JPET Fast Forward. Published on September 17, 2012 as DOI: 10.1124/jpet.112.197970

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EFFECTS OF THE NOVEL ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST ABT-107 ON SENSORY GATING IN DBA/2 MICE: PHARMACODYNAMIC CHARACTERIZATION.

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Running title; Alpha 7 agonist ABT-107 and Sensory Gating

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Number of text pages: 36

Number of tables: 0

Number of figures: 8

Number of references: 47

Word count in Abstract: 221

Word count in Introduction: 739

Word count in Discussion: 1450

Recommended Section Assignment: Drug Discovery and Translational Medicine

Abstract

Nicotinic acetylcholine receptor (nAChR) agonists improve sensory gating deficits in animal models and in schizophrenic patients. The aim of this study was to determine whether the novel and selective α7 nAChR full agonist ABT-107 improves sensory gating deficits in DBA/2 mice. Sensory gating was measured by recording hippocampal evoked potential P20-N40 waves and determining gating T:C ratios in a paired auditory stimulus paradigm. ABT-107 at 0.1 μmol/kg (average plasma concentration 1.1 ng/ml) significantly improved sensory gating by lowering T:C ratios during a 30 min period after administration in unanesthetized DBA/2 mice. ABT-107 at 1.0 μmol/kg was ineffective at 30 min after administration when average plasma levels were 13.5 ng/ml. However, the 1.0 μmol/kg dose was effective 180 min after administration when plasma concentration had fallen to 1.9 ng/ml. ABT-107 (0.1 μmol/kg) also improved sensory gating in anesthetized DBA/2 mice pretreated with α7 nAChR desensitizing doses of nicotine (6.2 μmol/kg), or ABT-107 (0.1 μmol/kg) itself. Moreover, repeated BID dosing of ABT-107 (0.1 μmol/kg) was as efficacious as a single dose. The acute efficacy of ABT-107 (0.1 μmol/kg) was blocked by the nAChR antagonist methyllycaconitine (MLA), but not by the α4β2 nAChR antagonist dihydro-β-erythroidine (DHβE). These studies demonstrate that ABT-107 improves sensory gating through activation of nAChRs, and that efficacy is sustained under conditions of repeated dosing, or with prior nAChR activation with nicotine.

Introduction

Sensory gating is a CNS function that inhibits responding to redundant auditory or visual stimuli, and is thought to facilitate the discrimination of relevant from irrelevant sensory input (Wan et al, 2008). Sensory gating deficits have been found in schizophrenic and Alzheimer's patients, and may contribute to the cognitive deficits associated with these diseases (Potter et al, 2006, Thomas et al, 2008). The non-selective neuronal nicotinic receptor (nAChR) agonist nicotine can transiently improve gating in schizophrenic patients, a finding that supports the concept of an important link between nAChRs and sensory gating function (Adler et al, 1998).

The homomeric α7 nAChR subtype is specifically implicated in having a role in sensory gating processes. For example, phamacological blockade of α7 receptors with α-bungarotoxin can induce sensory gating deficits in rodents (Luntz-Leybman et al 1992). Additionally, sensory gating deficits are found in C3H α7 receptor null mutant heterozygous mice that have significant reductions in hippocampal α7 receptor levels (Adams et al 2008). In humans, mutations in chromosome 15q14 locus, with single nucleotide polymorphisms in the promoter of the nAChR α7 gene, are found in schizophrenic patients with sensory gating deficits (Gault et al 1998, Leonard et al 2002, Raux et al 2002). These findings and others have prompted interest in developing selective α7 agonists for the treatment of the pre-attention and cognitive deficits of neuropsychiatric disorders. GTS-21 is a functionally selective α7 partial agonist with 20 % efficacy at the human α7 receptor (Briggs et al 1997, Kem et al 2004, Meyer et al 1998). GTS-21 improves gating in animal models, an effect that is blocked by the α7 antagonist α-bungarotoxin (Stevens et al 1998). GTS-21 improves P50

inhibitory gating in schizophrenic patients, and enhances cognition in the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) test (Olincy et al 2006). No significant improvement with GTS-21 was shown on the MATRICS Consensus Cognitive Battery test, but did have a significant effect on negative symptoms (Freedman et al 2008). The partial α7 agonist Tropisetron also attenuates sensory gating deficits in animal models and schizophrenic patients, and improves sustained visual attention on the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Hashimoto et al 2005, Koike et al 2005, Shiina et al 2010). A-582941, a selective α7 agonist with 52% efficacy at human α7 receptors, has effects in both animal sensory gating and cognition models (Bitner et al, 2007, Tietje et al 2008).

Optimizing new lead α7 agonists is directed toward improving the potency, selectivity, CNS penetration, and pharmacokinetic properties compared to existing compounds. A compound that has some of these characteristics is ABT-107 [5-(6-[(3R)-1-azabicyclo[2.2.2]oct-3-yloxy]pyridazin-3-yl)-1H-indole], which has 79% efficacy to activate human recombinant α7 receptors, and is at least 100-fold selective over non-α7 nAChR subtypes (Malysz et al, 2010). ABT-107 rapidly desensitizes native α7 receptors in rat hippocampal slices as measured by diminishing inward GABAergic inhibitory postsynaptic currents, a characteristic found with other α7 agonists (Malysz et al, 2010). ABT-107 produces cognitive efficacy across a variety of behavioral assays and displays a favorable pharmacokinetic profile with a 1:1 brain to plasma ratio or higher in mouse (Bitner et al, 2010). ABT-107 was characterized in this study for in vivo efficacy in DBA/2 mice, a strain which has altered α7 receptor expression in the hippocampus, and concomitant sensory gating deficits that are reversible with nicotine and

GTS-21 (Stevens et al 1996, Radek et al, 2006, Stevens and Wear, 1997). Desensitization of nAChRs after exposure to agonists, an effect that may limit efficacy, has been interrogated in the DBA/2 sensory gating model as well (Seguela et al, 1993; Stevens and Wear, 1997; Dajas-Bailador and Wonnacott, 2004). Thus, assessing sensory gating in DBA/2 mice represents an appropriate pre-clinical means to characterize the in vivo efficacy, selectivity, and pharmacodynamic properties of nAChR agonists. Moreover, the DBA/2 mouse is, to an extent, a disease relevant model, since sensory gating deficits and altered $\alpha 7$ expression resemble some aspects of schizophrenia (Olincy and Stevens, 2007).

This study sought to determine the acute and repeated dosing effects of ABT-107 on sensory gating in DBA/2 mice, as well as to determine the nAChR selectivity of the compound in this model. Sensory gating was assessed electrophysiologically by recording hippocampal P20-N40 evoked potential waves that were elicited with a paired auditory stimulus paradigm to derive gating ratios. Increased gating ratios, indicative of a deficit, are characteristic of DBA/2 mice and schizophrenic patients. Additionally, plasma concentrations were measured to establish the relationship between ABT-107 exposure and efficacy.

Materials and Methods

Subjects.

Animals were handled in accordance with scientific protocols approved by Abbott Laboratories and University of Colorado Institutional Animal Care and Use Committees (IACUC), and in accordance with the guidelines of the Association for Assessment and Accreditation of Animal Laboratory Care (AAALAC). Male DBA/2 mice (18–25 g) were obtained from Harlan SD (Indianapolis, IN) and group housed in home cages. Food (Purina Rodent Chow) and water were available ad libitum, and animals were kept on a 12-h light:dark cycle (lights on at 0600)

Sensory gating in unanesthetized DBA/2 mice.

Materials and procedure for surgical implantation of hippocampal electrodes into DBA/2 mice for recording auditory evoked potentials is described in detail in a previous paper (Radek et al, 2006). For these surgeries, mice were anesthetized with a ketamine/xylazine solution to provide 30-40 minutes of anesthesia. The following coordinates were used for placement of electrodes into the CA3 region of the hippocampus (relative to bregma): AP –1.8 mm, ML –2.6 mm. The electrode length was such that the tip was 1.65-1.70 mm below the dorsal surface of the brain. The electrodes were permanently anchored with dental acrylic, and four to seven days were allowed for recovery in the home cage before experimentation.

The procedure for acquiring hippocampal EEG signals for recording auditory evoked potentials in unanesthetized, freely moving DBA/2 mice has also been previously described in detail (Radek et al 2006). Electrical activity was amplified (differential AC EEG amplifiers, Grass Instrument Division, Astro-med Inc, West Warwick, RI) 1000 times, and 24 db bandpass filters

set to 1 and 300 Hz. Auditory evoked potentials were generated by presentation of 120 pairs of white noise bursts (5 msec duration), or clicks, of 70 dB sound pressure level (SPL), which was about 5 db above background. The noise bursts were presented in pairs with 500-msec between stimuli, and 15 sec between pairs. Data acquisition software (SciWorks, DataWave, Berthoud Co) recorded hippocampal EEG at a sampling rate of 1000 Hz while clicks were being delivered. The software averaged the 120-paired responses into one composite evoked response. Any section of EEG containing movement artifact was discarded, so in some cases fewer than 120 repetitions comprised the averaged evoked potential. The hippocampal sensory gating response to paired auditory stimuli was identified as the peak in the auditory evoked potential wave at a latency of 15-25 msec after the stimulus (P20 wave), followed by the peak of opposite polarity at 30-50 msec after the stimulus (N40 wave). The difference between these peaks was defined as the P20 - N40 amplitude (in microvolts). P20 - N40 amplitude was determined for the auditory evoked potential response to the first conditioning stimulus (C), and auditory evoked potential response to the second test stimulus (T). A ratio was derived between the two responses by dividing the test P20 – N40 amplitude by the conditioning P20 – N40 amplitude. This calculation, termed the T:C ratio, was the measure by which treatments were assessed for effects on sensory gating.

Drug administration unanesthetized DBA/2 mice

Drugs were administered 5 min before mice were placed into the recording chambers and initiation of evoked potential recording. Recording of paired auditory evoked potentials continued for 30 minutes after the recordings began. All pharmacological treatments were administered to unanesthetized DBA/2 mice by the intraperitoneal (ip) route of administration.

All compounds were diluted in 0.9% saline, which served as the vehicle control (1ml/kg). For a dose response experiment, separate cohorts of DBA/2 mice received vehicle or the 3 doses (0.01, 0.1, 1.0 μmol/kg) of ABT-107 (synthesized at Abbott Laboratories). In another set of studies, DBA/2 mice were pre-treated with either the nAChR antagonist methyllycaconitine citrate (MLA, Sigma Chemical Co, St. Louis MO) at 5.7 μmol/kg ip, or dihydro-β-erythroidine hydrobromide (DHβE, Sigma Chemical Co, St. Louis MO) at 2.8 μmol/kg ip, to determine the nAChR selectivity of ABT-107. ABT-107 (0.1 μmol/kg ip.) was administered 3-5 minutes after DHβE, or 45 minutes after MLA pretreatment. For these antagonist experiments, each mouse was administered all treatments including a control vehicle in random order on separate days with at least 72 hours between treatments. This within-subjects design allowed each mouse to serve as its own control in antagonist experiments.

Repeated dosing studies with ABT-107 were conducted by administering 0.1 μmol/kg for 4 days, twice a day, and once on the fifth and final day of administration. Sensory gating evoked potentials were recorded 5 min after administration of the first dose on day one (acute administration), and 5 min after the last dose on day 5. This repeated dosing study had a within subjects design, that is, one week mice would receive saline vehicle injections, and ABT-107 during the treatment week. Finally, an acute time course study was conducted by administering ABT-107 at two doses (0.1 and 1.0 μmol/kg ip) in two separate groups of mice. In one group, sensory gating evoked potentials were recorded 5 min after administration, and in another group, 180 min after administration. This study too involved mice either receiving saline vehicle injection on one day, and ABT-107 treatment on another. Thus, each mouse

would have a saline control to compare against the effect of either ABT-107 (0.1 or 1.0 μmol/kg) dose.

In a group of DBA/2 mice that were not implanted with hippocampal electrodes, plasma levels of ABT-107 were determined using analysis methods previously described (Bitner et al, 2010) for the 0.1 and 1.0 μmol/kg doses. The plasma samples were drawn at 30 and 180 min after administration to approximate ABT-107 levels present in the sensory gating time course study.

Sensory gating and drug treatments in anesthetized mice.

DBA/2 mice were anesthetized with chloral hydrate (400 mg/kg, IP) and pyrazole (400 mg/kg, IP) to retard the metabolism of the chloral hydrate. Anesthesia was supplemented periodically to maintain a surgical plane of anesthesia (2.0 mg/kg, IP, each of chloral hydrate and pyrazole as needed; at ~ 20 minute intervals). The animal was placed in a mouse adapter (Neuroprobe, Cabin John, MD) for a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA). Hollow ear bars, attached to miniature earphones that were connected to a sound amplifier (RadioShack), were placed adjacent to the externalization of the aural canal. Because the auditory evoked potentials are more consistent at a stable temperature of 36°C, body temperature was maintained at this level with a heating pad. The scalp was incised and a burr hole opened over the CA3 region of hippocampus [-1.8 mm anterior-posterior to bregma, +2.70 mm medial-lateral to midline (Paxinos and Franklin 2001)]. A teflon-coated, stainless steel wire microelectrode (0.127 mm diameter) was inserted into the CA3 pyramidal cell layer of the hippocampus (1.65–1.70 mm below the dorsal brain surface). Final electrode location was identified by the presence of complex action potentials typical of hippocampal pyramidal

neurons (Miller et al. 1995). A reference electrode, identical to the recording electrode, was placed on dura, anterior to bregma, contralateral to the recording electrode. The electrical activity was amplified 1000 times with bandpass 1 to 500 Hz (Miller et al. 1995) and led to an analog to digital converter (RC Electronics, Bakersfield, CA) for averaging by computer. Tones, 3000 Hz, 10-msec duration, 72 dB SPL generated as a sine wave were presented in pairs with a 500-msec intrapair interval and 10 sec between pairs. Although DBA/2 mice suffer hearing loss as they age, these tones were within the audible range for the mice (Willott et al. 1982). Responses to 16 pairs of tones were averaged at 5-min intervals. Each average was filtered digitally with bandpass between 10 and 250 Hz. The maximum negativity between 20 and 60 msec after the first stimulus was selected as the N40 wave and measured relative to the preceding positivity, a P20 wave. The full wave has been shown to be more stable than either component alone (Hashimoto et al 2005). Each of the waves (P20, N40) was also measured relative to the mean pre-stimulus activity and the P20 wave was also measured relative to the preceding N10.

Three parameters were assessed for each recording; the conditioning amplitude (response to the first stimulus), test amplitude (response to the second stimulus) and he ratio of the amplitudes of response to the test stimulus and the conditioning stimulus which provides a measure of sensory inhibition. The ratio of the test to the conditioning amplitude (T:C ratio) is 0.5 or less for most rodent strains and normal humans (Stevens et al. 1996). Four or 5 records were obtained before any drug injection to establish baseline sensory gating performance. Each mouse was drug naive at the time of experimentation.

Drug treatments in anesthetized mice.

Two doses of ABT-107 were tested, 0.1 μmol/kg and 0.01 μmol/kg. The second dose was deemed too low and only 2 mice were tested. All pharmacological treatments were administered to anesthetized DBA/2 mice by the intraperitneal (ip) route of administration. The 0.1 μmol/kg dose was tested in 6 mice using a double injection paradigm. The first injection was administered which was followed by 60 minutes of recording. After the 60 minute recording was completed, a second identical dose was administered and an additional 60 minutes of recordings were obtained. In order to determine if ABT-107 could stimulate nicotinic receptors which had been desensitized, (-)-nicotine hydrogen tartrate salt (Sigma Chemical Co, St. Louis MO) at 6.2 μmol/kg, ip was administered 60 minutes prior to 0.1 μmol/kg, ip, of ABT-107 and records collected for an additional 60 minutes. As a control, saline (1 ml/kg, ip) was also administered as 2 sequential injections, 60 minutes apart.

JPET Fast Forward. Published on September 17, 2012 as DOI: 10.1124/jpet.112.197970
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Results

Sensory gating in unanesthetized mice - ABT-107 dose response, antagonist, and repeated dosing studies. Figure 1 shows that ABT-107 produced a significant treatment effect on T:C ratios in unanesthetized DBA/2 mice (one-way ANOVA, $F(3,80)=3.387$, $p=0.022$). Newman Keuls post-hoc analysis revealed that the dose of 0.1 μmol/kg significantly decreased T:C ratios compared to vehicle. The doses of 0.01 and 1.0 μmol/kg did not differ significantly from vehicle. ABT-107 did not produce a statistically significant effect on either condition (one-way ANOVA, $F(3,80)=0.6492$, $p=0.5858$) or test amplitude $F(3,80)=0.9456$, $p=0.4229$).

Figure 2 shows that the effective ABT-107 dose of 0.1 μmol/kg was blocked by methyllycaconitine (5.7 μmol/kg). Main effects for ABT-107 (2-way repeated measures ANOVA, $F(1,46)= 3.799$, $p=0.0574$) and MLA (2-way repeated measures ANOVA, $F(1,46)= 0.2971$, $p=0.5884$) were not significant, however, a significant ABT-107 – MLA interaction was achieved (2-way repeated measures ANOVA, $F(1,46)= 4.057$, $p=0.0499$). Bonferroni post-tests show that ABT-107 was significantly different from vehicle treatment. Figure 2 also shows that the effective ABT-107 dose of 0.1 μmol/kg was not blocked by dihydro-β-erythroidine (DHβE). A main effect for ABT-107 was significant (2-way repeated measures ANOVA, $F(1,46)= 5.734$, $p=0.0231$), but not significant for DHβE (2-way repeated measures ANOVA, $F(1,46)= 0.001463$, $p=0.9697$), or for an ABT-107 – DHβE interaction (2-way repeated measures ANOVA, $F(1,46)= 0.04060$, $p=0.8417$).

Figure 3 shows the effects of acute and BID dosing of ABT-107 (0.1 μmol/kg) on T:C ratios in unanesthetized DBA/2 mice. A 2-way repeated measures ANOVA showed a significant overall ABT-107 treatment effect ($F(1, 44)=11.69$, $p=0.0014$), but no significant treatment day ($F(1, 44)=2.2$, $p=0.1452$), or ABT-107 – treatment day interaction ($F(1, 44)=0.00004991$, $p=0.9944$). ABT-107 significantly decreased T:C ratios compared to vehicle treatment on both day 1 after acute treatment, and on day 5 after the ninth injection ($p<0.05$, Bonferroni post hoc tests).

Figure 4 shows T:C ratio determinations and plasma concentrations in unanesthetized DBA/2 mice 30 and 180 minutes after ABT-107 (0.1, 1.0 μmol/kg) treatment. A significant one-way repeated measures AVOVA was obtained with 30 ($F(2, 44)=3.395$, $p=0.0478$) and 180 min ABT-107 pretreatment ($F(2, 44)= 4.127$, $p=0.0269$). Five min pretreatment with 0.1 μmol/kg ABT-107 significantly lowered T:C ratios, while the dose of 1.0 μmol/kg did not. This result is similar to that shown in Figure 1 for 0.1 and 1.0 μmol/kg, and in Figure 2 & 3 for 0.1 μmol/kg. In contrast, the 0.1 μmol/kg dose was ineffective after 180 min, while the 1.0 μmol/kg dose significantly lowered T:C ratios. In a satellite group of mice, mean $\pm$ SEM plasma concentrations of ABT-107 at 30 min after administration were 1.1 ± 0.2 ng/ml and 13.5 ± 6.3 ng/ml for the 0.1 and 1.0 μmol/kg doses, respectively. At 180 min, plasma concentrations were 0.2 ± 0.4 ng/ml and 1.9 ± 0.6 ng/ml for the 0.1 and 1.0 μmol/kg doses, respectively. Figure 5 is data derived from figure 4, but is depicted here as T:C ratios as a percent change from vehicle (vertical y-axis) versus ABT-107 plasma concentrations (horizontal x-axis). This graph shows that attaining plasma concentrations of 1-2 ng/ml, either

with a 0.1 μmol/kg dose with a 5 min pretreatment, or a 1.0 μmol/kg dose with a 180 min pretreatment, will significantly lower T:C ratios.

Sensory gating in anesthetized mice – sequential dosing studies.

As shown in Figure 6, sequential saline administration (60 min apart) did not produce any significant alterations on any parameter assessed (Conditioning amplitude $F(27, 108)=0.85$, $p=0.684$; Test amplitude $F(27, 108)=1.12$, $p=0.333$; TC ratio $F(27, 108)=0.76$, $p=0.793$). In contrast, the 0.1 μmol/kg dose of ABT-107 (Figure 7) did show significant changes in sensory gating parameters (conditioning amplitude $F(27, 189)=2.38$, $p<0.001$; Test amplitude $F(27, 189)=0.84$, $p=0.698$; T:C ratio $F(27, 189)=1.67$, $p=0.025$). Fisher's PLSD a posteriori analyses showed T:C ratio was significantly reduced from 30-45 minutes following the first injection and 10-15 following the second. This reduction in T:C ratio following the first injection of ABT-107 (0.1 μmol/kg) in anesthetized DBA/2 mice is similar to the effect seen in unanesthetized DBA/2 mice (Figure 1). The conditioning amplitude was significantly increased from 20-50 minutes following the first injection, and 15-20 minutes following the second.

When nicotine (6.2 μmol/kg) was administered 60 minutes prior to the ABT-107 injection (0.1 μmol/kg) (Figure 8), there were significant changes in conditioning amplitude and T:C ratio, while test amplitude just missed significance (conditioning amplitude $F(28, 140)=1.91$, $p=0.008$; Test amplitude $F(28, 140)=1.54$, $p=0.054$; T:C ratio $F(28, 140)=3.87$, $p<0.001$) when a full MANOVA was performed. However, if just the baseline and time points after nicotine administration were compared, a significant decrease in test amplitude was revealed ($F(16,$

80)=2.61, p=0.003), and the conditioning amplitude failed to achieve significance (F(16, 80)=1.70, p=0.063). Increased condition and decreased test amplitudes combined to produce a significant decrease in T:C ratio following nicotine administration (F(16, 80)=3.52, p<0.001). Fisher's PLSD a posteriori analyses for the full MANOVA showed T:C ratio was significantly reduced from 5-40 minutes following the nicotine injection, and for 5-30 following the ABT-107 injection. Significant increases in conditioning amplitude were apparent from 5-30 minutes following the nicotine injection and 5-20 minutes following the injection of ABT-107.

Discussion

Acute administration of the selective α7 full agonist ABT-107 attenuated the sensory gating deficits in DBA/2 mice as assessed by auditory evoked potentials in a paired stimulus paradigm. This ABT-107 effect is similar to that seen with other α7 nAChR agonists, as well as to atypical antipsychotics like olanzapine and clozapine (Simosky et al, 2003, Olincy and Stevens, 2007, Simosky et al, 2008). ABT-107 efficacy in the DBA/2 mouse model is suggestive of a beneficial therapeutic effect in treating sensory gating related pre-attention deficits in schizophrenia. The effect of ABT-107 appears to be α7-mediated, as pretreatment with the α7 nAChR antagonist MLA blocked the lowering of T:C ratios by ABT-107 at a systemic dose known to achieve brain levels that effectively inhibit α7 receptors (Turek et al, 1995). Consistent with our findings, MLA administered at a similar dose has been described to prevent the sensory gating effects of the α7 nAChR partial agonist Tropisetron (Hashimoto et al 2005). While MLA has been shown to block α4β2 at higher concentrations (Karadsheh et al. 2004), the inability of DHβE pretreatment to block ABT-107 gating efficacy here strongly suggests an α7-mediated effect. The 5-HT2a receptor has been implicated in sensorimotor gating function (Quednow et al 2009), and ABT-107 has moderate affinity for 5-HT2a and Sigma receptors. However, this compound is at least 100-fold more selective for α7 receptors over every other non-nicotinic receptor examined (Malysz et al 2010). This, together with the MLA blockade of ABT-107, tends to implicate a nAChR mediated mechanism rather than any other.

Improvement of sensory gating at the 0.1 μmol/kg dose of ABT-107 was maintained with repeated dosing, and acute and BID plasma levels were comparable (2.6 and 2.1 ng/ml, respectively). Therefore, efficacy is maintained with repeated treatment, but efficacy diminishes at higher plasma concentrations (i.e. higher dose). To investigate this further, the effects of ABT-107 on sensory gating at doses of 0.1 and 1.0 μmol/kg i.p. were examined at 30 and 180-min following drug administration. Consistent with the dose response study, 5-min pretreatment with the 0.1 μmol/kg dose of ABT-107 significantly reduced T:C ratios when plasma concentration was 1.1 ng/ml. The 0.1 dose μmol/kg was ineffective 180 min after treatment when plasma concentration had fallen to 0.2 ng/ml. The 1.0 μmol/kg dose administered 5-min prior to evoked potential recording was ineffective when plasma concentration was 13.1 ng/ml, but was effective 180-min after administration when plasma concentration had fallen to 1.9 ng/ml. The plasma concentrations of 0.1 μmol/kg (30 min after treatment) and 1.0 μmol/kg (180 min after treatment) are similar, as is the efficacy to attenuate the sensory gating deficit of DBA/2 mice. This supports the concept that efficacy can be sustained within plasma range even with continuous exposure of the drug to receptors.

It is unclear why the highest plasma concentration of ABT-107 (13.1 ng/ml) did not improve gating in these studies. nAChRs of the α7 subtype rapidly desensitize upon exposure to agonists, and desensitization has been demonstrated for ABT-107 in hippocampal GABA IPSCs in vitro (Malysz et al, 2010). Desensitization of receptors with the 1.0 μmol/kg dose of ABT-107 may be a one explanation for a lack of in vivo efficacy in the DBA/2 sensory gating model. Activation of α7 nAChRs on GABA containing hippocampal interneurons is thought

to be a neuronal substrate for sensory gating (Miller and Freedman, 1995, Moxon et al, 2003), and desensitizing this inhibitory system would result in reduced control over excitatory pyramidal neuron firing. Populations of nAChRs may exist between the activated and desensitized state at the same time (Picciotto et al, 2008), and perhaps a net activation is being produced by ABT-107 at plasma concentrations of ~1-2 ng/ml. The partial α7 agonists GTS-21 and Tropisetron have somewhat wider efficacy ranges in the anesthetized DBA/2 mouse sensory gating model compared to ABT-107 (Hashimoto et al, 2005, Stevens et al, 1998). However, it is not entirely clear that partial agonists will consistently provide a wider efficacy range, since only one dose of GTS-21 improved gating in another study (Simosky et al 2001). Nonetheless, potent and full agonists such as ABT-107 may be effective at driving α7 desensitization in vivo, and therefore narrow the efficacious dose range.

The possible influence of prior nAChR activation on ABT-107 efficacy was also investigated in a sequential dosing paradigm in anesthetized DBA/2 mice. Measuring auditory sensory gating in anesthetized mice is an established technique that has been used to evaluate drug time course, as well as to demonstrate putative desensitization of nAChRs in vivo (Stevens and Wear, 1997). ABT-107 (0.1 μmol/kg) significantly lowered T:C ratios in anesthetized DBA/2 mice, an effect similar to that obtained in unanesthetized mice. By 60 minutes after injection, the effect of ABT-107 was diminished, but a second injection again significantly reduced T:C ratios. This suggests that ABT-107 itself did not induce an insensitivity to subsequent nAChR activation under these treatment conditions. In another experiment to examine potential desensitization of receptors, an efficacious dose of nicotine (6.2 μmol/kg i.p.) was administered 60-min before ABT-107 (0.1 μmol/kg i.p.). Nicotine improved gating

as indicated by a significant decrease in the T:C ratio, and, as with ABT-107, the effect was diminished by 60 minutes. ABT-107 administration at 60 minutes after nicotine resulted in a second lowering of T:C ratios that was comparable in efficacy to ABT-107 (0.1 μmol/kg) without nicotine pretreatment. Thus, there is no overt in vivo evidence for loss of ABT-107 efficacy due to prior acute nicotine or ABT-107 activation of nAChRs. The half-life of nicotine in mice is about 7-10 min (Petersen et al, 1984), and it would have been desirable to test ABT-107 at an earlier time point as well as one hour after nicotine injection. However, nicotine is fully efficacious to improve gating during, and well after maximal plasma concentrations have been attained (Stevens and Wear, 1997). Thus, there would be no window to see any additional improvement in gating after administration of a second compound. In a sequential dosing paradigm similar to the one used in the present study, an initial efficacious dose of nicotine renders a second, identical nicotine dose ineffective to improve DBA/2 mouse gating (Stevens and Wear, 1997). Therefore, the dosing approach taken in the present experiments appear to be a reasonable in vivo way to determine the efficacy of agonists with prior nicotine exposure, at least with acute dosing. The ability of ABT-107 to maintain efficacy after nicotine administration is particularly important since, for the treatment of schizophrenia, many patients are exposed to significant levels of nicotine through cigarette smoking (Lohr and Flynn, 1992, Griffith et al, 1998). It must be noted, however, that while acute pre-activation of the nAChRs did not occlude the efficacy ABT-107, these experiments may not entirely model receptor characteristics under chronic nicotine exposure that is seen with heavy cigarette smoking.

In anesthetized DBA/2 mice, the decrease of T:C ratios by ABT-107 was driven largely by increased conditioning stimulus P20-N40 amplitude. In individual unanesthetized DBA/2 mice, there was a tendency for ABT-107 to induce small increases in conditioning and/or decreases in test amplitudes, which together, were sufficient to significantly lower T:C ratios. Nicotine and the α4β2 selective agonist A-85380 both increase condition stimulus P20-N40 amplitude, an effect that is blocked by the α4β2 antagonist DHβE (Radek et al 2006, Wildeboer and Stevens, 2008). Therefore, the effect of ABT-107 to increase conditioning stimulus P20-N40 amplitude in anesthetized DBA/2 mice is suggestive of an α4β2 activation. ABT-107 is reported to increase extracellular acetylcholine in the pre-frontal cortex (Bitner et al, 2010), but as α7 receptors are present in the hippocampus (Stevens et al, 1996), it is conceivable that ABT-107 similarly increases hippocampal acetylcholine as well, the site for assessing sensory gating in the present studies. Thus, ABT-107 may activate α4β2 nAChRs indirectly through the release of acetylcholine, which has greater than 100-fold higher binding Ki for α4β2 over α7 (Marks et al, 1986). The α4β2 antagonist DHβE did not attenuate the effect of ABT-107 on gating in the experiment using unanesthetized DBA/2 mice. Nevertheless, it is possible that, in addition to α7, ABT-107 is activating α4β2 nAChRs and is affecting the sensory gating response through acetylcholine release. Eliciting inhibitory GABA transmission is a likely function of both α7 and α4β2 nAChRs, and co-activation of these subtypes may result in a net augmentation of inhibitory gating function (McClure-Begley et al, 2009, Radek et al, 2010).

In summary, these studies demonstrate that the selective α7 full agonist ABT-107 improves sensory gating in DBA/2 mice, and an optimal plasma concentration was determined that

produced consistent efficacy, either with acute or repeated administration. Furthermore, prior activation of nAChRs with nicotine does not decrease the acute efficacy of ABT-107, which may suggest a favorable profile for treating schizophrenic patients that smoke.

Acknowledgements

The authors thank Dr. Kennan Marsh of the Abbott Discovery Pharmacokenetics Group for determination of ABT-107 plasma concentrations in DBA/2 mice.

Author Contributions

Participated in research design: R.J. Radek, H.M. Robb, K.E. Stevens, M. Gopalakrishnan, R.S. Bitner

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Wrote or contributed to the writing of the manuscript: R.J. Radek, H.M. Robb, K.E. Stevens, M. Gopalakrishnan, R.S. Bitner

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Legends for Figures

Figure 1. A, ABT-107 (0.1 μmol/kg ip.) significantly decreased T:C ratios (improved sensory gating) in unanesthetized DBA/2 mice. Higher and lower doses were ineffective. B & C, Statistically significant effects of ABT-107 were not achieved on condition or test P20-N40 amplitudes. 120 auditory evoked potential responses were acquired and averaged over a period of 30 minutes following treatments. 0.0 (vehicle) n=34, 0.01 μmol/kg n=18, 0.1μmol n=22, 1.0 μmol/kg n=10. Data are mean ± SEM, *p<0.05 vs. vehicle, Newman-Keuls *a posteriori* test.

Figure 2. A, The nAChR antagonist MLA (5.7 μmol/kg i.p.) blocks the lowering of T:C ratios produced by ABT-107 (0.1 μmol/kg i.p.). MLA alone (5.7 μmol /kg i.p.) had no effect on T:C ratios. MLA experiment, within subject treatment, n=24. B, The α4β2 antagonist DHβE (2.8 μmol /kg i.p.) does not block ABT-107 (0.1 μmol/kg i.p.) lowering of T:C ratios. DHβE alone (2.8 μmol i.p.) had no effect on T:C ratios. DHβE experiment within subject treatment, n=16. Data are mean ± SEM, *p<0.05, Bonferroni *a posteriori* tests vs. vehicle.

Figure 3. Repeated BID administration of ABT-107 in unanesthetized DBA/2 mice. Sensory gating was assessed after the first injection (acute) on day 1, and after the ninth injection on day 5. T:C ratio was decreased after injection on day 1, as well as after the ninth injection on day 5. Within subjects treatment, n=23. Data are mean ± SEM, *p<0.05, Bonferroni *a posteriori* test.

Figure 4. Time course study for the effects of ABT-107 in unanesthetized DBA/2 mice. A, ABT-107 at 0.1 μmol/kg when administered 5-min before sensory gating assessment significantly reduced the T:C ratio, while 180 min pretreatment at this dose did not. B, 5-min pretreatment with ABT-107 at 1.0 μmol/kg did not improve gating, while 180 min pretreatment did result in a significant lowering of T:C ratios. As determined in satellite mice, ABT-107 (0.1 μmol/kg) plasma levels at 30 and 180 min were 1.1±0.2 and 0.2±0.4 ng/ml, respectively. ABT-107 (1.0 μmol/kg) plasma levels at 30 and 180 min were 13.1±6.5 and 1.9±0.6 ng/ml, respectively. Within subject treatment n=15. Data are mean ± SEM, **p*<0.05, Newman-Keuls. *a posteriori* test.

Figure 5. T:C ratios as a percent change from vehicle versus ABT-107 plasma concentrations. Plot derived from T:C ratios and plasma concentrations shown in figure 4. T:C ratios are significantly decreased from vehicle with ABT-107 plasma concentrations of ~1-2 ng/ml. Horizontal bar highlights plasma concentration range that elicits significant reduction of T:C ratios. Data are mean ± SEM. Significance markers are from statistical analysis conducted on T:C ratios in figure 4.

Figure 6. Anesthetized DBA/2 mice received saline injections (1 ml/kg, ip) at the first arrow. This was followed by recordings every 5 minutes for 60 minutes, at which time a second identical injection of saline was administered and second 60 minutes of recordings were made. A & B, Saline injection had no significant effects on condition, test, or T:C ratios. Data are mean ± SEM, *n*=5. B refers to pre-injection baseline recordings.

Figure 7. ABT-107 was administered to anesthetized DBA/2 mice at 0.1 μmol/kg, ip, twice, 60 minutes apart. A, This dose of the compound produced significant increases in conditioning amplitude after both the first and second injections. B, The increase in conditioning amplitude produced significant decreases in T:C ratio after both injections. Data are mean ±SEM, n=6. *p<0.05, **p<0.01, Fisher's PLSD.

Figure 8. Nicotine (6.2 μmol/kg, ip) was injected into anesthetized DBA/2 mice, and 60 minutes later, ABT-107 (0.1 μmol/kg, ip) was injected and data collected for an additional 60 minutes. A, When a full MANOVA of all time points was performed, significant increases in conditioning amplitude and decreases in T:C ratio were found. When only the time after nicotine administration was analyzed, the increase in conditioning amplitude did not reach significance. B, Decreases in test amplitude and T:C ratio did achieve statistical significance. Data are mean ± SEM, n=6. *p<0.05, **p<0.01, Fisher's PLSD.

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Figure 1

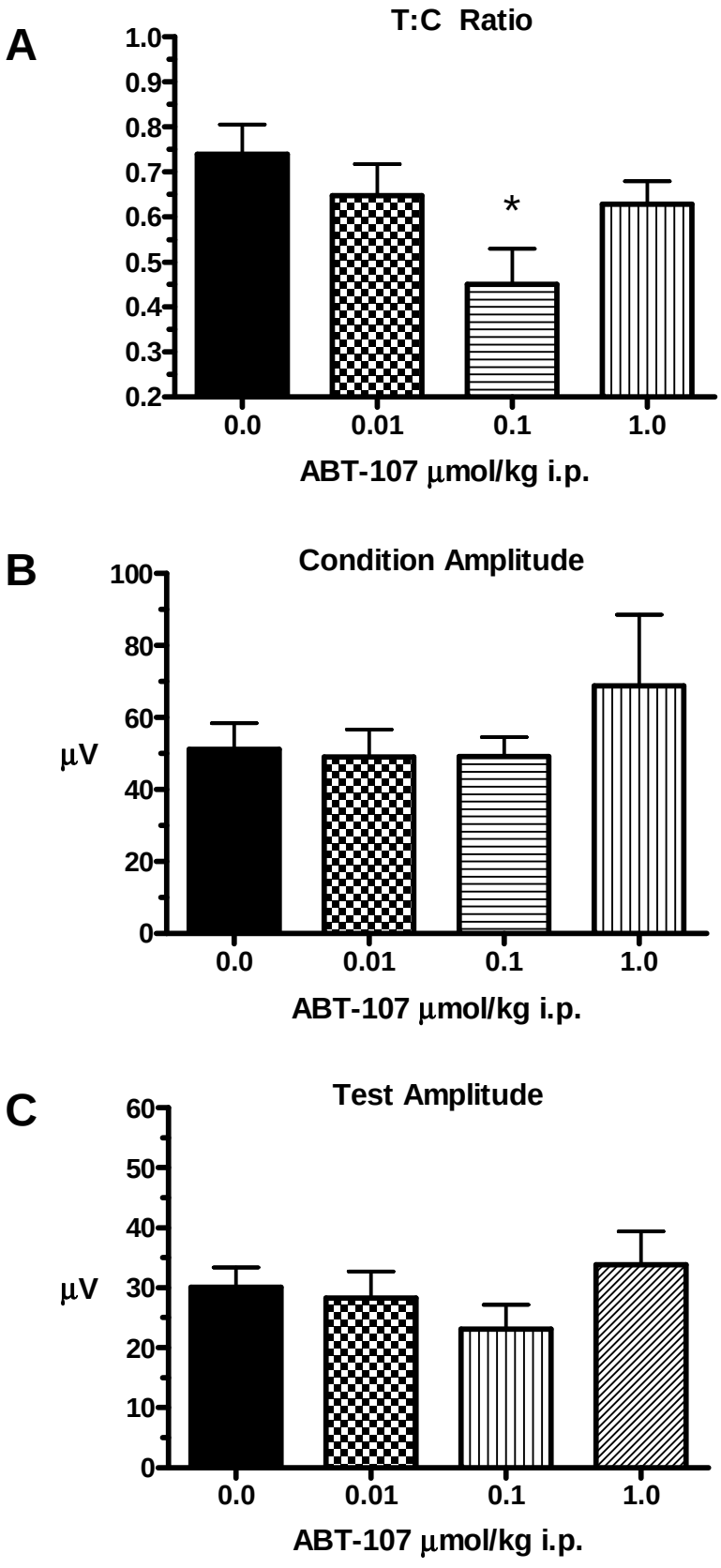

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Figure 2

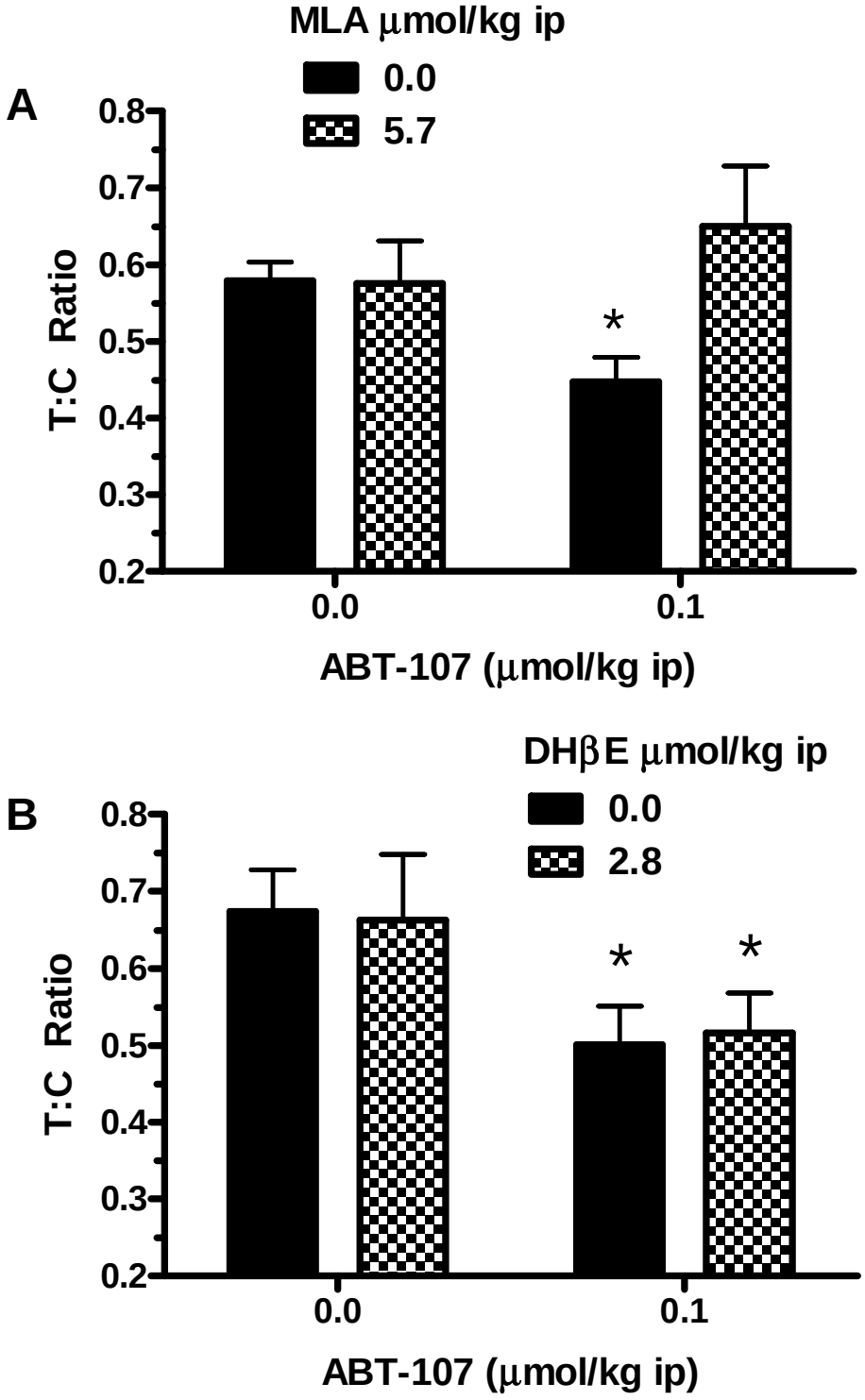

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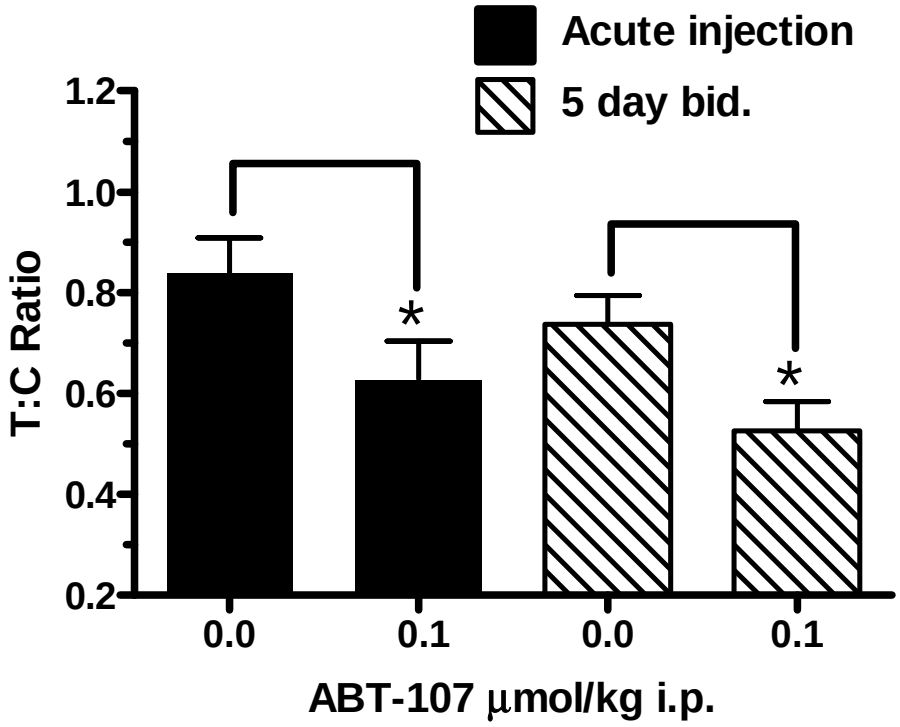
Figure 3



plasma concentration 30 min after acute injection = 2.6±0.3 ng/ml

plasma concentration 30 min after 5 day BID dosing = 2.1±0.2 ng/ml

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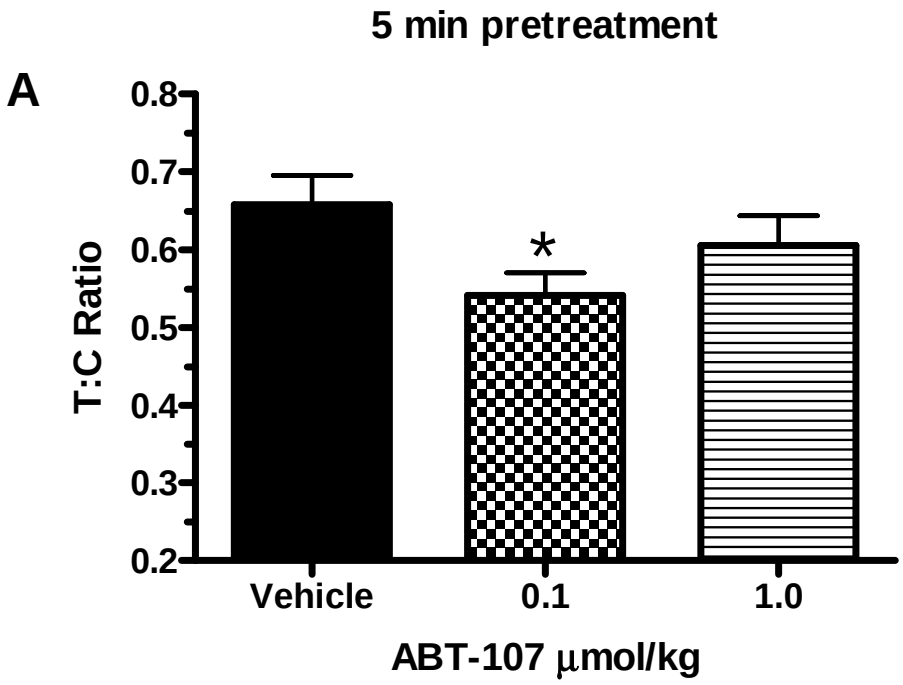
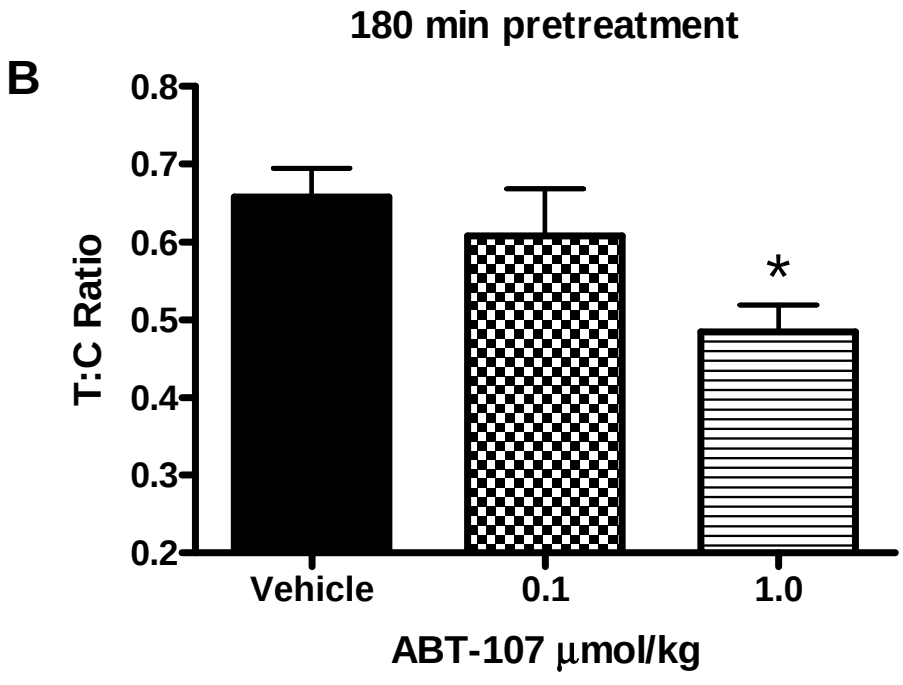
Figure 4



0.1 μmol/kg ABT-107 in plasma @ 30 min = 1.1 ± 0.2
1.0 μmol/kg ABT-107 in plasma @ 30 min = 13.1 ± 6.5



0.1 μmol/kg ABT-107 in plasma @ 180 min = 0.2 ± 0.4
1.0 μmol/kg ABT-107 in plasma @ 180 min = 1.9 ± 0.6

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Figure 5

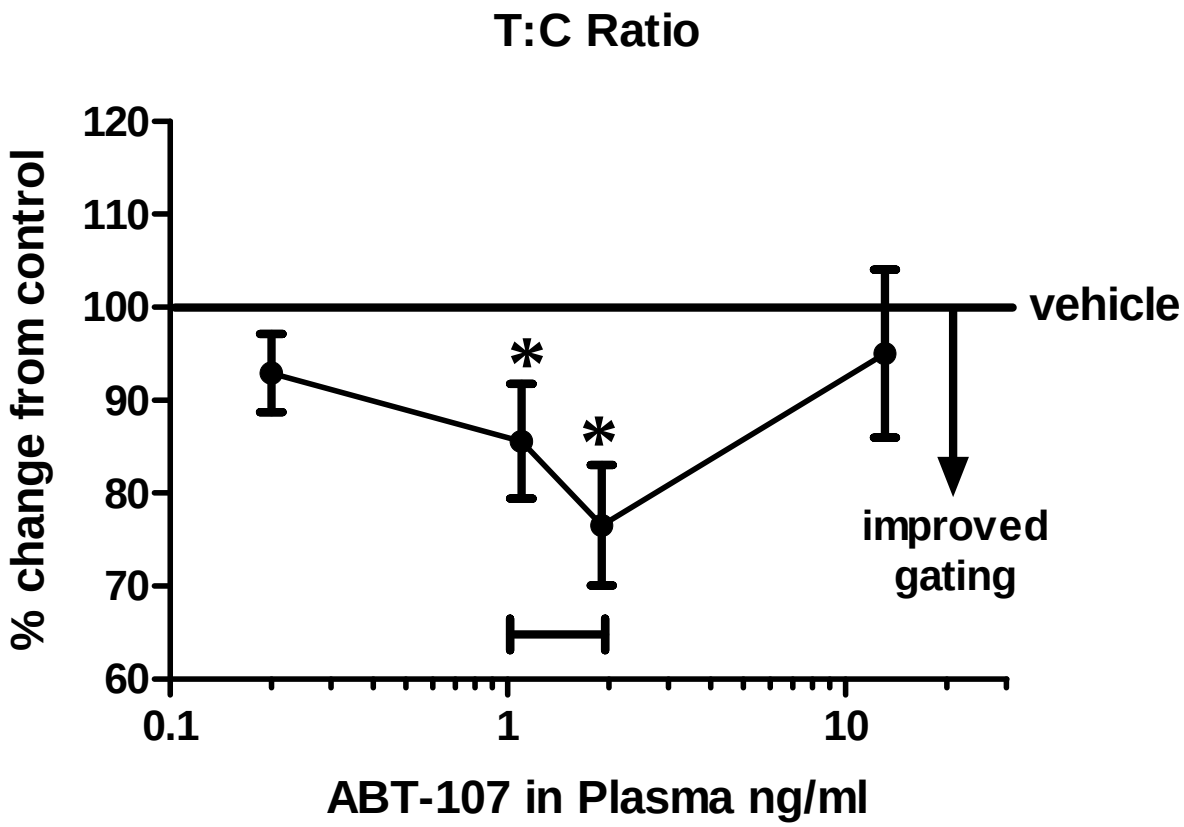

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Figure 6

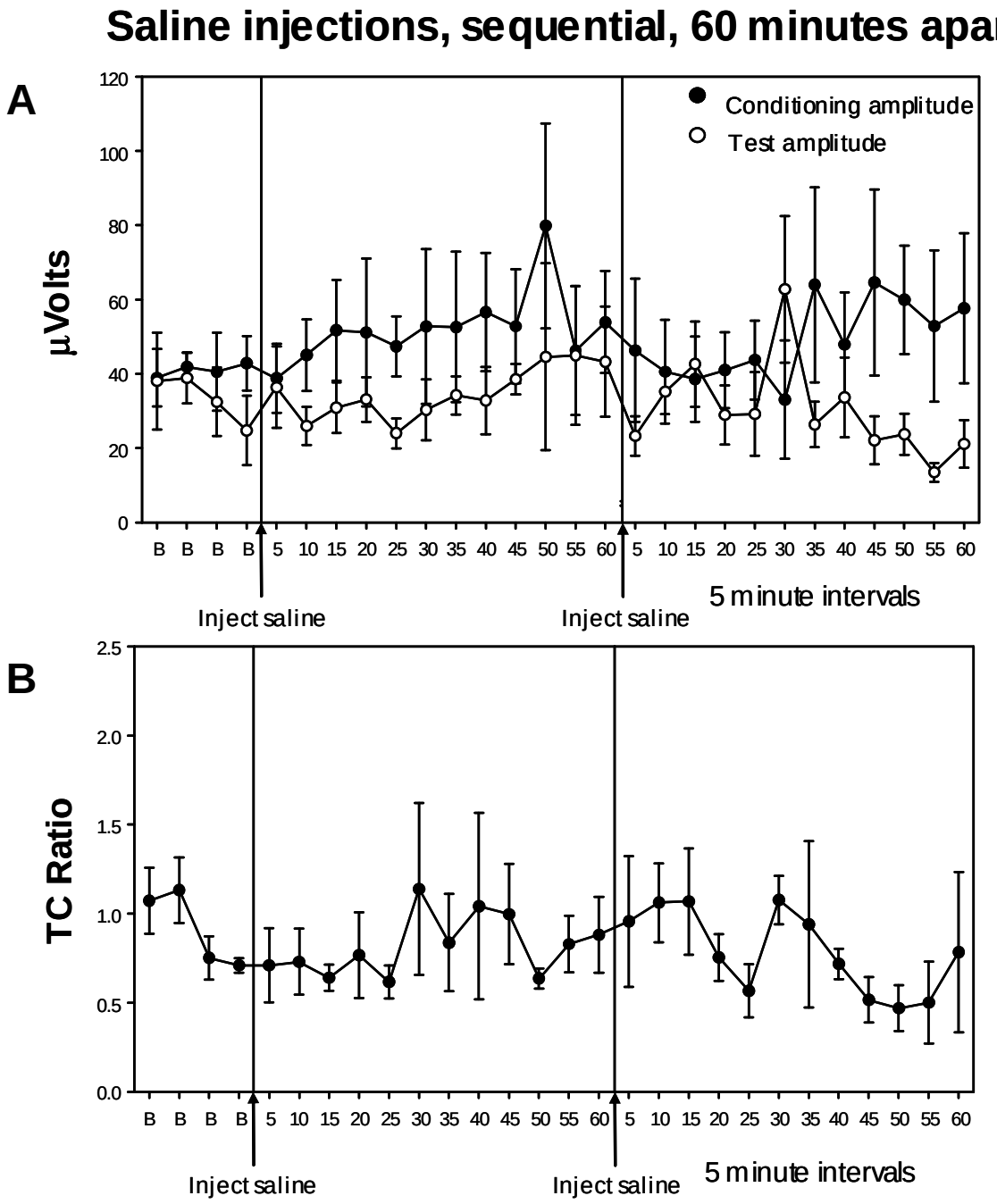

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Figure 7

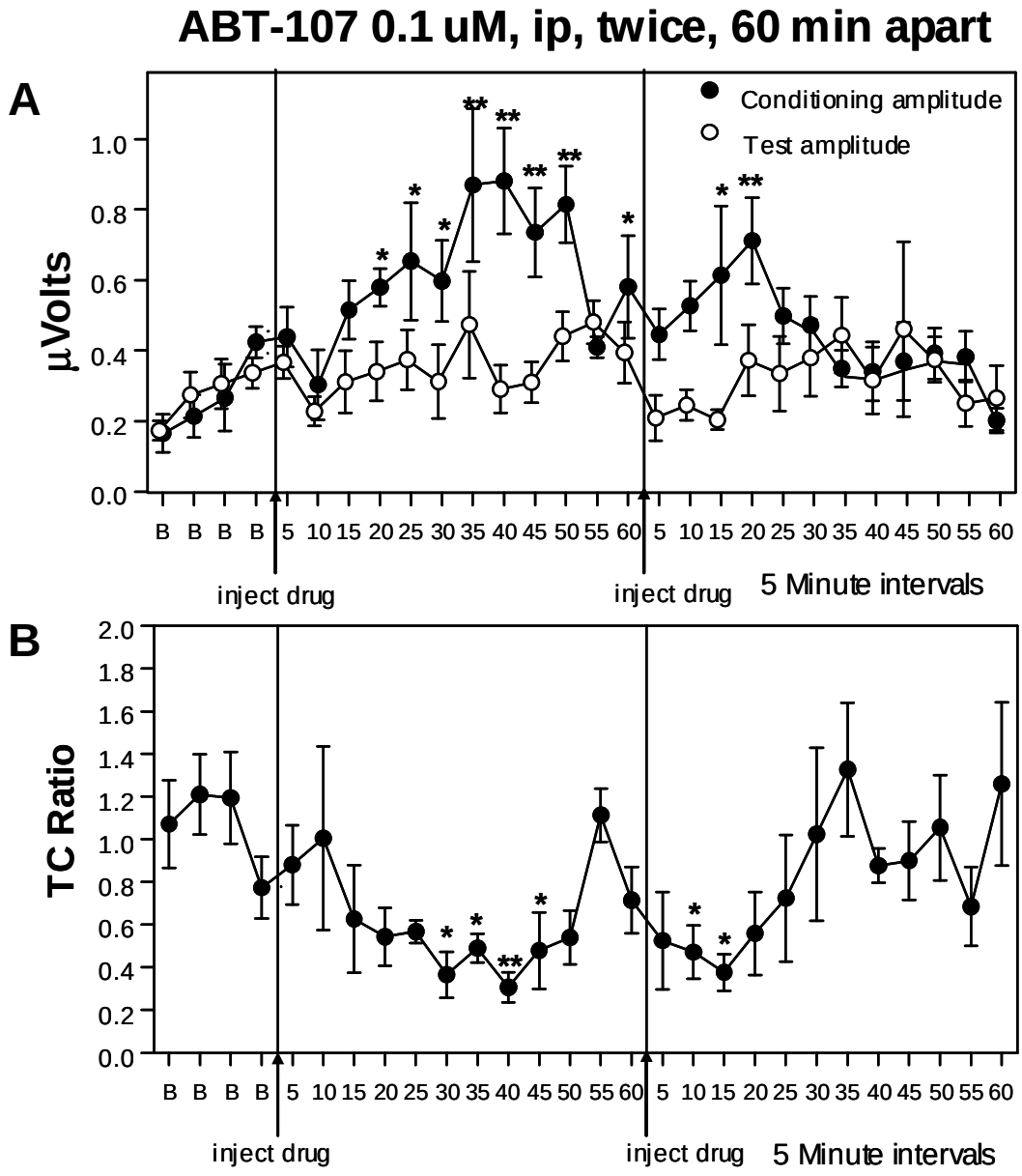

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Figure 8