective antagonist methyllycaconitine into the VTA reduced the NAcc DA response to systemic nicotine (Schilstrom et al., 1998b). However, depending on effective tissue concentration, MLA may inhibit nAChRs containing subunits other than $\alpha 7$. Thus, studies with α -bungarotoxin would further clarify the role of $\alpha 7$ -containing nAChRs. In addition to the potential effects of nicotine on presynaptic glutamatergic afferents, nicotine-induced Glu release may depend on the enhanced secretion of DA within the VTA. Nicotine stimulates DA release in the VTA to an extent similar to that seen in the NAcc (our unpublished data). Although unproven, this DA secretion may stimulate the release of Glu through D_1 dopamine receptors, in a manner similar to that postulated

for the pars reticulata of the substantia nigra (Rosales et al., 1997).

In summary, this study demonstrates that Glu secretion in the VTA is involved both tonically and phasically in nicotine-stimulated DA secretion within the NAcc. Lower doses of systemic nicotine (e.g., 0.065 mg/kg) do not enhance VTA Glu release, but these levels of Glu, acting through NMDA receptors, are required to maintain the responsiveness of DA neurons to nicotine. In contrast, higher doses of nicotine (e.g., 0.09 mg/kg) do release Glu in the VTA, and this is correlated significantly with the enhanced release of DA in the NAcc. Although nicotine stimulates Glu secretion to a similar degree in both the VTA and NAcc, these tonic and phasic effects of Glu appear to affect the basal activity of DA neurons and their responsiveness to nicotine only through glutamatergic-dopaminergic interactions within the VTA.

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