

Galantamine Enhances Dopaminergic Neurotransmission *In Vivo* Via Allosteric Potentiation of Nicotinic Acetylcholine Receptors

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Clinical studies suggest that adjunct galantamine may improve negative and cognitive symptoms in schizophrenia. These symptoms may be related to impaired dopaminergic function in the prefrontal cortex. Indeed, galantamine has been shown to increase dopamine release *in vitro*. Galantamine is an allosteric modulator of nicotinic acetylcholine receptors (nAChRs) and, at higher doses, an acetylcholine esterase (AChE) inhibitor. We have previously shown that nicotine, through stimulation of nAChRs in the ventral tegmental area (VTA), activates midbrain dopamine neurons and, hence, potentiation of these receptors could be an additional mechanism by which galantamine can activate dopaminergic pathways. Therefore, the effects of galantamine (0.01–1.0 mg/kg s.c.) on dopamine cell firing were tested in anaesthetized rats. Already at a low dose, unlikely to result in significant AChE inhibition, galantamine increased firing activity of dopaminergic cells in the VTA. The effect of galantamine was prevented by the nAChR antagonist mecamylamine (1.0 mg/kg s.c.), but not the muscarinic receptor antagonist scopolamine (0.1 mg/kg s.c.), and it was not mimicked by the selective AChE inhibitor donepezil (1.0 mg/kg s.c.). Our data thus indicate that galantamine increases dopaminergic activity through allosteric potentiation of nAChRs. Galantamine's effect was also prevented by the $\alpha 7$ nAChR antagonist methyllycaconitine (6.0 mg/kg i.p.) as well as the *N*-methyl-D-aspartate antagonist CGP39551 (2.5 mg/kg s.c.), indicating a mechanism involving presynaptic facilitation of glutamate release. In parallel microdialysis experiments, galantamine was found to increase extracellular levels of dopamine in the medial prefrontal cortex. These results may have bearing on the enhancement of negative and cognitive symptoms in schizophrenia.

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INTRODUCTION

The acetylcholine esterase (AChE) inhibitor galantamine is currently used for symptomatic treatment of Alzheimer's disease, the pathophysiology of which involves degeneration of cholinergic neurons (Davies and Maloney, 1976). Recent clinical data suggest that galantamine might also be useful as adjunct therapy in schizophrenia, as it has been found to improve both negative and cognitive symptoms of the disease (Rosse and Deutsch, 2002; Allen and McEvoy, 2002; Bora *et al*, 2005). It has been hypothesized that increasing cholinergic neurotransmission in the cortex may be a generally desirable effect of antipsychotic drugs since those that are most effective against cognitive symptoms also increases the extracellular levels of cortical acetylcholine (Ichikawa *et al*, 2002). However, clinical data

suggest that while the weak AChE inhibitor galantamine has beneficial effects in schizophrenia, the more potent and specific AChE donepezil has not (Friedman *et al*, 2003). These studies suggest that a mechanism separate from acetylcholine esterase inhibition may be involved in the effect of galantamine on schizophrenia. In fact, galantamine has two separate mechanisms of action which can both enhance cholinergic neurotransmission in the brain. At low doses, it binds allosterically to nicotinic acetylcholine receptors (nAChRs) and potentiates their function, and at high doses it acts as a weak AChE inhibitor (Schrattenholz *et al*, 1996; Maelicke *et al*, 2000). Thus, while selective AChE inhibitors may increase cholinergic neurotransmission at both muscarinic acetylcholine receptors (mAChRs) and nAChRs, galantamine has the potential of preferentially potentiating endogenous cholinergic neurotransmission occurring at nAChRs and this difference may explain the drugs' differing clinical profile.

Both clinical and preclinical studies suggest that negative and cognitive symptoms in schizophrenia may involve dysfunctional dopaminergic neurotransmission in the prefrontal cortex (for a recent review, see Svensson, 2003). Among the cognitive deficits reliably observed in schizophrenic patients are impairments in working memory, that

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is, an impaired ability to retain and manipulate information over a short period of time. Working memory is largely executed in various regions of the prefrontal cortex and depends on an accurate level of dopamine D1 receptor stimulation (Arnsten *et al*, 1994). A recent PET study in schizophrenic patients reported increased dopamine D1 receptor availability in the prefrontal cortex (Abi-Dargham *et al*, 2002). Furthermore, a negative correlation between the D1 receptor availability and performance on a task requiring working memory was observed. Based on these findings it was suggested that dopamine D1 receptors are upregulated to compensate for decreased release of dopamine. Thus, a dopamine-dependent working memory deficit in schizophrenia is not likely due to a dopamine deficiency at the receptor level, but rather to upstream mechanisms that control the release or degradation of dopamine (Egan *et al*, 2001).

Recently, galantamine was shown to increase nerve terminal release of dopamine *in vitro* (Zhang *et al*, 2004), but because of the *in vitro* preparation used any action of galantamine within the dopaminergic cell body region could not be detected. However, earlier data from our laboratory has shown that nicotine activates dopamine cell firing rate as well as burst firing (Grenhoff *et al*, 1986) and that nAChRs in the ventral tegmental area (VTA) are critically involved in nicotine's stimulatory effect on terminal dopamine release (Nisell *et al*, 1994; Schilström *et al*, 1998b). Specifically, burst firing induced by nicotine is in all probability due to presynaptic enhancement of afferent glutamate release. Nicotine has been shown to activate presynaptic $\alpha 7$ -subunit containing nAChRs in the VTA and enhance glutamate release that leads to stimulation of N-methyl-D-aspartate (NMDA) receptors on dopamine cells (Schilström *et al*, 1998a, b, 2000; Mansvelder and McGehee, 2000; Schilström *et al*, 2003). Moreover, heteromeric $\beta 2$ -containing nAChRs on dopamine cell bodies and γ -amino-butyric acid (GABA)-ergic interneurons also influence the activity of dopaminergic neurons (Calabresi *et al*, 1989; Picciotto *et al*, 1998; Mansvelder *et al*, 2002; Schilström *et al*, 2003). These studies indirectly suggest additional mechanisms by which galantamine can improve negative and cognitive symptoms in schizophrenia. Thus, through its action as an allosteric modulator of nAChRs, galantamine may, accordingly, facilitate dopamine release by increasing dopaminergic firing through potentiation of nAChR function in the VTA. Therefore, using *in vivo* single unit recordings, we investigated the effect of galantamine on dopamine cell firing. Galantamine's effect was analyzed pharmacologically using the subtype nonselective nAChR antagonist mecamylamine, the mAChR antagonist scopolamine, the selective AChE inhibitor donepezil as well as the $\alpha 7$ -selective nAChR antagonist methyllycaconitine (MLA), and the NMDA receptor antagonist CGP39551. Moreover, using *in vivo* microdialysis, the effect of galantamine on dopamine output in the prefrontal cortex was examined.

MATERIALS AND METHODS

All experiments were approved by, and conducted in accordance with, the Stockholm North Committee on

Ethics of Animal Experimentation (ethical approval numbers N18/01, N18/04, and N340/02). Efforts were made to minimize the number of animals used and their suffering.

Extracellular Single Cell Recording of Dopaminergic Neurons of the VTA *In Vivo*

The procedures for extracellular single cell recording have been described previously (Grenhoff *et al*, 1986; Schilström *et al*, 2003). Rats (250–300 g; male Sprague–Dawley, BK Universal, Sollentuna, Sweden) were anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic frame (David Kopf, Tujunga, CA). Anesthesia was maintained throughout the experiment with periodical injections of chloral hydrate (approximately 150 mg/kg/h i.p.) and body temperature was kept at 37°C with a rectal thermometer connected to an electrical heating pad. Recording electrodes were pulled in a Narishige (Tokyo, Japan) vertical puller from borosilicate glass capillaries (Clark Electromedical Instruments, Reading, UK) with outer and inner diameters of 1.50 and 1.17 mm, respectively, and filled with 2% Pontamine Sky Blue in 2 M NaCl. The tips of the electrodes were broken to yield an impedance of 2–4 M Ω at 135 Hz and then slowly lowered into a hole drilled in the skull above the VTA (3.2 ± 0.3 mm anterior of the interaural line and 0.7 ± 0.2 mm lateral to the midline; Paxinos and Watson, 1998) by using a hydraulic microdrive (David Kopf, Tujunga, CA). Presumed dopamine neurons were found 7.5–8.5 mm from the brain surface and were recognized by their characteristic triphasic action potential waveform of more than 2.0 ms duration, basal firing rates of 1–10 Hz, and frequent occurrence of burst firing (Wang, 1981). Moreover, the 'Ungless-filter', consisting of tail- or toe-pinch, was applied and only cells that responded with a transient inhibition of firing were included in the study (Ungless *et al*, 2004). Extracellular electrical activity was amplified, filtered (band pass 0.3–3 kHz), discriminated, and monitored on an oscilloscope (TDS 310, Tektronix, Beaverton, OR) and an audiometer (Grass, AM8B/C, West Warwick, RI). Discriminated spikes were fed via a CED 1401 interface (Cambridge Electronic Design, Ltd, Cambridge, UK) to a computer running CED Spike 2 software. After completion of the experiment, the position of the electrode was marked by iontophoresis of Pontamine Sky Blue into the tissue (5 μ A for 5 min). The rat was killed with an overdose of anesthetic, and the brain was removed and placed in 25% sucrose, 10% paraformaldehyde solution. Sections of the VTA were cut (50 μ M) and stained with neutral red and the recording sites were verified by light microscopy.

Microdialysis

Rats (male Wistar, BK Universal, Sollentuna, Sweden) were anaesthetized with a cocktail containing Hypnorm[®] (Fentanyl 0.315 mg/ml + Fluanison 10 mg/ml, Janssen-Cilag) and Dormicum[®] (Midazolam 5 mg/ml, Roche) diluted in distilled water (1:1:2; 5 ml/kg i.p.) and mounted in a stereotaxic frame. Dialysis probes were implanted in the medial PFC (AP: +3.0; ML \pm 0.6; DV: –5.2, relative to bregma and the dural surface according to the atlas of

Paxinos and Watson, 1998). Dialysis occurred through a semipermeable membrane (Filtral AN69, Hospal Industrie, France) with an active surface length of 4 mm. Dialysis experiments were conducted approximately 48 h after surgery in freely moving rats. The dialysis probe was perfused with a physiological perfusion solution (Apoteksbolaget, Sweden) at a rate of 2.5 μ l/min set by a microinfusion pump (Harvard Apparatus, Holliston, MA). Dialysate was collected over 30 min intervals and automatically injected into a high-performance liquid chromatography system. On-line quantification of dopamine in the dialysate was accomplished by electrochemical detection (ESA, Chelmsford, MA). Following experiment, the rat was killed with an overdose of anesthetic, the brain was removed and placed in 25% sucrose, 10% paraformaldehyde solution. Sections of the VTA were cut (50 μ M) and stained with neutral red and the probe placements sites were verified by light microscopy.

Drugs

Galantamine and donepezil were generously provided by Janssen Cilag AB, and CGP39551 was a generous gift from Novartis. Scopolamine, mecamlamine, and MLA were purchased from the local Sigma-Aldrich supplier (Stockholm, Sweden). All drugs were dissolved in physiological saline solution and, besides MLA, which was injected intraperitoneally, drugs were injected subcutaneously. In electrophysiological experiments, when applicable, drugs were administered approximately 10 min apart.

Data Analysis

Electrophysiology. Dopamine cell firing was analyzed with respect to the average firing rate and the percentage of action potentials fired in bursts, calculated over consecutive periods of 500 inter-spike time intervals (ISI). Since the time period during which spikes are analyzed will depend on firing frequency, in cells with firing frequencies lower than 3.0 Hz, consecutive periods of 250 ISI intervals were used to yield better time resolution. In experiments in which only one drug was administered, the last analyzed period before drug injection was compared to the period within 15 min after injection representing the median effect. In experiments in which two drugs were administered, the last period before any drug injection was compared to the median effect observed within 15 min of injection of galantamine (or donepezil). Firing rate data were statistically analyzed with two-way analysis of variance (ANOVA) followed by Newman-Keul's test for multiple comparisons or Student's paired *t*-test when appropriate. Values for all the individual cells are presented in figures with lines indicating mean \pm SEM. Analysis of burst firing was performed on the percentage of spikes fired in bursts in the last analyzed period before injection and was compared to the period within 15 min after injection representing the median effect. Burst firing is defined as a series of spikes starting when the interval between two spikes is lower than 80 ms and terminating when the interval exceeds 160 ms (Grace and Bunney, 1984). Since burst firing was not normally distributed, the nonparametric Kruskal-Wallis

ANOVA or the Mann-Whitney *U*-test was used to analyze differences between treatments and Wilcoxon matched pairs signed ranks test was used to evaluate within effects of the different treatments. Data are presented in figures as absolute pre- and postinjection values for all the individual cells and the median across cells is indicated with a horizontal line.

Microdialysis. Dopamine levels were expressed and statistically analyzed as percent of basal levels. Baseline was defined as the average of the two samples immediately preceding treatment. The mean percent changes were then calculated for each 30 min sample or all rats in each group. Three experimental groups were included. The effect of galantamine on dopamine output in the prefrontal cortex was tested at 0.1 and 1.0 mg/kg s.c. and to control for injection effects a vehicle group was included. Data were analyzed by one- and two-way (treatment \times time) ANOVA with repeated measures followed by the Newman-Keuls test for multiple comparisons. All statistical analyses were performed using the STATISTICA software (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

Effects of Galantamine on Dopamine Cell Firing

Galantamine was tested in the doses 0.01, 0.1, and 1.0 mg/kg s.c. (Figure 1). Two-way ANOVA analysis of the firing rate data revealed a significant time ($F_{1,25} = 16.114$, $p < 0.001$), but not a group or interaction effect. Further statistical analysis showed that the mean firing rate increases from 4.61 ± 0.71 Hz under baseline conditions to 4.90 ± 0.68 Hz following injection of 0.01 mg/kg (s.c.) galantamine ($p < 0.05$, Figure 1b) and from 5.51 ± 0.58 Hz to 5.95 ± 0.52 Hz following 0.1 mg/kg (s.c.) galantamine ($p < 0.01$, Figure 1a and b) were significant, but the slight elevation from 5.36 ± 0.53 Hz to 5.55 ± 0.46 Hz following 1.0 mg/kg (s.c.) galantamine was not ($p > 0.05$, Figure 1b). Burst firing is a variable that tends not to follow a normal distribution. Descriptive statistics revealed that this was the case also in our experiments (skewness < -1 or skewness > 1) and therefore the results were analyzed by Kruskal-Wallis ANOVA and Wilcoxon's matched pairs signed ranks test. There were no significant differences between the groups before or after galantamine injection but all doses increased burst firing when compared to its own baseline. At 0.01 mg/kg, galantamine increased median burst firing from 7.2 to 10.8% ($p < 0.05$, $n = 10$, Figure 1c), at 0.1 mg/kg median burst firing increased from 24.4 to 46.3% ($p < 0.01$, $n = 9$, Figure 1a and b), and at 1.0 mg/kg galantamine the median burst firing increased from 4.4 to 27.2% ($p < 0.05$, $n = 9$, Figure 1c). Since galantamine activates dopamine cells already at low doses that have been shown to result in very low AChE inhibition (Geerts *et al*, 2005), the present data suggest that the mechanism by which galantamine increases dopamine cell firing may be allosteric potentiation of nAChRs. Galantamine was most effective at activating dopaminergic cells at 0.1 mg/kg s.c. Therefore, our pharmacological characterization of galantamine's effect was performed using this dose.

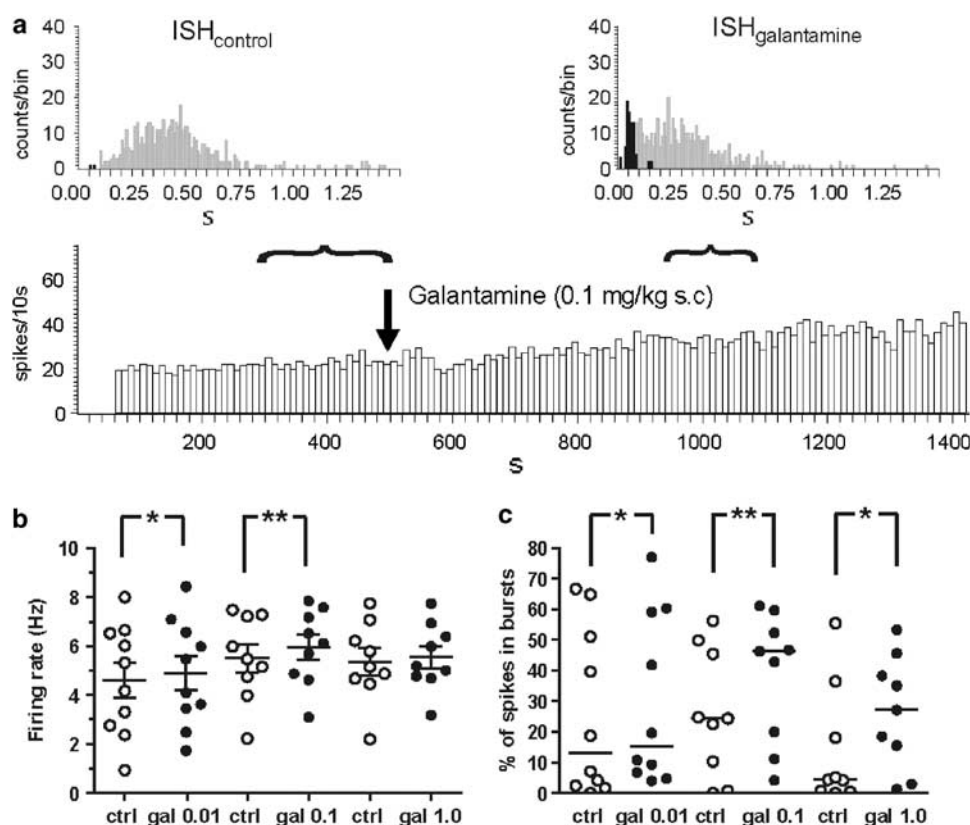


Figure 1 (a) Ratemeter recording and interspike time interval histograms (ISHs) of a representative experiment showing the effects of galantamine (0.1 mg/kg s.c.) on firing rate and the percentage of spikes fired in bursts (as indicated by black bars in ISHs). Arrow indicates the time of injection. The time periods from which the spike analyses were performed are indicated above the ratemeter recording with lying curly brackets. (b) Summary graph showing the effects of varying doses of galantamine on firing rate. The firing rate for each cell is represented by an open circle (ctrl) before and a filled circle after galantamine injection. Horizontal lines indicate mean \pm SEM. (c) Summary graph showing the effects of varying doses of galantamine on burst firing. Each cell recorded is represented by an open circle before and a filled circle after galantamine injection. Horizontal line indicates the median. * = $p < 0.05$, ** = $p < 0.01$, ctrl = control, gal = galantamine.

Galantamine Activates Dopamine Cell Firing Via Activation of nAChRs

If the effect of galantamine is due to inhibition of AChE, it would increase acetylcholine levels that could activate both nAChRs and mAChRs. Therefore, the effect of galantamine was tested in the presence of an antagonist at nAChRs and mAChRs, respectively. Thus, we first established a dose of the nAChR antagonist mecamylamine and the mAChR antagonist scopolamine that by themselves had no effect. Mecamylamine, when given at 1.0 mg/kg (s.c.), a dose that we have previously used to precipitate nicotine-withdrawal in chronically nicotine-treated rats (Hildebrand *et al*, 1998), produced no effects on firing activity of dopamine neurons. The frequency was 4.76 ± 0.74 Hz before and 4.68 ± 0.78 after injection (paired *t*-test, $p > 0.05$, $n = 8$, data not shown). Burst firing was unchanged from 2.2% before and 6.6% after injection ($p > 0.05$, $n = 8$, Wilcoxon's matched pairs signed ranks test). Scopolamine was first tested at 1.0 mg/kg s.c., but this dose clearly decreased firing rate (for this reason, experiments were not pursued to an n high enough for statistical analysis, data not shown) and a lower dose was chosen. When given at 0.1 mg/kg, scopolamine had no effect on firing activity. Basal firing rate was 5.34 ± 0.76 Hz before and 5.15 ± 0.75 after scopolamine

injection (paired *t*-test, $p > 0.05$, $n = 7$, data not shown). Numerically, median burst firing increased from 2.2 to 9.5%, but since there was large variation the effect was not statistically significant ($p > 0.05$, $n = 7$, data not shown).

Two-way ANOVA comparing the effects of galantamine (0.1 mg/kg) in pretreated vs nonpretreated animals revealed that there were no significant differences between the various groups, but there were time and interaction effects ($F(\text{interaction})_{4,40} = 3.628$, $p < 0.05$). *Post hoc* analysis revealed that although galantamine produced a significant effect in nonpretreated animals, it did not in mecamylamine-pretreated animals (basal 4.73 ± 0.40 Hz, postinjection 4.58 ± 0.40 Hz, $p > 0.05$, $n = 6$, Figure 2a and b). Moreover, in mecamylamine-pretreated rats, galantamine did not increase burst firing significantly (basal burst firing was 2.9% and post-galantamine injection 3.8%, $p > 0.05$, $n = 6$, Figure 2a and c). In rats pretreated with scopolamine, galantamine increased firing rate in a majority of the nine cells tested (5.30 ± 0.61 Hz before and 5.93 ± 0.60 Hz after injection, $p < 0.001$, Figure 2b and d) and also burst firing increased in response to galantamine (from the basal value 28.7 to 51.1% postinjection, $p < 0.05$, Figure 2c and d). These data suggest that nAChRs, but not mAChRs, mediate the stimulatory effect of galantamine on

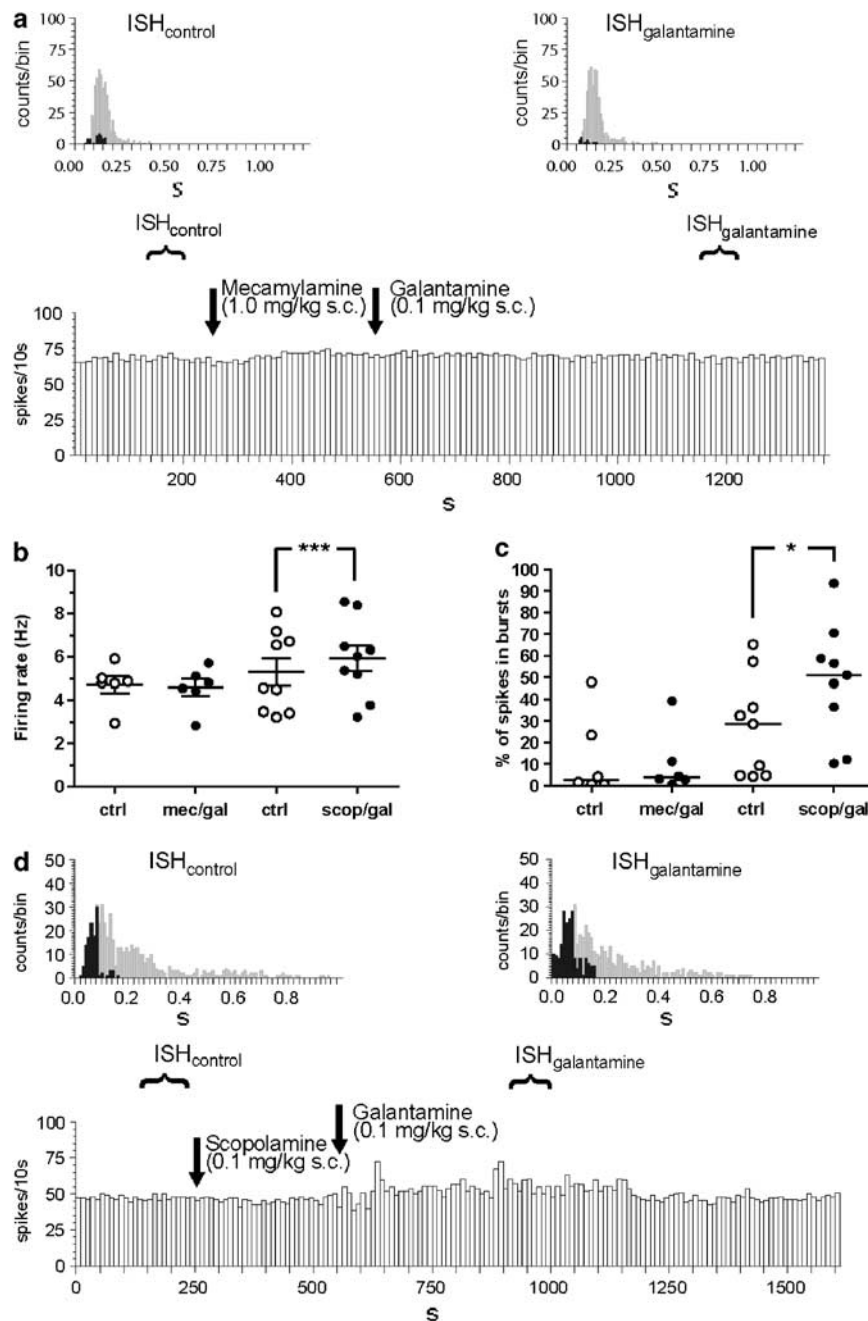


Figure 2 (a and d) Ratemeter recordings and ISHs showing the effects of (a) galantamine in the presence of the nAChR antagonist mecylamine (1.0 mg/kg s.c.) and (d) galantamine in the presence of the muscarinic receptor antagonist scopolamine. Arrows indicate the time of injections. The time periods from which the spike analyses were performed are indicated above the ratemeter recording with lying curly brackets. Effects of galantamine on firing rate in mecylamine and scopolamine-pretreated animals are summarized in (b) in which the basal level (ctrl) for each cell is represented by an open circle and the effect of galantamine by a filled circle. Mean \pm SEM are indicated by horizontal lines. (c) Summary graph showing the effects on burst firing. Basal burst firing for each cell (ctrl) is indicated by an open circle and the effect of galantamine by a filled circle. Horizontal line indicates the median. gal = galantamine, mec = mecylamine, scop = scopolamine, * = $p < 0.05$, *** = $p < 0.001$.

dopamine cell firing. Previous *in vitro* experiments have suggested that the long-lasting excitatory effects of nicotine on dopaminergic cells may be due to nicotine-induced synaptic plasticity (Mansvelder and McGehee, 2000). In order to test whether the effects of galantamine are reversible, we performed a separate set of experiments in which galantamine was administered first and then followed 5–10 min later by mecylamine. Although mecylamine

was not able to reduce firing rate in naïve animals, administration of mecylamine to galantamine-treated rats decreased firing rate of dopaminergic neurons from 4.76 ± 0.76 Hz to 4.08 ± 0.80 Hz ($p < 0.05$, $n = 6$, paired *t*-test, data not shown). Moreover, mecylamine reduced median burst firing in galantamine-treated rats from 36.8 to 13.7% ($p < 0.05$, $n = 6$, Wilcoxon's matched pairs signed ranks test, data not shown).

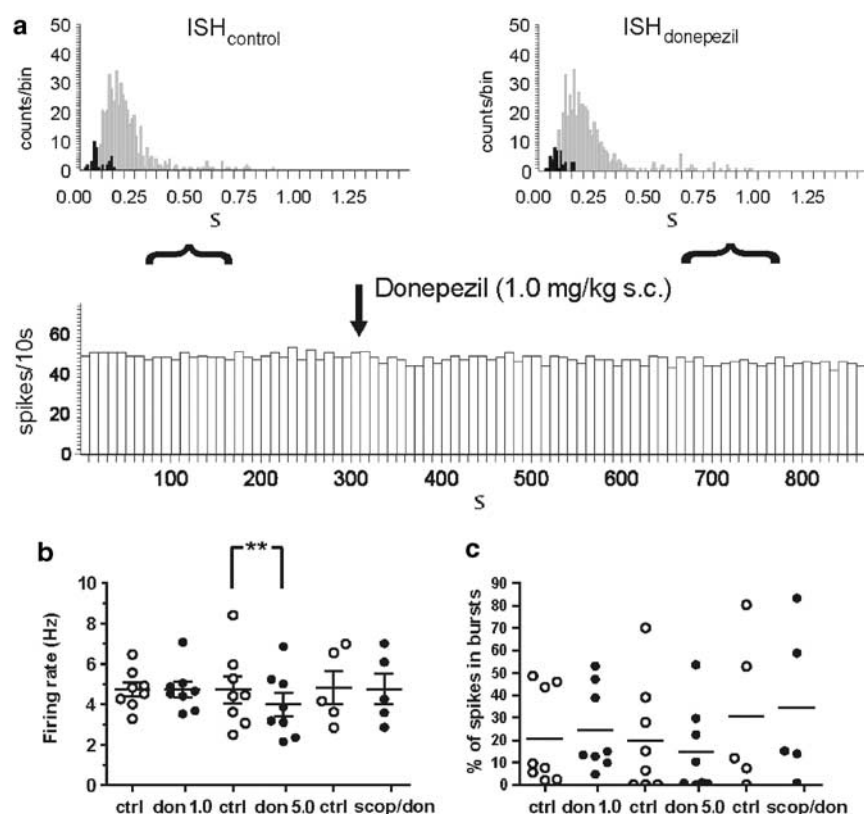


Figure 3 (a) Ratemeter recording and interspike time interval histograms (ISHs) of a representative experiment showing the effects of donepezil (1.0 mg/kg s.c.) on firing rate and the percentage of spikes fired in bursts (as indicated by black bars in ISHs). Arrow indicates the time of injection. The time periods from which the spike analyses were performed are indicated above the ratemeter recording with lying curly brackets (b) Summary graph showing the effects of 1.0 mg/kg, 5.0 mg/kg of donepezil and 5.0 mg/kg of donepezil in scopolamine pretreated animals on firing rate. Each cell is represented by an open circle indicating the basal firing rate (ctrl) and a filled circle after donepezil injection. Mean \pm SEM are indicated by horizontal lines. (c) Summary graph showing the effects of 1.0 mg/kg, 5.0 mg/kg of donepezil and 5.0 mg/kg of donepezil in scopolamine pretreated animals on burst firing. Each cell recorded is represented by an open circle indicating basal burst firing and a filled circle indicating burst firing after donepezil injection. Horizontal line indicates the median. don = donepezil, scop = scopolamine, ** = $p < 0.01$.

The Selective AChE Inhibitor Donepezil does not Affect Dopamine Cell Firing

In order to test whether inhibition of AChE could contribute to the effects of galantamine, we tested the selective AChE inhibitor donepezil at 1.0 and 5.0 mg/kg (s.c.). Two-way ANOVA of firing rate data revealed no significant differences between the groups, but there was a significant interaction effect ($F(\text{interaction})_{1,14} = 7.877$, $p < 0.05$). *Post hoc* analysis revealed that whereas the lower dose had no effect on firing rate (4.75 ± 0.35 Hz before and 4.73 ± 0.39 Hz after injection, $p > 0.05$, $n = 8$, Figure 3a and b), the higher dose significantly decreased firing rate (4.73 ± 0.66 Hz before and 3.98 ± 0.57 Hz after injection, $p < 0.01$, $n = 8$, Figure 3b). There were no statistical differences in burst firing between the two groups (Mann-Whitney *U*-test) and there was no significant effect of either dose on burst firing when compared to basal (median 8.6% before and 14.6% after injection of 1.0 mg/kg donepezil and 10.8% before and 5.7% after 5.0 mg/kg, $p > 0.05$, $n = 8$, Wilcoxon's matched pairs signed ranks test, Figure 3c). Since a higher dose of donepezil had an inhibitory effect on dopamine cell firing, these results argue in favor of the notion that galantamine increases dopamine cell firing through allosteric potentiation of nAChRs. As a conse-

quence, the inhibitory effect of donepezil may rather be mediated by mAChRs and therefore we tested the effect of donepezil in the presence of scopolamine. Two-way ANOVA of firing rate (scopolamine alone, donepezil 5.0 mg/kg alone, and scopolamine + donepezil groups included) revealed no significant treatment effect, but significant time and interaction effects ($F(\text{interaction})_{2,17} = 4.393$, $p < 0.05$). Newman-Keuls test for multiple comparisons revealed that donepezil did not decrease firing rate significantly in scopolamine-pretreated rats (4.84 ± 0.82 before and 4.77 ± 0.78 after donepezil injection, Figure 3b). As presented above, neither scopolamine nor donepezil had significant effects on burst firing and there was no significant effect on burst firing when the two were combined ($p > 0.05$, Figure 3c).

The Effects of Galantamine on Dopamine Cell Firing are Prevented by an $\alpha 7$ nAChR Antagonist and an NMDA Receptor Antagonist, Respectively

We have recently shown that $\alpha 7$ nAChRs within the VTA are critically involved in nicotine-induced burst firing (Schilström *et al*, 2003). Previous data demonstrate that presynaptic $\alpha 7$ -containing nAChR in the VTA can enhance glutamate release and activate NMDA receptors on

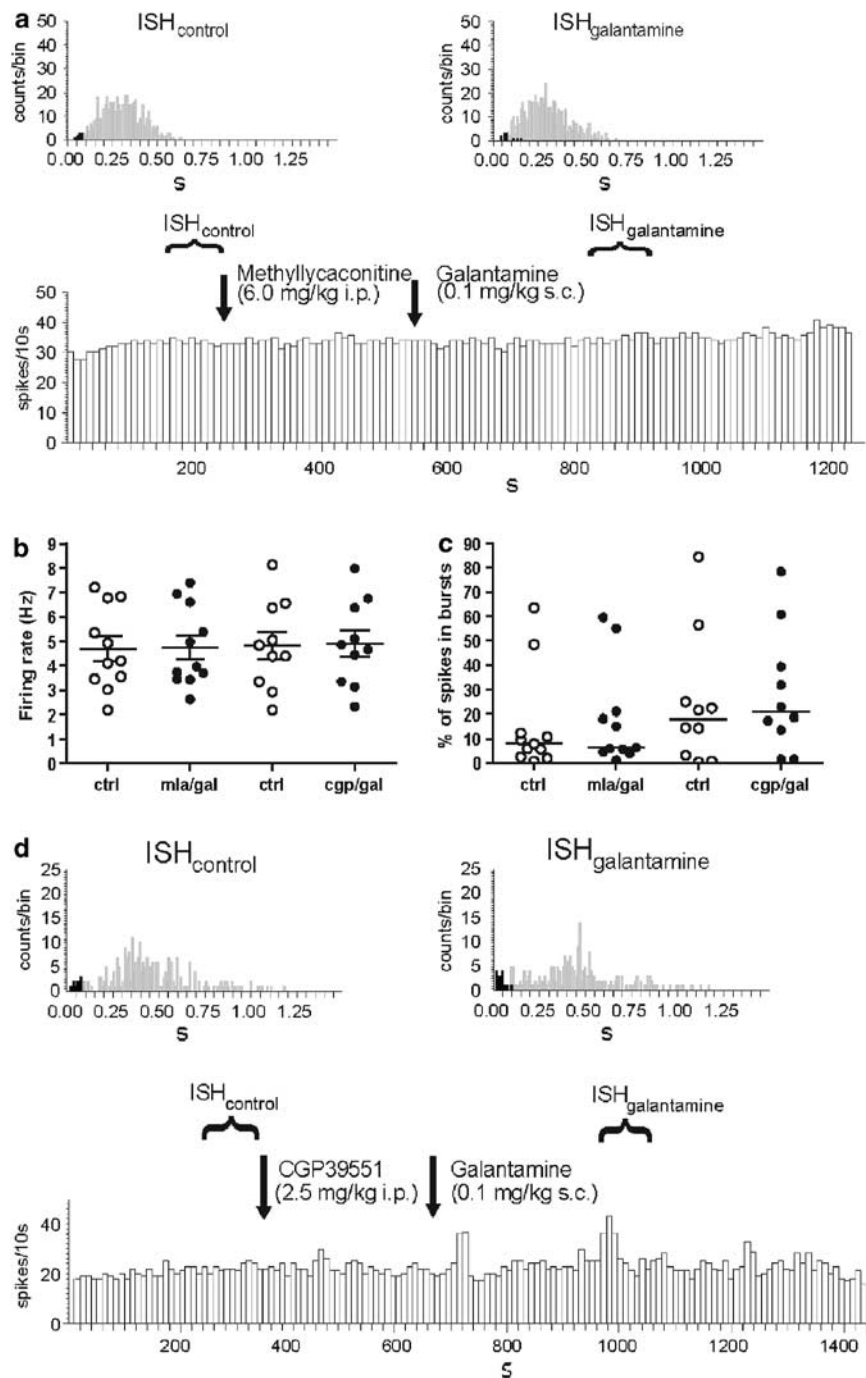


Figure 4 Ratemeter recordings and ISHs showing the effects of galantamine in (a) the presence of the $\alpha 7$ selective nAChR antagonist methyllycaconitine (6.0 mg/kg s.c.) and (d) the presence of the competitive NMDA receptor antagonist CGP39551 (CGP). Arrows indicate the time of injection and the time periods from which the spike analyses were performed are indicated above the ratemeter recording with lying curly brackets. Effects of galantamine on firing rate in methyllycaconitine and CGP39551-pre-treated animals are summarized in (b) in which the basal level (ctrl) for each cell is represented by an open circle and a filled circle after galantamine injection. Mean \pm SEM are indicated by horizontal lines. (c) Summary graph showing the effects on burst firing. Basal burst firing for each cell (ctrl) is indicated by an open circle and the effect of galantamine by a filled circle. Horizontal line indicates the median. gal = galantamine, mla = methyllycaconitine, cgp = CGP39551.

dopamine cells (Mansvelder and McGehee, 2000; Schilström *et al*, 1998a,b, 2000). Therefore, in order to test the involvement of putative $\alpha 7$ nAChRs and NMDA receptors, the effect of galantamine was tested in animals pretreated with the $\alpha 7$ nAChR antagonist methyllycaconitine (MLA, 6.0 mg/kg i.p.) or the NMDA receptor antagonist CGP39551

(2.5 mg/kg s.c.), respectively. Here, in accordance with our and others previous results (Schilström *et al*, 2003; Schwieler *et al*, 2004), we observed that although MLA had variable effects in individual cells there were no overall significant effects on firing rate (4.94 ± 0.58 Hz before and 4.78 ± 0.60 Hz after MLA, $p > 0.05$, paired t -test, $n = 10$, data

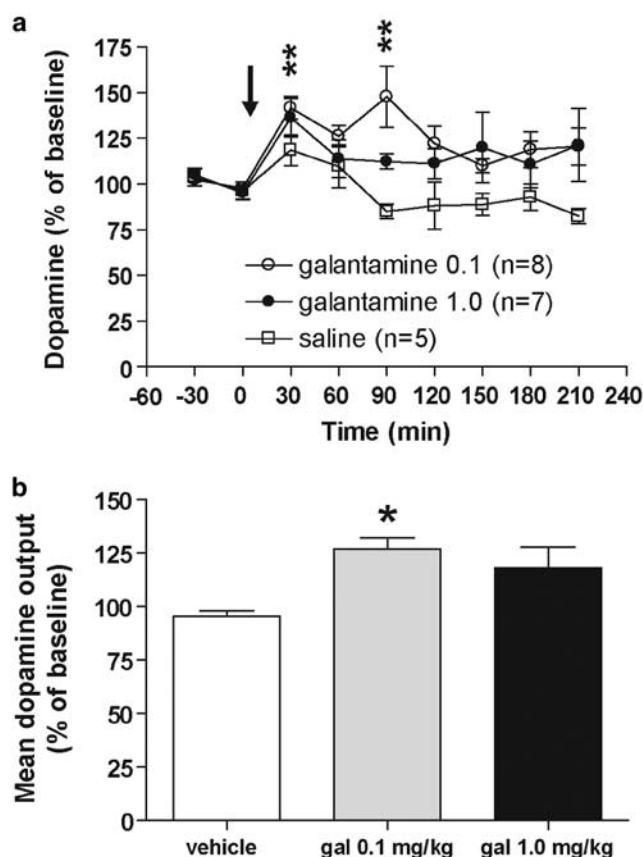


Figure 5 Effects of galantamine on extracellular levels of dopamine in the medial prefrontal cortex. (a) Temporal effects of 0.1 mg/kg and 1.0 mg/kg galantamine compared to a vehicle injection on extracellular levels of dopamine in the medial prefrontal cortex. Data are expressed as per cent of baseline and presented as mean \pm SEM. ** = $p < 0.01$ compared to baseline. (b) Mean dopamine output during the whole 3.5 hours following injection of vehicle, galantamine 0.1 mg/kg and galantamine 1.0 mg/kg, respectively. * = $p < 0.05$ compared with vehicle.

not shown) or burst firing (median before 25.5% and median after MLA 35.1%, $p > 0.05$, Wilcoxon's matched pairs signed ranks test, $n = 10$, data not shown). In MLA-pretreated animals, galantamine had no significant effect on firing rate (4.69 ± 0.51 Hz before and 4.75 ± 0.49 Hz after galantamine injection, $p > 0.05$, $n = 11$, Figure 4a and b) or burst firing (basal value 8.0% post-galantamine injection 6.4%, $p > 0.05$, $n = 11$, Figure 4a and c).

CGP39551 (2.5 mg/kg i.p.) by itself had no significant effects on firing rate (basal 5.62 ± 0.52 Hz before and 5.57 ± 0.73 after CGP39551, $p > 0.05$, $n = 9$, paired t -test, data not shown) or burst firing (from basal value of 22–34.1% postinjection, $p > 0.05$, $n = 9$, data not shown). Although the overall effect of CGP39551 on burst firing was nonsignificant, CGP39551 produced rather large effects in individual cells. When galantamine was tested in CGP39551-pretreated animals, there were no dramatic effects on firing rate or burst firing. Basal firing rate was 4.82 ± 0.57 Hz and after galantamine injection 4.90 ± 0.55 Hz ($p > 0.05$, $n = 10$, Newman–Keul's test for multiple comparisons, Figure 4b and d). Median burst firing was 18.1% under basal conditions and 20.8% after galantamine injection ($p > 0.05$, $n = 10$, Wilcoxon's matched pairs signed ranks test, Figure 4c and d).

Galantamine Increases Extracellular Levels of Dopamine in the Prefrontal Cortex

The effects of galantamine 0.1 mg/kg (s.c.) and 1.0 mg/kg (s.c.) on dopamine output in the prefrontal cortex were compared to a saline injection. Basal levels of dopamine did not differ between the three different groups ($p > 0.05$, one-way ANOVA). The overall basal level of dopamine in the mPFC was 0.29 ± 0.03 fmol/min. Statistical analysis (two-way ANOVA) of the data revealed both time ($F(\text{time})_{7,119} = 3.825$, $p < 0.001$) and treatment ($F(\text{treatment})_{2,17} = 4.837$, $p = 0.022$) effects, but no interaction effect ($F(\text{interaction})_{7,119} = 1.303$, $p = 0.216$). Further statistical analysis (one-way ANOVA followed by Newman–Keuls test for multiple comparisons) revealed that only the lower dose of galantamine increased dopamine levels in the PFC compared to baseline ($n = 8$, Figure 5a). Galantamine produced a significant elevation of dopamine levels in two different time points after injection ($p < 0.01$), while neither vehicle ($n = 5$) nor the higher dose of galantamine ($n = 7$) produced any significant effects. Analysis of treatment effects (one-way ANOVA followed by Newman–Keuls multiple comparison test) revealed that 0.1 mg/kg, but not 1.0 mg/kg or saline, increased the mean dopamine output significantly ($p < 0.05$, Figure 5b).

DISCUSSION

The main findings of the present study are that galantamine increases firing activity of dopaminergic cells in the VTA and, in addition, that galantamine increases dopamine output *in vivo* in the prefrontal cortex. Our pharmacological analysis strongly suggests that galantamine activates dopamine cell firing through allosteric potentiation of nAChRs. This conclusion is based on the observations herein that the effect of galantamine is not dose dependent, that it is antagonized by the nAChR antagonists mecamylamine, but not the mAChR antagonist scopolamine, and that it is not mimicked by the selective AChE inhibitor donepezil. Our results also propose that the effect of galantamine involves an $\alpha 7$ nAChR-mediated presynaptic facilitation of glutamate release that activates NMDA receptors, since it was prevented by the $\alpha 7$ nAChR antagonist MLA and the NMDA receptor antagonist CGP39551. In addition, the finding that the effect of galantamine is reversible suggests that, even if $\alpha 7$ nAChRs and NMDA receptors are involved, galantamine does not induce plasticity the way nicotine does (Mansvelder and McGehee, 2000).

As mentioned previously (cf. Introduction), emerging clinical data indicate that adjunctive galantamine can improve negative and cognitive symptoms in schizophrenia. We hypothesized that this beneficial effect of galantamine may be due to allosteric potentiation of nAChRs in the VTA, which might generate increased dopamine efflux in the mPFC. Here, galantamine was shown to increase extracellular levels of dopamine in the prefrontal cortex, suggesting that following galantamine administration there is an increased release of dopamine as a result of increased neuronal activity. Galantamine's effect on prefrontal dopamine efflux was significant at 0.1 mg/kg but not 1.0 mg/kg, an observation that parallels our electrophysiological data

showing that 0.1 mg/kg of galantamine increased both firing rate and burst firing of dopaminergic neurons. Thus, it appears that galantamine enhances dopaminergic activity most effectively at a dose that results only in minor AChE inhibition (Geerts *et al*, 2005), and the effect may therefore rather be due to allosteric potentiation of nAChRs. In support of this conclusion, a recent study reported that a higher dose of galantamine (3.0 mg/kg s.c.) did not increase dopamine efflux in the nucleus accumbens (Sharp *et al*, 2004).

Being an allosteric potentiating ligand, galantamine does not activate nAChRs directly but instead enhances the ability of the endogenous ligand, acetylcholine, to activate the receptor (Schrattenholz *et al*, 1996; Maelicke *et al*, 2000). Consequently, since neither of the nAChR antagonists, mecamylamine or MLA, affected firing rate or burst firing when given alone, it appears that even if acetylcholine is available, its concentration may be functionally insufficient under basal conditions to activate nAChRs. Thus, an acetylcholine-mediated activation of nAChRs may require higher levels of acetylcholine and rather be involved in phasic activations of dopaminergic neurons. In support of this notion, it has been shown that acetylcholine levels in the VTA increase in response to feeding and drinking (Rada *et al*, 2000). Moreover, electrical or chemical stimulation of the pedunculopontine and laterodorsal tegmental nuclei (PPTg and LdTg), the brainstem nuclei containing acetylcholinergic neurons that send afferents to the VTA, induces burst firing of midbrain dopaminergic cells (Lokwan *et al*, 1999; Floresco *et al*, 2003) and increases dopamine release in the nucleus accumbens, in part by activating nAChRs and NMDA receptors in the VTA (Forster and Blaha, 2000). Our data suggest that under conditions of high cholinergic activity, acetylcholine may activate $\alpha 7$ nAChRs located on glutamatergic terminals to enhance glutamate release. This mechanism is supported by our previous demonstration that local administration of MLA in the VTA reduces food-induced dopamine release in the nucleus accumbens (Schilström *et al*, 1998b). Moreover, a recent anatomical study demonstrated that $\alpha 7$ nAChRs in the VTA may be located presynaptically at sites indicative of a paracrine mode of signaling (Jones and Wonnacott, 2004).

In contrast to galantamine, donepezil did not activate dopamine cell firing. In fact, the higher dose produced a significant attenuation of firing rate. This finding is surprising since both mAChRs and nAChRs are present on dopaminergic neurons and stimulation of both has been shown to produce excitation (Calabresi *et al*, 1989; Lacey *et al*, 1990). The inhibitory effect of donepezil was blocked by the mAChR antagonist scopolamine, indicating that it is mediated by acetylcholine acting at mAChRs. Although most studies have reported excitatory effect of mAChR activation in the VTA (Lacey *et al*, 1990; Gronier and Rasmussen, 1998), some reports demonstrate inhibitory effects as well (Fiorillo and Williams, 2000; Zheng and Johnson, 2003). Another possibility could be that inhibition involving mAChRs occurs at the level of the LdTg. Increased levels of acetylcholine due to AChE inhibition in the LdTg might thus activate autoinhibitory mAChRs and, thereby, inhibit the activity of these cells (Luebke *et al*, 1993; Forster and Blaha, 2000) with a resulting decrease in the release of acetylcholine in the VTA. The fact that following the highest

dose of galantamine there was no significant effect on firing rate or prefrontal dopamine output may be related to a similar mechanism. At higher doses, galantamine is indeed an AChE inhibitor and therefore an mAChR-mediated inhibitory effect may counteract the stimulatory effects seen at lower doses. Another, slightly different explanation may be derived from the recent demonstration that donepezil can inhibit nAChR-mediated currents in dopaminergic cells independent of AChE inhibition (Di Angelantonio *et al*, 2004). Thus, the inhibitory effect mediated by mAChRs could be the result of reduced excitation rather than increased inhibition. Nevertheless, the fact that we observe an inhibitory effect on dopamine cell firing mediated by mAChRs is interesting and remains elusive.

Galantamine was particularly effective at increasing burst firing of dopaminergic cells. It is not known exactly how burst firing may be specifically related to cognitive functions in the prefrontal cortex but since phencyclidine (PCP), a drug that induces a schizophrenia-like syndrome including negative, positive, and cognitive symptoms (Javitt and Zukin, 1991), blocks burst firing of dopaminergic neurons (Svensson, 2000), burst firing is likely to play a critical role. Burst firing is unique in the sense that it results in larger amounts of dopamine release and it induces gene expression in postsynaptic neurons in a dopamine D1 receptor-dependent manner (Gonon and Buda, 1985; Chergui *et al*, 1996). Burst firing induced by nicotine, which can also enhance cognition (Levin and Simon, 1998), is largely dependent on activation of $\alpha 7$ nAChRs (Schilström *et al*, 2003), suggesting that galantamine may preferentially modulate $\alpha 7$ nAChRs. Galantamine is known to bind to the α -subunit of nAChRs (Schrattenholz *et al*, 1996) and, obviously, there are more binding sites for galantamine on the homopentameric $\alpha 7$ nAChRs (Couturier *et al*, 1990) than on the heteropentameric nAChRs which contain only two or three α -subunits (Anand *et al*, 1991; Cooper *et al*, 1991). This notion is supported by the observation that in HEK-293 cells galantamine appeared to potentiate $\alpha 7$ nAChR-mediated currents to a greater extent than $\alpha 4\beta 2$ nAChR-mediated currents (Maelicke *et al*, 2001). Thus, galantamine may not only possess the ability to selectively enhance endogenous acetylcholine transmission occurring at nAChRs but may also provide a preference for $\alpha 7$ nAChRs. Importantly, clinical and preclinical data indicate that $\alpha 7$ nAChRs are implicated in the pathophysiology of schizophrenia and that $\alpha 7$ agonists may provide a novel treatment alternative (Martin *et al*, 2004).

Even if our data clearly indicate that allosteric potentiation of nAChRs is the mechanism by which galantamine increases dopamine cell firing, and that $\alpha 7$ - as well as NMDA receptors are implicated, the definite location of the receptors involved remains to be determined. Galantamine was administered systemically and nAChRs in other parts of the brain may be activated and indirectly stimulate dopamine neurons. The notion that galantamine's effect may be mediated by nAChRs located in the VTA is, however, supported by our previous work, showing that the ability of systemic nicotine to enhance nerve terminal dopamine release is specifically antagonized by local administration of nAChR and NMDA receptor antagonists into the VTA (Nisell *et al*, 1994; Schilström *et al*, 1998a,b).

In conclusion, we have shown that galantamine, but not donepezil, increases the firing activity of dopaminergic cells in the VTA and, in parallel, enhances terminal dopamine efflux in the prefrontal cortex. This difference between galantamine and donepezil may be related to their reported differential effects in schizophrenia. Galantamine has been shown to improve negative and cognitive symptoms in schizophrenia while donepezil has not (Rosse and Deutsch, 2002; Allen and McEvoy, 2002; Bora et al, 2005; Friedman et al, 2003). As schizophrenia is associated with a very high prevalence of cigarette smoking (Hughes et al, 1986; Ziedonis et al, 1994), a phenomenon that has been suggested to represent an attempted self-medication with nicotine (Hughes et al, 1986; Svensson et al, 1990; Adler et al, 1993), an additional benefit with adjunctive galantamine treatment might be a reduced cigarette smoking in schizophrenia.

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