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The smoking cessation drug varenicline improves deficient P20–N40 inhibition in DBA/2 mice

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ABSTRACT

Varenicline, an FDA approved smoking cessation pharmacotherapy, is an α4β2* nicotinic acetylcholine receptor (nAChR) partial agonist and an α7* nAChR full agonist. Both subtypes of nAChR are involved in modulating auditory evoked responses in rodents. In DBA/2 mice, an inbred strain, auditory evoked responses to paired auditory stimuli fail to inhibit to the second stimulus. This mouse strain replicates the auditory evoked response inhibition deficit experienced by the majority of schizophrenia patients. In this current study, we examined the effects of five different doses of varenicline (0.06, 0.3, 0.6, 3 and 6 mg/kg) on auditory evoked responses in anesthetized DBA/2 mice. We also administered α4β2* and α7* nAChR selective antagonists prior to varenicline administration to determine which nAChR subtypes mediate the effects of varenicline. Four of the five doses of varenicline produced improvements in auditory evoked response inhibition deficits. Selective blockade of either the α4β2* or α7* nAChR in competition with 0.6 mg/kg varenicline prevented varenicline induced improvements. In competition with a higher dose of varenicline (3 mg/kg) only blockade of the α4β2* nAChR prevented varenicline induced improvement in auditory evoked response inhibition. These data indicate the importance of α4β2* nAChRs and the potential involvement of the α7* subtype in varenicline's effects on auditory evoked responses in DBA/2 mice.

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1. Introduction

The smoking cessation pharmacotherapy, varenicline, is currently the most successful FDA approved drug for achieving abstinence from smoking, as compared to placebo, bupropion and nicotine replacement therapies (Cahill et al., 2008; Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006; Tonstad et al., 2006; Williams et al., 2007). Varenicline is a partial agonist for heteromeric α4β2* nicotinic acetylcholine receptors (nAChRs) and full agonist for homomeric α7* nAChRs, relative to the endogenous nAChR ligand, acetylcholine (Mihalak et al., 2006; Papke et al., 2010). Varenicline also possesses efficacy for α3β2 and α3β4 heteromeric nAChRs, however, its potencies for these receptor subtypes are at least 14-fold less than for the α4β2* subtype (Mihalak et al., 2006; Papke et al., 2010). It is proposed that varenicline's clinical efficacy is via mesolimbic dopamine release following nAChR activation. Dopamine release in rodents subsequent to varenicline administration is proposed to occur due to varenicline's interaction with α4β2* nAChRs in the ventral tegmental area (Coe et al., 2005; Reperant et al., 2010; Rollema et al., 2007a). Varenicline induced dopamine release alleviates decreased levels of dopamine experienced during nicotine withdrawal. In addition, as an α4β2* partial agonist, varenicline inhibits additional dopamine release in the presence of nicotine, therefore reducing the reinforcing effects of nicotine (Coe et al., 2005; Cohen et al., 2003; Rollema et al., 2007b).

Varenicline is a compound of interest in our laboratory as both α7* and α4β2* nAChRs mediate aspects of sensory inhibition of auditory evoked responses, a measure of sensory processing (Adler et al., 1999; Radek et al., 2006; Stevens et al., 1996; Wildeboer and Stevens, 2008). Among persons diagnosed with schizophrenia, the majority experience deficits in the inhibition of repeated auditory evoked responses (Adler et al., 2004). These deficits manifest as a lack of inhibition of response to repeated auditory stimuli, such that persons report an inability to focus attention and experience a "flooding" of auditory stimuli (Venables, 1964). In humans, these auditory evoked responses are measured by electroencephalograms (EEGs) with a conditioning-test paradigm. Measured neuronal inhibition to repeated auditory stimuli originates in the hippocampus (Grunwald et al., 2003). In response to a pair of auditory stimuli, a positive voltage inflection, termed the P50 waveform, is observed in response to each stimuli. During normal inhibition, decreases in amplitude in response to the second (test) stimulus as compared to the amplitude of the first (conditioning) stimulus are observed. Deficient inhibition is defined as the ratio of P50 amplitudes (test/conditioning) with a value of 0.5 or greater (Freedman et al., 1997; Leonard et al., 1996). The ratios of test/conditioning amplitudes are referred to as T/C ratios. In humans this deficit has been genetically linked to chromosome 15 locus q13.3 (van Bon et al., 2009), the location

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of the α7 nAChR gene (CHRNA7). In post-mortem brain tissue from persons with schizophrenia, the level of α7* nAChR binding, as determined by radiolabeled alpha-bungarotoxin (BTX), is decreased compared to tissue from non-schizophrenia persons, as is the level of α4β2* nAChR binding, as determined by radiolabeled cytisine (Freedman et al., 1995).

In rodents, the P20–N40 waveform complex is analogous to the P50 waveform in humans (Hashimoto et al., 2005). The DBA/2 mouse, an inbred strain, models the auditory evoked response inhibition deficit experienced among persons diagnosed with schizophrenia (Stevens et al., 1996). The DBA/2 mouse has endogenously low levels of α7* nAChR binding as compared to other mouse strains, such as C57BL/6 with intermediate levels or C3H with levels higher than DBA/2 or C57BL/6 (Marks et al., 1989). Both C57BL/6 and C3H mice, with higher levels of α7 binding, exhibit normal auditory evoked responses whereas low levels of α7* binding observed for DBA/2 mice has been correlated with the auditory evoked response inhibition deficit (Stevens et al., 1996). In both humans and rodents expressing the phenotype for deficient inhibitory auditory evoked responses, nicotine transiently improves the deficit (Adler et al., 1993; Stevens and Wear, 1997), as does the selective α7* nAChR partial agonist, DMXB-A (Olincy et al., 2006; Stevens et al., 1998). Rodent studies also suggest a role for the α4β2* nAChR in modulation of auditory evoked responses (Radek et al., 2006; Rudnick et al., 2009). Agonists for α4β2* nAChRs produce improvements in the deficient phenotype (Stevens and Wear, 1997; Wildeboer and Stevens, 2008). It had been previously determined that DMXB-A produces improvements in the auditory evoked response inhibition deficit of DBA/2 mice by decreasing the amplitude of the test response (Simosky et al., 2001; Stevens et al., 1998), whereas an agonist for α4β2*, 5-I A-85380, produces improvements by increasing the amplitude of the conditioning response (Wildeboer and Stevens, 2008). Thus, varenicline, with its dual receptor agonist profile, was tested for its potential impact in our DBA/2 mouse model for deficient inhibition of repeated auditory evoked responses. In this study, we examined the effect of varenicline in male DBA/2 mice alone and following pre-administration of selective α4β2* or α7* nAChR antagonists. Based on previously published literature of varenicline's interaction with α4β2* nAChRs (Coe et al., 2005; Mihalak et al., 2006; Papke et al., 2010; Reperant et al., 2010; Rollema et al., 2007a), we hypothesized that varenicline would transiently improve DBA/2 deficient auditory evoked response inhibition and that the effects would be produced primarily via α4β2* nAChRs.

2. Materials and methods

2.1. Animals

Male DBA/2 mice (20–25 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed five to a cage in ventilated cage racks at the Center for Comparative Medicine at the University of Colorado Denver, School of Medicine (UCD-SOM). The mice were provided water and food (Harlan Teklad, Indianapolis, IN) ad libitum. Lighting was cycled at 12 hour intervals with lights on at 6:00 AM. All animal procedures were approved by the Institutional Animal Care and Use Committee of UCD-SOM and conform to the Principles of Laboratory Animal Care (Institute of Laboratory Animal Resources, 1996).

2.2. Surgery

For electrophysiological recording of auditory evoked responses, mice were anesthetized by intraperitoneal (IP) injection of the anesthetic chloral hydrate (400 mg/kg) followed by an injection (IP) of pyrazole (400 mg/kg) to retard the metabolism of chloral hydrate. Once a surgical plane of anesthesia was attained, mice were placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA) on a heating pad (35°C) to maintain a stable core temperature. Hollow earbars, attached to miniature earphones connected to an audio amplifier, were placed adjacent to the externalization of the aural canal. During recording, chloral hydrate and pyrazole were supplemented as necessary (5 mg/kg, IP) to maintain a surgical plane of anesthesia as evidenced by lack of reflexive limb withdrawal in response to toe pinch.

The scalp was incised and burr holes drilled over the dorsal CA3 region of the hippocampus [1.8 mm posterior from bregma, 2.5 mm lateral from midline] (Paxinos and Franklin, 2001) and the contralateral anterior cortex. The recording electrode, a Teflon-coated stainless-steel cut wire (0.127 mm diameter), was inserted into the CA3 pyramidal cell layer of the hippocampus (1.5 to 1.7 mm ventral from the dorsal brain surface). Final placement of the recording electrode was determined by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller and Freedman, 1995). The reference electrode, identical in composition to the recording electrode, was placed on dura through the burr hole over the contralateral cortex. Antagonist experiments involving dihydro-β-erythroidine (DHβE) or α-bungarotoxin (BTX) required a third burr hole to be drilled over the anterior lateral ventricle (0.8 mm anterior from bregma, 0.5 mm lateral from midline (Paxinos and Franklin, 2001)) ipsilateral to the recording of electrode for placement of an injection cannula. A 26-gage needle attached to a 10 μl Hamilton syringe (Hamilton, Reno, NV) was inserted into the anterior lateral ventricle (2.0 mm below the dura) for intracerebroventricular (ICV) administration of antagonist.

2.3. Experimental protocol

Tones (3000 Hz, 10 ms, 70 dB), as the auditory stimuli, were presented in pairs separated by a 500-millisecond interval with 10-seconds between pairs of stimuli. Responses to 16 pairs of tones were filtered with a bandpass between 10 and 5000 Hz. The N40 wave was defined as the maximum negativity between 20 and 60-milliseconds after stimulus onset and measured relative to the proceeding positivity, the P20 wave. The measure of the animals' auditory evoked response inhibition is reported as a T/C ratio. The T/C ratio is defined as the ratio of the amplitudes of the response to the second tone, the test amplitude, to the response to the first tone, the conditioning amplitude. A decrease in T/C ratio after drug administration, as compared to pre-drug baseline indicates improved inhibition of auditory processing. Five baseline records, at 5-minute intervals, were obtained prior to compound administration. Previous studies in our lab confirmed that vehicle control injections had no impact upon T/C ratios in DBA/2 mice (Hashimoto et al., 2005; Stevens and Wear, 1997). Electrical responses were amplified 1000 times with analog to digital conversion (SciWorks, DataWave, Loveland, CO) and averaged by computer. Data were collected, stored and analyzed with the SciWorks computer program (DataWave, Loveland, CO).

Varenicline was dissolved in 0.9% NaCl and administered IP at five doses (0.06 mg/kg, $n=6$; 0.3 mg/kg, $n=11$; 0.6 mg/kg, $n=12$; 3 mg/kg, $n=11$; 6 mg/kg, $n=11$). After injection, recordings continued for up to 40 min, at 5 minute intervals.

For antagonist experiments, either 27 nM DHβE or 1.25 nM BTX was administered at a volume of 1 μl into the anterior lateral ventricle following baseline recordings (Simosky et al., 2003). After injection of antagonist, four records at 5-minute intervals were obtained to verify that the antagonist alone did not affect the test amplitude, the conditioning amplitude or T/C ratio. After these four records of antagonist alone, an injection of either 0.6 mg/kg or 3 mg/kg IP varenicline was administered. Records at 5-minute intervals were obtained for 40-minutes of records, thereafter.

2.4. Compounds

Varenicline tartrate was graciously provided by Pfizer (Groton, CT), BTX and DHβE hydrobromide were obtained from Tocris (Ellisville,

MO). Varenicline, DHβE and BTX doses were calculated as free base weight. All compounds were dissolved in 0.9% NaCl.

2.5. Statistical analysis

The time course of varenicline alone or in conjunction with antagonist was analyzed, for each dose, using repeated measures MANOVA. A significant p-value ($p<0.05$) obtained from a MANOVA was followed by Fisher's protected least-significant difference (PLSD) a posteriori analysis to compare individual post-injection time points to collapsed average baseline values.

3. Results

Varenicline was administered to DBA/2 mice at five different doses (0.06, 0.3, 0.6, 3 and 6 mg/kg). All but one of the doses tested (0.06 mg/kg) produced improvements in inhibitory processing of the P20–N40 auditory evoked response. The improvements were evidenced by significant decreases in the T/C ratios (Fig. 1), defined as the ratio of amplitudes of the evoked potential of the test response to that of the conditioning response (Fig. 2). Of the five doses tested, 0.3, 0.6, 3 and 6 mg/kg IP produced significant changes on T/C ratio [0.3 mg/kg: $F_{(13,130)}=2.27$, $p=0.010$; 0.6 mg/kg: $F_{(13,143)}=3.22$, $p<0.001$; 3 mg/kg:$F_{(13,130)}=3.26$, $p<0.001$; 6 mg/kg: $F_{(13,130)}=2.98$, $p=0.001$]. Fisher's PLSD a posteriori analysis revealed multiple time points, at each dose, with significant decreases in T/C ratio, at least 10-min following varenicline administration, relative to the average of baseline measurements (Fig. 1).

Analyses of the conditioning and test amplitudes indicated that there were significant changes in both parameters, depending on the dose of varenicline administered (Fig. 1). At the second lowest dose, 0.3 mg/kg (IP), there was no statistically significant effect on either the conditioning or test amplitudes. However, the conditioning amplitude did trend towards an increase as compared to baseline following varenicline administration [$F_{(13,130)}=1.76$, $p=0.056$]. The 0.6 mg/kg dose produced a significant effect on the test amplitude [$F_{(13,143)}=2.24$, $p=0.011$]. Fisher's PLSD a posteriori analysis revealed significant decrease in the test amplitude at the 10 and 15 minute time points following varenicline administration. There was no significant effect on the conditioning amplitude at this dose. The 3 mg/kg dose of varenicline produced no effect on the test amplitude whereas there was a significant effect on conditioning amplitude [$F_{(13,130)}=2.88$, $p=0.001$]. Fisher's PLSD a posteriori analysis showed that the 5 and 10 minute time points following varenicline administration were significantly increased. The highest dose of varenicline tested, 6 mg/kg (IP), produced no significant effect on the test amplitude but again significantly impacted the conditioning amplitude [$F_{(13,130)}=3.02$, $p=0.001$]. Fisher's PLSD a posteriori analysis detected significant increases in conditioning amplitude at the 15, 25, 30 and 40 minute time points following varenicline administration.

Although varenicline is proposed to work via the α4β2* nAChR subtype, it also has agonist activity at the α7* subtype (Mihalak et al., 2006; Papke et al., 2010). In order to determine the relative impact of activation of either α4β2* or α7* nAChRs following varenicline administration, selective antagonists for either receptor subtype were centrally administered 20 min prior to the injection of varenicline but following five baseline recordings. Interestingly, antagonism of the α7* subtype by BTX (1.25 nM, ICV) also blocked varenicline (0.6 mg/ml) induced improvement in T/C ratio compared to administration of varenicline alone (Fig. 3). Blockade of the α4β2* subtype by DHβE (27 nM, ICV) prevented significant decreases in T/C ratio following varenicline administration (0.6 mg/kg, IP) (Fig. 4). Antagonism of each receptor subtype was also examined in competition with 3 mg/kg varenicline in order to determine a possible concentration dependent mechanism of varenicline at each receptor subtype. At the 3 mg/kg dose of varenicline, the α7* receptor antagonist (BTX) failed to prevent varenicline induced significant decreases in T/C ratio (Fig. 5). Antagonism of the α4β2* subtype by DHβE (27 nM, ICV) prevented significant decreases in T/C ratio following 3 mg/kg varenicline administration (Fig. 6), similar to the 0.6 mg/kg varenicline dose (Fig. 4). There were no significant changes in either the test or conditioning amplitude following antagonist plus varenicline administration for either BTX or DHβE (Figs. 3, 4, 5 and 6).

4. Discussion

The findings of our study indicate increased inhibition of auditory evoked responses to repeated auditory stimuli and overall improvement of P20–N40 inhibition in DBA/2 mice with administration of varenicline. Auditory evoked response inhibition, as a measure of sensory processing, is proposed to be a pre-attentional aspect of early sensory processing (Rollema et al., 2009) or the habituation of the auditory evoked responses to repeated stimuli (Rudnick et al., 2010). In rats expressing normal auditory evoked responses, varenicline does not impact P20–N40 inhibition (Rollema et al., 2009). However, in rats with amphetamine induced deficits in P20–N40 inhibition, varenicline transiently reduced the deficit recorded in the CA3 region of the hippocampus, indicating modulation of auditory evoked responses via α4β2* nAChRs (Rollema et al., 2009). Our results in DBA/2 mice are consistent with the previous finding that varenicline improves P20–N40 inhibition in a deficient model.

Whereas previous reports indicate a significant role of α4β2* nAChRs in varenicline's mechanism of action (Rollema et al., 2009; Rudnick et al., 2010), our results suggest a possible role for α7* nAChRs in varenicline induced improvement of deficient P20–N40 inhibition, via modulation of the test amplitude, of DBA/2 mice. At 0.6 mg/kg, varenicline significantly decreased the test amplitude with no significant effect on the conditioning amplitude (Fig. 1). A significant effect on the test amplitude is thought to be mediated via α7* nAChRs (Stevens et al., 1998) whereas a significant effect on the conditioning amplitude is proposed to occur via α4β2* nAChRs (Radek et al., 2006; Wildeboer and Stevens, 2008). Thus, varenicline may be activating α7* nAChRs at 0.6 mg/kg in our DBA/2 mouse model. Although varenicline is a full agonist for α7* nAChRs, previous studies quantify the affinity of varenicline to be much greater for the α4β2* subtype as compared to the α7* subtype (Rollema et al., 2009). In addition, the functional EC_{50} value of varenicline is higher for α7* than for α4β2* (Mihalak et al., 2006). A recent study examined the effect of varenicline on mouse α4β2 and α7 nAChRs expressed in *Xenopus* oocytes. The EC_{50} of varenicline for the C57BL/6 mouse α7 was 0.8 μM as compared to 2.6 μM for C57BL/6 α4β2 (Papke et al., 2010). These results suggest that α7 nAChRs may be functionally activated at a lower dose of varenicline than α4β2 in mice. However, it should be noted that the EC_{50} value determinations of varenicline on mouse nAChRs were performed on C57BL/6 mouse α4 subunits expressed in conjunction with β2 nAChR subunits in *Xenopus* oocytes (Papke et al., 2010). This mouse strain expresses the more common functional genetic variant of the α4 nAChR subunit, a threonine at position 529 rather than an alanine (DBA/2 mice) (Dobelis et al., 2002; Kim et al., 2003; Stitzel et al., 2001). Because the functional responses of these two variants differ, it is possible that the functional response of DBA/2 mice to varenicline may differ from those of C57BL/6 mice.

Previously, the effect of varenicline on auditory evoked responses of C57BL/6 mice was determined to produce an overall increase in P20 amplitude in response to both the first and second auditory stimuli (Rudnick et al., 2010). This study examined only the peak response, P20, and not the entire P20–N40 waveform. Because they observed increased P20 amplitudes to both the first and second stimuli, they did not observe an effect on sensory inhibition. Our results demonstrate that varenicline produces a significant decrease in T/C ratio, indicating an improvement in P20–N40 inhibition. Differences in outcome between the current and former study are most likely due to several factors, including the

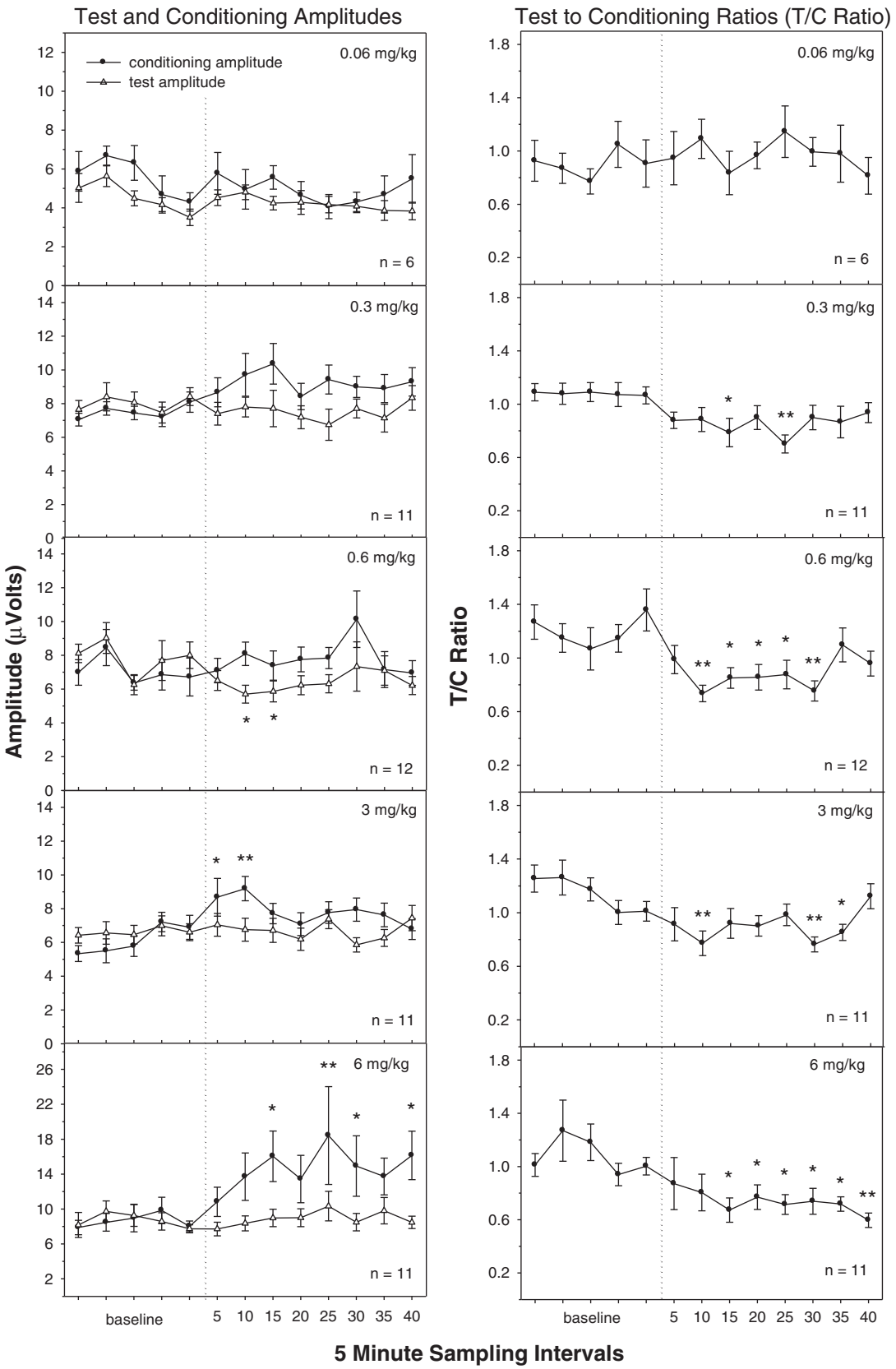

Fig. 1. Conditioning and test amplitudes (left) and T/C ratios (right) before and after varenicline administration at four different doses. Varenicline injection (IP) occurred at the dotted line. Records were obtained at 5 minute intervals following injection. Varenicline produced improvements in P20–N40 inhibition at each dose tested as indicated by the significant decreases in T/C ratios (left) as compared to baseline values. Asterisks indicate time points following varenicline administration where a significant effect, as determined by Fisher's PLSD, on either the conditioning amplitude (upper asterisks) or test amplitude (lower asterisks) were found as compared to an averaged baseline for the corresponding dose. Data are mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$.

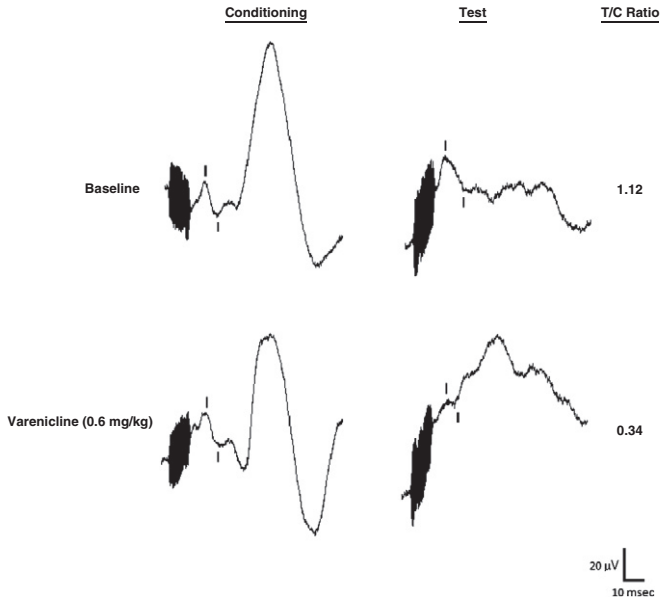

Fig. 2. Representative P20–N40 waveforms averaged from an individual DBA/2 mouse. Waveforms obtained prior to drug administration (Baseline) and after 0.6 mg/kg varenicline administration. Varenicline administration (0.6 mg/kg, IP) resulted in a decreased T/C ratio indicating an improvement in P20–N40 inhibition. Tick marks denote the waveform and arrows indicate audio stimulus onset. The noise at the start of the waveform represents the audio stimulus artifact. Calibration 20 μV/10 ms.

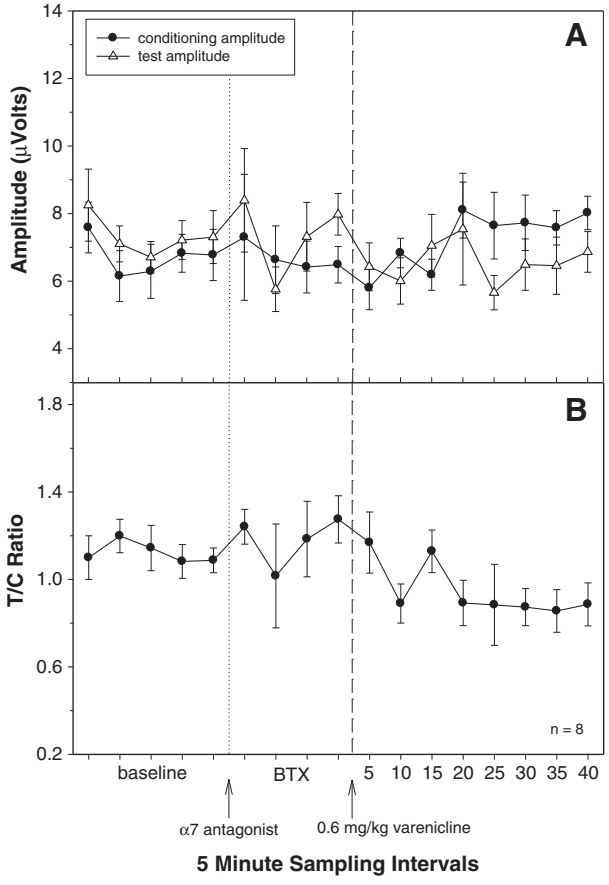

Fig. 3. Inhibition of varenicline (0.6 mg/kg, IP) induced improvement of P20–N40 inhibition by BTX (1 μl of 1.25 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to varenicline injection (0.6 mg/kg, IP). Injection of the α7 antagonist BTX (dotted line) occurred 20 min prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean ± SEM; $n = 8$.

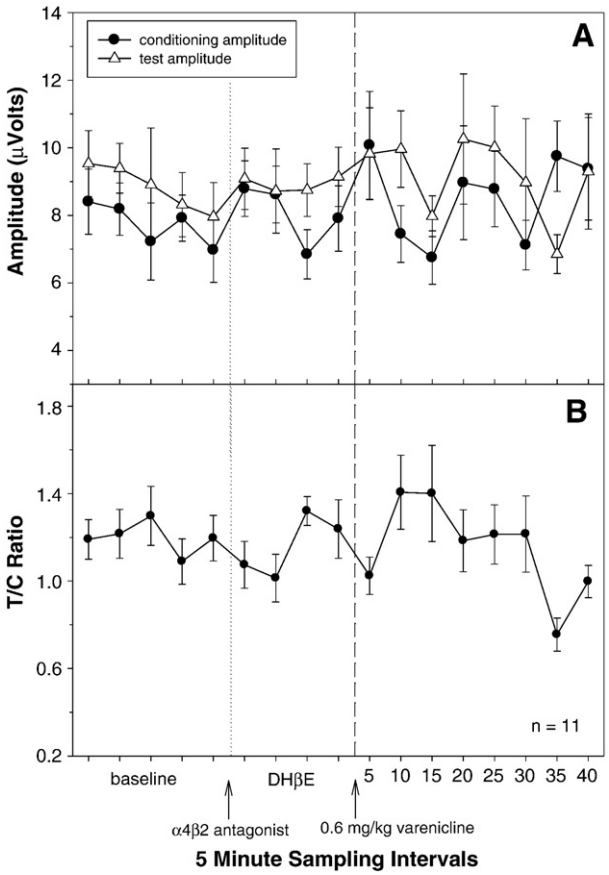

Fig. 4. Inhibition of varenicline (0.6 mg/kg, IP) induced improvement of P20–N40 inhibition by DHβE (1 μl of 27 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to drug injection. Injection of the α4β2 antagonist DHβE (dotted line) occurred 20 min prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean ± SEM; $n = 11$.

difference in mouse strains, and differences in recording and analysis methods. Our study examined an anesthetized P20–N40 deficient model whereas the former study examined a model of normal auditory evoked response in the non-anesthetized state. The present study assessed the full P20–N40 wave complex, which has been found to be less variable than either individual component (Hashimoto et al., 2005), while the Rudnick et al. (2010) study only assessed the P20 component.

In order to further examine the involvement of α7* and α4β2* nAChRs in varenicline's action on P20–N40 inhibition, we administered selective antagonists prior to administration of varenicline. Selective antagonists for either α7* or α4β2* have been reported to produce no effects on the test or conditioning amplitudes or T/C ratio in DBA/2 mice (Simosky et al., 2003). In competition with 0.6 mg/kg varenicline, both α7* and α4β2* selective antagonists independently prevented any improvements in P20–N40 inhibition, as noted by the lack of significant decrease of T/C ratio (Figs. 3 and 4). The antagonist data indicates that at 0.6 mg/kg varenicline, antagonism of either α7* or α4β2* nAChRs prevents varenicline induced improvements in P20–N40 inhibition in DBA/2 mice. This data suggests that in a deficient P20–N40 endophenotype model, both receptor subtypes may be involved in the effect of varenicline at a low dose (0.6 mg/kg). In competition with 3 mg/kg varenicline, only the α4β2* selective antagonist prevented improvement in P20–N40 inhibition (Fig. 6). The α7* selective antagonist failed to prevent improvement in P20–N40 inhibition following 3 mg/kg varenicline administration, as noted by significant decreases of T/C ratios (Fig. 5). These results indicate a possible dose dependent activation of either receptor subtype by varenicline, such that α4β2* nAChRs are involved in varenicline induced responses at higher doses

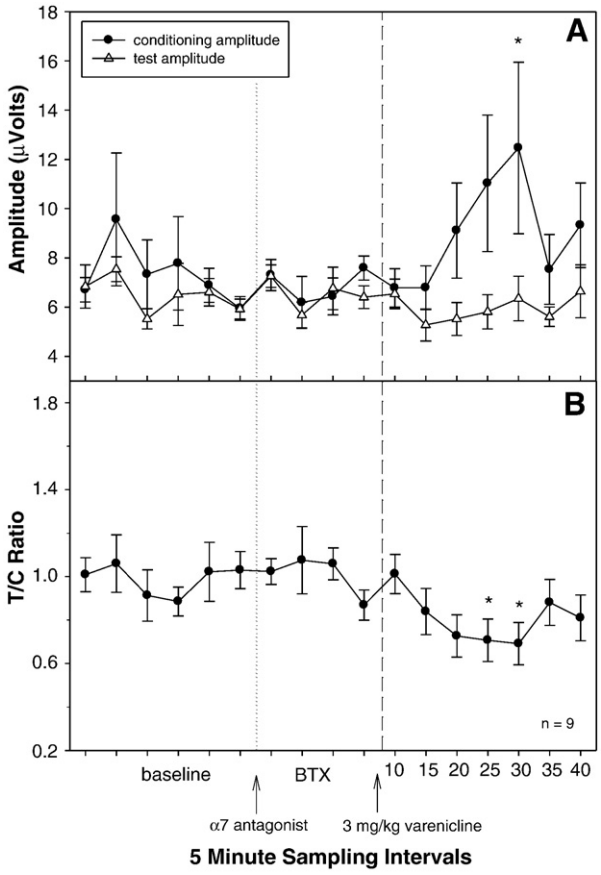

Fig. 5. Inhibition of varenicline (3 mg/kg, IP) induced improvement of P20–N40 inhibition by BTX (1 μl of 1.25 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to varenicline injection. Injection of the α7 antagonist BTX (dotted line) occurred 20 min prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean ± SEM; $n = 9$.

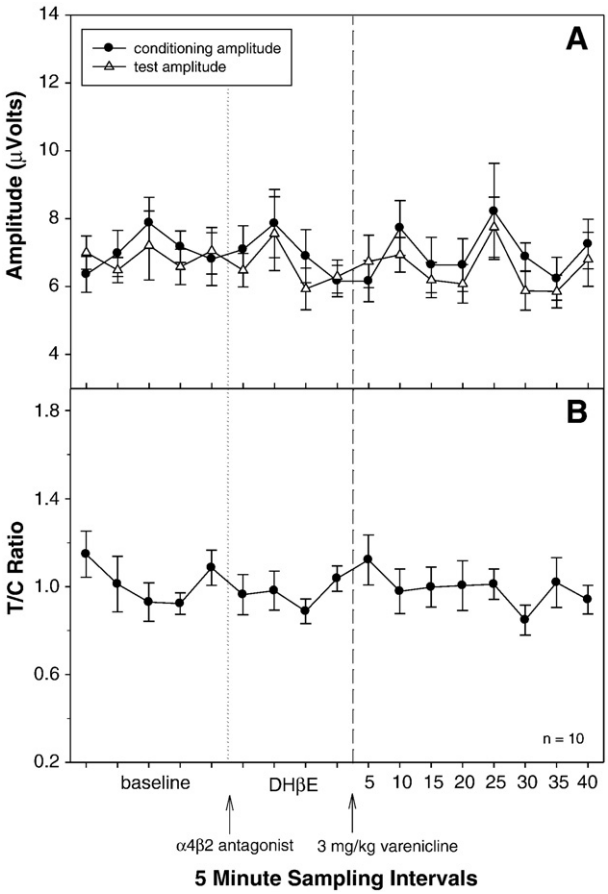

Fig. 6. Inhibition of varenicline (3 mg/kg, IP) induced improvement of P20–N40 inhibition by DHβE (1 μl of 27 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to drug injection. Injection of the α4β2 antagonist DHβE (dotted line) occurred 20 min prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean ± SEM; $n = 10$.

and α7* nAChRs may be involved in mediating responses to lower doses of varenicline.

The effect of varenicline on T/C ratio was relatively long lasting with effects observed 25–40 min following administration. At 6 mg/kg varenicline, it is possible that other nAChR subtypes, including α3β4, are being activated. It is also possible that at the higher varenicline doses, or at the later time points measured, α4β2* and α7* nAChRs are desensitized. Both a brief exposure to a high concentration of agonist as well as prolonged exposure of a nicotinic agonist can lead to desensitization of the receptors (Colquhoun and Ogden, 1988; Papke and Thinschmidt, 1998; Fenster et al., 1999; Gentry and Lukas, 2002; Katz and Thesleff, 1957; Vann et al., 2006). Desensitization of nAChRs, as one conformational state of the protein, may present as a lack of inhibition to repetitive auditory stimuli in our paradigm. This would be reflected as an increase in T/C ratio. Although there are significant decreases in T/C ratio at later time points for the 3 and 6 mg/kg doses, we cannot rule out downstream processes following nAChR activation and desensitization. It is possible that dopamine release, following varenicline induced activation of nAChRs on dopaminergic neurons, could also be modulating auditory evoked responses (Coe et al., 2005; Reperant et al., 2010; Rollema et al., 2007a).

Whereas activation of α7* nAChRs result in inhibition to the second auditory stimulus (Stevens et al., 1996), activation of α4β2* may increase a pre-attentional state with the increase of the conditioning amplitude following the first auditory stimulus. The α4β2* subtype has been observed in the molecular layer of the dentate gyrus (Clarke et al., 1985; Pauly et al., 1989). The mossy fiber axons of dentate granule cells synapse onto both pyramidal neurons and interneurons in the CA3 region of the hippocampus (Henze et al., 2000). Therefore, it is possible that varenicline acting on α4β2* nAChRs within the dentate gyrus, may alter conditioning amplitude responses within the hippocampal CA3 region. In addition, α4β2* nAChRs on neurons within the VTA, which projects diffusely to the dentate gyrus (Clarke et al., 1985; Swanson et al., 1987; Pauly et al., 1989) may cause activation of neurons in dentate gyrus resulting in increase of the conditioning amplitude. The α7* subtype are reported to be located on inhibitory interneurons in various regions of the hippocampus as well as in the dentate gyrus, which are involved in the sensory inhibition response (Gray et al., 1996; Alkondon et al., 1999). Activation of this subtype, by varenicline or other agonists, ultimately inhibits pyramidal neuron response to repeated auditory stimuli, via GABA release which activates GABA receptors on excitatory inputs to pyramidal neurons (Alkondon et al., 1999; Alkondon and Albuquerque, 2001). The inhibition of pyramidal neuron response to repetitive auditory stimuli, following α7* nAChR activation, is reflected in decreased test amplitudes.

There are possible limitations for the use of varenicline in persons with a diagnosed mental illness. Recent reports reveal a potential for a varenicline-induced exacerbation of psychiatric symptoms in patients with a diagnosed mental illness, including major depressive disorder, depression, bipolar disorder, attention deficit hyperactivity disorder, post-traumatic stress disorder and schizophrenia. The side effects produced in this population include visual hallucinations, mania, psychosis, agitation, anxiety, depression and suicidal ideation (Alhatem and Black, 2009; DiPaula and Thomas, 2009; Freedman, 2007; Kohen and Kremen, 2007; Morstad et al., 2008; Popkin, 2008; Pumariega et al.,

2008; Raidoo and Kutscher, 2009; Spirling et al., 2008). In contrast to these cases, other published reports indicate no exacerbation of psychiatric symptoms in persons with a diagnosed mental illness (Evins and Goff, 2008; Fatemi, 2008; McClure et al., 2010; Ochoa, 2009).

5. Conclusions

In conclusion, our study provides support for the involvement of $\alpha4\beta2^*$ nAChRs, and a possible role for the $\alpha7^*$ subtype, in varenicline induced improvement of P20–N40 inhibition in DBA/2 mice. Whereas prior studies examined the importance of β2-containing nAChRs in varenicline induced improvements on normal sensory inhibition, our study examined mice deficient in sensory inhibition and also determined that the functional activity of varenicline on $\alpha4\beta2^*$ nAChRs produces improvements in sensory inhibition. This study is the first to administer selective nAChR antagonists prior to varenicline administration to examine specific involvement of $\alpha4\beta2^*$ and $\alpha7^*$ nAChRs in the effect of varenicline on P20–N40 inhibition. Because of the genetic association between the $\alpha7^*$ nAChR and sensory inhibition in persons with schizophrenia, it is important to understand the effect of this drug in the DBA/2 mouse model.

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References

Adler LE, Freedman R, Ross RG, Olincy A, Waldo MC. Elementary phenotypes in the neurobiological and genetic study of schizophrenia. Biol Psychiatry 1999;46: 8–18.
Adler LE, Hoffer LD, Wiser A, Freedman R. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. Am J Psychiatry 1993;150:1856–61.
Adler LE, Olincy A, Cawthra EM, McRae KA, Harris JG, Nagamoto HT, et al. Varied effects of atypical neuroleptics on P50 auditory gating in schizophrenia patients. Am J Psychiatry 2004;161:1822–8.
Alhatem F, Black JE. Varenicline-induced mania in a bipolar patient. Clin Neuropharmacol 2009;32:117–8.
Alkondon M, Albuquerque EX. Nicotinic acetylcholine receptor alpha7 and alpha4beta2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. J Neurophysiol 2001;86:3043–55.
Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. J Neurosci 1999;19:2693–705.
Cahill K, Stead LF, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane database of systematic reviews (Online); 2008. CD006103.
Clarke PBR, Schwartz D, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [3H]acetylcholine, [3H]nicotine, and [125I]-alpha-bungarotoxin. J Neurosci 1985;5:1307–15.
Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. J Med Chem 2005;48:3474–7.
Cohen C, Bergis OE, Galli F, Lochead AW, Jegham S, Biton B, et al. SSR591813, a novel selective and partial alpha4beta2 nicotinic receptor agonist with potential as an aid to smoking cessation. J Pharmacol Exp Ther 2003;306:407–20.
Colquhoun D, Ogden DC. Activation of ion channels in the frog end-plate by high concentrations of acetylcholine. J Physiol 1988;395:131–59.
DiPaula BA, Thomas MD. Worsening psychosis induced by varenicline in a hospitalized psychiatric patient. Pharmacotherapy 2009;29:852–7.
Dobelis P, Marks MJ, Whiteaker P, Balogh SA, Collins AC, Stitzel JA. A polymorphism in the mouse neuronal alpha4 nicotinic receptor subunit results in an alteration in receptor function. Mol Pharmacol 2002;62:334–42.
Evins AE, Goff DC. Varenicline treatment for smokers with schizophrenia: a case series. J Clin Psychiatry 2008;69:1016.
Fatemi SH. Varenicline efficacy and tolerability in a subject with schizophrenia. Schizophr Res 2008;103:328–9.
Fenster C, Beckman ML, Parker JC, Sheffield EB, Whitworth TL, Quick MW, et al. Regulation of alpha4beta2 nicotinic receptor desensitization by calcium and protein kinase C. Mol Pharmacol 1999;55:432–43.
Freedman R. Exacerbation of schizophrenia by varenicline. Am J Psychiatry 2007;164: 1269.
Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A, et al. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci USA 1997;94:587–92.
Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biol Psychiatry 1995;38:22–33.
Gentry CL, Lukas RJ. Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. Curr Drug Targets CNS Neurol Disord 2002;1:359–85.
Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 2006;296:47–55.
Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA. Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature 1996;383:713–6.
Grunwald T, Boutros NN, Pezer N, von Oertzen J, Fernandez G, Schaller C, et al. Neuronal substrates of sensory gating within the human brain. Biol Psychiatry 2003;53:511–9.
Hashimoto K, Iyo M, Freedman R, Stevens KE. Tropisetron improves deficient inhibitory auditory processing in DBA/2 mice: role of alpha 7 nicotinic acetylcholine receptors. Psychopharmacology 2005;183:13–9.
Henze DA, Urban NN, Barrionuevo G. The multifarious hippocampal mossy fiber pathway: a review. Neuroscience 2000;98:407–27.
Institute of Laboratory Animal Resources. CoLS, National Research Council. Guide for the care and use of laboratory animals. Washington, D.C: National Academy Press; 1996.
Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, et al. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA 2006;296:56–63.
Katz B, Thesleff S. A study of the desensitization produced by acetylcholine at the motor end-plate. J Physiol 1957;138:63–80.
Kim H, Flanagin BA, Qin C, Macdonald RL, Stitzel JA. The mouse Chrna4 A529T polymorphism alters the ratio of high to low affinity alpha 4 beta 2 nAChRs. Neuropharmacology 2003;45:345–54.
Kohen I, Kremen N. Varenicline-induced manic episode in a patient with bipolar disorder. Am J Psychiatry 2007;164:1269–70.
Leonard S, Adams C, Breese CR, Adler LE, Bickford P, Byerley W, et al. Nicotinic receptor function in schizophrenia. Schizophr Bull 1996;22:431–45.
Marks MJ, Romm E, Campbell SM, Collins AC. Variation of nicotinic binding sites among inbred strains. Pharmacol Biochem Behav 1989;33:679–89.
McClure JB, Swan GE, Catz SL, Jack L, Javitz H, McAfee T, et al. Smoking outcome by psychiatric history after behavioral and varenicline treatment. J Subst Abuse Treat 2010;38:394–402.
Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Mol Pharmacol 2006;70:801–5.
Miller CL, Freedman R. The activity of hippocampal interneurons and pyramidal cells during the response of the hippocampus to repeated auditory stimuli. Neuroscience 1995;69:371–81.
Morstad AE, Kutscher EC, Kennedy WK, Carnahan RM. Hypomania with agitation associated with varenicline use in bipolar II disorder. Ann Pharmacother 2008;42: 288–9.
Nides M, Oncken C, Gonzales D, Rennard S, Watsky EJ, Anziano R, et al. Smoking cessation with varenicline, a selective alpha4beta2 nicotinic receptor partial agonist: results from a 7-week, randomized, placebo- and bupropion-controlled trial with 1-year follow-up. Arch Intern Med 2006;166:1561–8.
Ochoa EL. Varenicline reduced smoking behaviour in a mentally ill person. J Psychopharmacol 2009;23:340–1.
Olincy A, Harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, et al. Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. Arch Gen Psychiatry 2006;63: 630–8.
Oncken C, Gonzales D, Nides M, Rennard S, Watsky E, Billing CB, et al. Efficacy and safety of the novel selective nicotinic acetylcholine receptor partial agonist, varenicline, for smoking cessation. Arch Intern Med 2006;166:1571–7.
Papke RL, Thinschmidt JS. The correction of alpha7 nicotinic acetylcholine receptor concentration–response relationships in *Xenopus oocytes*. Neurosci Lett 1998;256: 163–6.
Papke RL, Wecker L, Stitzel JA. Activation and inhibition of mouse muscle and neuronal nicotinic acetylcholine receptors expressed in *Xenopus oocytes*. J Pharmacol Exp Ther 2010;333:501–18.
Pauly JR, Stitzel JA, Marks MJ, Collins AC. An autoradiographic analysis of choinergic receptors in mouse brain. Brain Res Bull 1989;22:453–9.
Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2001.
Popkin MK. Exacerbation of recurrent depression as a result of treatment with varenicline. Am J Psychiatry 2008;165:774.
Pumariega AJ, Nelson R, Rotenberg L. Varenicline-induced mixed mood and psychotic episode in a patient with a past history of depression. CNS Spectr 2008;13:511–4.
Radek RJ, Miner HM, Bratcher NA, Decker MW, Gopalakrishnan M, Bitner RS. Alpha4beta2 nicotinic receptor stimulation contributes to the effects of nicotine in the DBA/2 mouse model of sensory gating. Psychopharmacology 2006;187: 47–55.
Raidoo BM, Kutscher EC. Visual hallucinations associated with varenicline: a case report. J Med Case Rep 2009;3:7560.
Reperant C, Pons S, Dufour E, Rollema H, Gardier AM, Maskos U. Effect of the alpha4beta2* nicotinic acetylcholine receptor partial agonist varenicline on dopamine release in beta2 knock-out mice with selective re-expression of the beta2 subunit in the ventral tegmental area. Neuropharmacology 2010;58:346–50.

Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, et al. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology 2007a;52:985–94.
Rollema H, Coe JW, Chambers LK, Hurst RS, Stahl SM, Williams KE. Rationale, pharmacology and clinical efficacy of partial agonists of alpha4beta2 nACh receptors for smoking cessation. Trends Pharmacol Sci 2007b;28:316–25.
Rollema H, Hajos M, Seymour PA, Kozak R, Majchrzak MJ, Guanowsky V, et al. Preclinical pharmacology of the alpha4beta2 nAChR partial agonist varenicline related to effects on reward, mood and cognition. Biochem Pharmacol 2009;78:813–24.
Rudnick ND, Koehler C, Picciotto MR, Siegel SJ. Role of beta2-containing nicotinic acetylcholine receptors in auditory event-related potentials. Psychopharmacology 2009;202:745–51.
Rudnick ND, Strasser AA, Phillips JM, Jepson C, Patterson F, Frey JM, et al. Mouse model predicts effects of smoking and varenicline on event-related potentials in humans. Nicotine Tob Res 2010;12:589–97.
Simosky JK, Stevens KE, Adler LE, Freedman R. Clozapine improves deficient inhibitory auditory processing in DBA/2 mice, via a nicotinic cholinergic mechanism. Psychopharmacology 2003;165:386–96.
Simosky JK, Stevens KE, Kem WR, Freedman R. Intragastric DMXB-A, an alpha7 nicotinic agonist, improves deficient sensory inhibition in DBA/2 mice. Biol Psychiatry 2001;50:493–500.
Spirling LI, Stapleton JA, Sutherland G. Suicidal ideation and varenicline: a possible case of mistaken adverse drug reaction? J Clin Psychiatry 2008;69:1834–5.
Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks MJ, et al. Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. Neuropsychopharmacology 1996;15:152–62.
Stevens KE, Kem WR, Mahnir VM, Freedman R. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. Psychopharmacology 1998;136:320–7.
Stevens KE, Wear KD. Normalizing effects of nicotine and a novel nicotinic agonist on hippocampal auditory gating in two animal models. Pharmacol Biochem Behav 1997;57:869–74.
Stitzel JA, Dobelis P, Jimenez M, Collins AC. Long sleep and short sleep mice differ in nicotine-stimulated 86Rb + efflux and alpha4 nicotinic receptor subunit cDNA sequence. Pharmacogenetics 2001;11:331–9.
Swanson LW, Simmons DM, Whiting PJ, Lindstrom J. Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. J Neurosci 1987;7:3334–42.
Tonstad S, Tonnesen P, Hajek P, Williams KE, Billing CB, Reeves KR. Effect of maintenance therapy with varenicline on smoking cessation: a randomized controlled trial. JAMA 2006;296:64–71.
van Bon BW, Mefford HC, Menten B, Koolen DA, Sharp AJ, Nillesen WM, et al. Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. J Med Genet 2009;46:511–23.
Vann RE, James JR, Rosecrans JA, Robinson SE. Nicotinic receptor inactivation after acute and repeated in vivo nicotine exposures in rats. Brain Res 2006;1086:98–103.
Venables PH. Input dysfunction in schizophrenia. Prog Exp Pers Res 1964;72:1–47.
Wildeboer KM, Stevens KE. Stimulation of the alpha4beta2 nicotinic receptor by 5-I A-85380 improves auditory gating in DBA/2 mice. Brain Res 2008;1224:29–36.
Williams KE, Reeves KR, Billing Jr CB, Pennington AM, Gong J. A double-blind study evaluating the long-term safety of varenicline for smoking cessation. Curr Med Res Opin 2007;23:793–801.