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RESEARCH PAPER

α7 nicotinic ACh receptor-deficient mice exhibit sustained attention impairments that are reversed by β2 nicotinic ACh receptor activation

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BACKGROUND AND PURPOSE

Disruptions of executive function, including attentional deficits, are a hallmark of a number of diseases. ACh in the prefrontal cortex regulates attentive behaviour; however, the role of α7 nicotinic ACh receptor (α7nAChR) in attention is contentious.

EXPERIMENTAL APPROACH

In order to probe attention, we trained both wild-type and α7nAChR knockout mice on a touch screen-based five-choice serial reaction time task (5-CSRT). Following training procedures, we then tested sustained attention using a probe trial experiment. To further differentiate the role of specific nicotinic receptors in attention, we then tested the effects of both α7nAChR and β2nAChR agonists on the performance of both wild-type and knockout mice on the 5-CSRT task.

KEY RESULTS

At low doses, α7nAChR agonists improved attentional performance of wild-type mice, while high doses had deleterious effects on attention. α7nAChR knockout mice displayed deficits in sustained attention that were not ameliorated by α7nAChR agonists. However, these deficits were completely reversed by the administration of a β2nAChR agonist. Furthermore, administration of a β2nAChR agonist in α7nAChR knockout mice elicited similar biochemical response in the prefrontal cortex as the administration of α7nAChR agonists in wild-type mice.

CONCLUSIONS AND IMPLICATIONS

Our experiments reveal an intricate relationship between distinct nicotinic receptors to regulate attentional performance and provide the basis for targeting β2nAChRs pharmacologically to decrease attentional deficits due to a dysfunction in α7nAChRs.

Abbreviations

5-CSRT, five-choice serial reaction time task; VAChT, vesicular ACh transporter

Tables of Links

TARGETS		
Ligand-gated ion channels[a]	**Transporters**[b]	**Enzymes**[c]
α7nAChR (CHRNA7)	VAChT	AChE
β2nAChR		ChAT
CHRNA2		ERK1
CHRNA4		ERK2

LIGANDS
ACh
Methyllycaconitine
Nicotine
PHA-543,613

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

Attentional performance can be severely compromised in different neuropsychiatric and neurodegenerative diseases, including schizophrenia and Alzheimer's disease (Mega and Cummings, 1994; Buckner, 2004). ACh release in the prefrontal cortex (PFC), a brain area known to play a central role in attention (Kastner and Ungerleider, 2000; Corbetta and Shulman, 2002; Buschman and Miller, 2007), is important for the regulation of attentive behaviour (Elliott, 2003). Schizophrenic patients present severe physiological and molecular dysfunctions in the PFC (Weinberger *et al.*, 1986; Mirnics *et al.*, 2000; Guillozet-Bongaarts *et al.*, 2014). One of the more profound molecular changes is the loss of the α7 nicotinic ACh receptor (α7nAChR), encoded by the *CHRNA7* gene. Although the genetic linkage between the *CHRNA7* gene and schizophrenia is complex, with studies pointing towards and against *CHRNA7* as a risk gene for the disease (Xu *et al.*, 2001; Zammit *et al.*, 2007), robust decreases in protein and mRNA expression of the α7nAChR have been shown in the PFC of patients with schizophrenia (Guan *et al.*, 1999; Guillozet-Bongaarts *et al.*, 2014). Moreover, in Alzheimer's disease, Aβ peptides can bind to α7nAChRs (Wang *et al.*, 2000) and disrupt their function (Chen *et al.*, 2006).

There is accumulating evidence demonstrating that cue detection during attentional efforts is mediated by nicotinic receptor signalling (McGaughy *et al.*, 1999a; Grottick and Higgins, 2000; Parikh *et al.*, 2007, 2010). β2nAChRs are both necessary and sufficient to regulate attention in mice using a non-demanding five-choice serial reaction time task (5-CSRT) paradigm (Guillem *et al.*, 2011). On the other hand, the role of α7nAChRs in attention is still not completely understood. Initial studies suggest that CHRNA7$^{-/-}$ mice present deficits in sustained attention (Hoyle *et al.*, 2006; Young *et al.*, 2007). However, it has been reported that for less demanding tasks no deficits were observed in α7nAChR-null mice (Guillem *et al.*, 2011). Taken together, these results suggest that attentional deficits in CHRNA7$^{-/-}$ mice may depend on the attentional load. Pharmacological manipulations of α7nAChRs have also produced conflicting results, most likely because of the poor selectivity of the drugs used (Grottick and Higgins, 2000; Pichat *et al.*, 2007; Rezvani *et al.*, 2009; Wallace *et al.*, 2011a). Interestingly, studies in humans have shown that agonists for the α7nAChR can improve the performance of patients suffering from schizophrenia on neurocognitive tests (Olincy *et al.*, 2006; Olincy and Stevens, 2007).

Here, we report that α7nAChR-null mice present deficits in their ability to sustain attention in a demanding paradigm. Moreover, we found that activation of α7nAChRs increased biochemical signalling and attention in wild-type (WT) mice, but not in CHRNA7$^{-/-}$ mice. Interestingly, activation of β2nAChRs triggered similar biochemical pathways as α7nAChR agonists and reversed attentional deficits in α7nAChR-null mice. These results suggest that α7nAChRs may contribute to attention performance, but activation of β2nAChRs can bypass the deficits triggered by deficient α7nAChR signalling. Our results suggest that the α7nAChR plays a role in sustained attention during demanding tasks and that β2nAChR drugs may be of potential use for correcting cognitive and molecular signalling deficits seen in psychiatric or neurological disorders in which α7nAChRs are affected.

Methods

Animals

CHRNA7$^{-/-}$ mice (B6.129S7 nAChR Chrna7tm1Bay/J) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were housed in groups of three or four per cage in a temperature-controlled room with 12/12 h light/dark cycle (07:00–19:00 h), and water was provided *ad libitum*. Only male mice were used in these studies. For the 5-CSRT studies, mice were housed in pairs and restricted to 85% of their free-feed weight and maintained on 85% of their weight for the duration of the studies as described (Kolisnyk *et al.*, 2013a,b). All behavioural experiments were conducted between 12:00 and 17:00 h. We followed the ARRIVE guidelines (Kilkenny *et al.*, 2010); hence, mice were randomized for behavioural tests, and the experimenter was blind to the genotype. All procedures were conducted in accordance with

guidelines from the Canadian Council of Animal Care at the University of Western Ontario with an approved institutional animal protocol (2008-127).

Five-choice serial reaction time task training

A cohort of WT and CHRNA7$^{-/-}$ mice ($n = 7$ per genotype, 5–6 months old) was trained in the 5-CSRT task using the automated Bussey–Saksida Mouse Touch Screen System model 81426 (Campden Instruments, Lafayette, IN, USA). Schedules were designed, and data were collected using the ABET II TOUCH software v.2.15 (Lafayette Instruments, Lafayette, IN, USA). Mice were trained to respond to the touch screen chambers using a previously described operant training procedure (Kolisnyk *et al.*, 2013a, b).

Training on the 5-CSRT task was performed as previously described (Romberg *et al.*, 2011). Once the performance of a mouse reached criterion (80% accuracy, 20% omissions for three consecutive days) at 4 s stimulus duration, stimulus duration was reduced to 2 s. After reaching criterion at a 2 s stimulus duration, the mouse was tested on probe trials.

Probe trial

To probe attention in mice, we used a previously described probe trial schedule with reduced stimulus durations (Romberg *et al.*, 2011; Kolisnyk *et al.*, 2013a). Mice were tested for 2 days at a given stimulus duration (1.5, 1, 0.8 or 0.6 s). Each day, sessions lasted 50 trials or 1 h. After each test, the animal was retested at the 2 s stimulus duration for 2 days, until the mice had been tested at all stimulus durations. The order of the probe trial sessions was semi-randomized using a Latin square method. Behavioural data were averaged over the 2 days of each stimulus duration.

Drug injections

For all drug experiments, mice were tested at the 0.6 s stimulus duration. The same mice used for the initial 5-CSRT experiments were used for the drug studies. Animals were injected 30 min before testing for the PHA-543,613 (Sigma-Aldrich, St Louis, MO, USA) and PNU-228,927 (Tocris Bioscience, Bristol, UK) experiments and 15 min before testing for the ABT-418 (Sigma-Aldrich) experiment (McGaughy *et al.*, 1999b). Doses of PHA-543,613 [0.33, 1 and 3 mg kg^{-1}, i.p. (Acker *et al.*, 2008)], PNU-282,927 [1, 3 and 5 mg kg^{-1}, i.p. (Hajos *et al.*, 2005; Vicens *et al.*, 2013)] and ABT-418 [0.04, 0.13 and 0.39 mg kg^{-1}, i.p. (McGaughy *et al.*, 1999a)] were chosen based on previous studies. In control experiments, vehicle (saline) was injected. The order of drug injections was semi-randomized using a Latin square method. Between different doses in the drug injection experiments, mice were given two washout days during which they were baselined with a 2 s stimulus duration.

Analysis of 5-CSRT task

On all 5-CSRT task sessions, accuracy was calculated as the number of correct responses divided by the number of correct and incorrect responses (touches to a wrong window while the correct stimulus was still displayed). Omissions were calculated as the total number of omitted trials divided by the number of total trials. Response latency was the time the mouse took to touch the correct stimulus after the onset of its display. Reward collection latency was defined as the time it took the mouse to enter the reward magazine following a correct response. A premature response was counted when the mouse touched one of the windows prior to the stimulus being displayed. Finally, a perseverative response was any identical response that occurred following a correct, incorrect or premature response.

Food intake in food-deprived mice

Feeding behaviour was analysed as previously described (Semenova and Markou, 2007). Naive groups of WT and CHRNA7$^{-/-}$ mice ($n = 8$ per genotype) were housed individually and were deprived of food overnight before the test. During the test, mice were placed in a clean cage and given 20 g of standard chow. Food intake was measured 20, 40, 60 and 80 min after the start of the test. Food intake was normalized to the body weight of the animals.

Quantitative PCR

Total RNA was extracted from freshly dissected PFC tissue, using the Aurum Total RNA for fatty and fibrous tissue kit (Bio-Rad Laboratories, Hercules, CA, USA); cDNA synthesis and quantitative PCR (qPCR) analysis of nicotinic receptor expression were performed as previously described (Guzman *et al.*, 2011; Kolisnyk *et al.*, 2013a).

Western blotting

Western blotting was performed as previously described (Martins-Silva *et al.*, 2011). For analysis of phospho-proteins in the PFC, mice were given i.p. drug injections and were killed 30 min later. Tissue was then homogenized in lysis buffer supplemented with protease and phosphatase inhibitor cocktails (Thermo Fisher Scientific, Waltham, MA, USA). The antibodies used were anti-vesicular ACh transporter (VAChT) (catalogue #139103; Synaptic Systems, Göttingen, Germany), anti-ChAT (catalogue #1DB-001-0000849693; Millipore, Billerica, MA, USA), anti-synaptophysin (catalogue #S5768; Sigma-Aldrich), anti-ERK1/2 (catalogue #4695; Cell Signaling Technology, Danvers, MA, USA), anti-phospho-ERK1/2 (catalogue #4372; Cell Signaling Technology), anti-cFos (catalogue #4384; Cell Signaling Technology) and anti-β-actin (catalogue #ab49900; Abcam, Cambridge, UK). Band intensity was quantified using FLUOROCHEMQ software (Thermo Fisher Scientific).

Statistical analyses

All data are expressed as mean ± SEM. SIGMASTAT 3.5 (Systat Software, San Jose, CA, USA) was used for all statistical analysis. Comparisons between two experimental groups were made by Student's *t*-test. When several experimental groups or treatments were analysed, one-way ANOVA or two-way ANOVA with repeated-measures tests were used as required. Statistically significant effects were further analysed using Tukey's honestly significant difference *post hoc* tests. In all analyses, $P < 0.05$ was considered statistically significant.

Results

α7nAChR-null mice present normal acquisition on the 5-CSRT task

No difference between CHRNA7$^{-/-}$ mice and WT controls was observed in the number of sessions required to reach criterion at any of the pre-training phases for the 5-CSRT task [Supporting Information Fig. 1; RM-ANOVA: no effect of genotype, $F_{(1,14)} = 2.814$, $P = 0.1156$; main effect of training phase, $F_{(4,14)} = 104.3$, $P < 0.0001$; no interaction effect, $F_{(4,14)} = 1.126$, $P = 0.3535$]. During training on the 5-CSRT task as well, CHRNA7$^{-/-}$ mice took as many sessions as WT controls to achieve criterion at both the 4 and 2 s stimulus durations [RM-ANOVA: no effect of genotype, $F_{(1,14)} = 2.552$, $P = 0.1325$; main effect of stimulus duration, $F_{(1,14)} = 57.78$, $P < 0.0001$; no interaction, $F_{(1,14)} = 4.472$, $P = 0.0529$; Supporting Information Fig. 1B]. It should be noted that there was a strong tendency for the CHRNA7$^{-/-}$ mice to take longer to learn the task at the 4 s stimulus duration, which may reflect previously documented impairments in procedural learning in these mice (Young *et al.*, 2011).

α7nAChR-null mice have impaired sustained attention

Once mice reached criterion at 2 s stimulus duration, we assessed attention performance by using a probe trial, with reduced stimulus durations (1.5, 1, 0.8 and 0.6 s stimulus durations) as previously described (Romberg *et al.*, 2011). Across all four stimulus durations, CHRNA7$^{-/-}$ mice performed similarly to controls in both total measures of omissions [RM-ANOVA: no effect of genotype, $F_{(1,36)} = 3.235$, $P = 0.0972$; main effect of stimulus duration, $F_{(3,36)} = 14.50$, $P < 0.001$; no interaction, $F_{(3,36)} = 0.5136$, $P = 0.6755$; Figure 1A] and accuracy [RM-ANOVA: no effect of genotype, $F_{(1,36)} = 0.06134$, $P = 0.8086$; main effect of stimulus duration, $F_{(3,36)} = 9.208$, $P < 0.001$; no interaction, $F_{(3,36)} = 0.6347$, $P = 0.5975$; Figure 1B]. To assess sustained attention, we analysed rate of omissions and response accuracy over blocks of 25 trials across the various stimulus durations of the probe trial experiment. As each probe trial session ends after 50 trials or 1 h, analysing blocks of 25 trials divided the performance between two halves: block A and block B. This procedure allowed us to determine if mice can sustain attention during the full period of the probe trial and maintain performance between the first and second periods of testing. Performance of control WT mice did not differ across the probe trial in terms of omissions [RM-ANOVA: no difference between blocks A and B, $F_{(1,6)} = 1.904$, $P = 0.2168$; main effect of stimulus duration, $F_{(3,18)} = 8.661$, $P < 0.001$; and no interaction, $F_{(3,18)} = 0.4736$, $P = 0.7045$; Figure 1C) or accuracy [RM-ANOVA: no effect of blocks, $F_{(1,6)} = 4.319$, $P = 0.0829$; main effect of stimulus duration, $F_{(3,18)} = 4.897$, $P = 0.0166$; and no interaction, $F_{(3,18)} = 0.4947$, $P = 0.6905$; Figure 1D]. In contrast, CHRNA7$^{-/-}$ mice displayed increased omission errors in the second half of the probe trial experiment compared with the first half [RM-ANOVA: main effect of block, $F_{(1,6)} = 20.59$, $P < 0.001$; main effect of stimulus duration, $F_{(3,18)} = 9.471$, $P < 0.001$; and main interaction effect, $F_{(3,18)} = 12.13$, $P < 0.001$; Figure 1E). *Post hoc* analysis confirmed that CHRNA7$^{-/-}$ mice had significantly more omission errors during the second half of the task at both the 0.8 and 0.6 s stimulus durations, suggesting that these mice display impaired ability to sustain attention. Interestingly, CHRNA7$^{-/-}$ mice did not present accuracy impairments across the two blocks [RM-ANOVA: no effect of block, $F_{(1,6)} = 1.348$, $P = 0.2897$; main effect of stimulus duration, $F_{(3,18)} = 5.877$, $P = 0.0056$; and no interaction effect, $F_{(3,18)} = 0.6404$, $P = 0.5989$; Figure 1F].

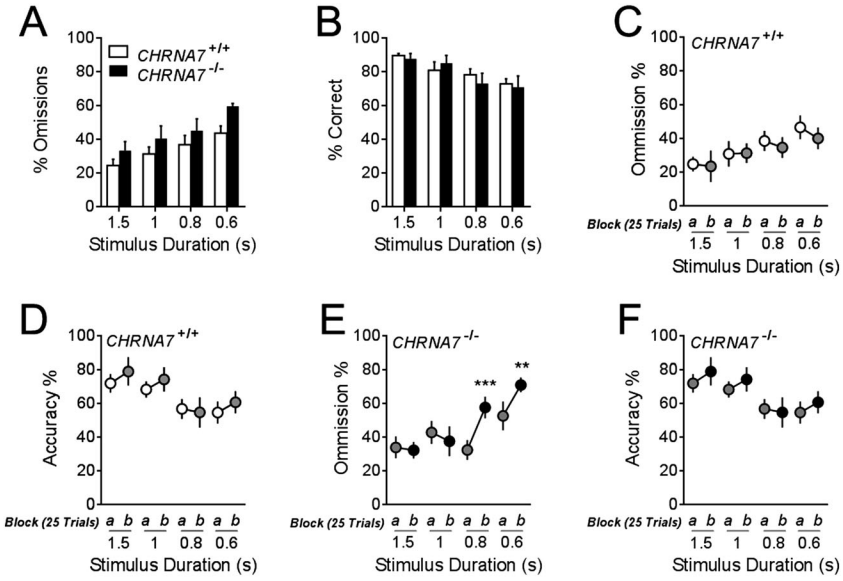

Figure 1

CHRNA7$^{-/-}$ mice have impaired sustained attention. Comparison between genotypes of (A) omissions and (B) accuracy during the probe trial experiment using the 5-CSRT task. (C) Omissions and (D) accuracy across bins of 25 trials in WT mice. (E) Omissions and (F) accuracy across bins of 25 trials in CHRNA7$^{-/-}$ mice (data are presented as mean ± SEM; *$P < 0.05$,**$P < 0.01$, ***$P < 0.001$).

Increases in omission on the 5-CSRT task have been proposed to be due to lack of attention, lack of motivation or motor impairments (Robbins, 2002). Given that CHRNA7$^{-/-}$ mice were no different from controls in terms of latency to respond to stimulus or latency to collect the reward, it is unlikely that motivation or motor impairments are causing the deficits in sustained attention (Robbins, 2002; Spinelli *et al.*, 2004). To address this, we measured several other parameters to test for motivational or aberrant behaviour in CHRNA7$^{-/-}$ mice. α7nAChR-null mice showed no difference in latency to respond to the stimulus [RM-ANOVA: no effect of genotype, $F_{(1,36)} = 0.01533$, $P = 0.9035$; no effect of stimulus duration, $F_{(3,36)} = 2.003$, $P = 0.1310$; no interaction, $F_{(3,36)} = 1.223$, $P = 0.3154$; Figure 2A]. We then assessed response latency across blocks of trials to determine if the sustained attention deficits in CHRNA7$^{-/-}$ mice may be due to delayed responsiveness. Response latency did not differ across blocks for either the WT [RM-ANOVA: no effect of block, $F_{(1,6)} = 0.07218$, $P = 0.7972$; no effect of stimulus duration, $F_{(3,18)} = 2.749$, $P = 0.0792$; and no interaction effect, $F_{(3,18)} = 0.7886$, $P = 0.5159$; Figure 2B] or CHRNA7$^{-/-}$ mice [RM-ANOVA: no effect of block, $F_{(1,6)} = 0.2481$, $P = 0.6362$; no effect of stimulus duration, $F_{(3,18)} = 2.2557$, $P = 0.1166$; and no interaction effect, $F_{(3,18)} = 1.490$, $P = 0.2501$; Figure 2C]. We also assessed the time to retrieve their reward following a correct response [RM-ANOVA, no effect of genotype, $F_{(1,36)} = 0.2025$, $P = 0.6607$; no effect of stimulus duration, $F_{(3,36)} = 1.153$, $P = 0.3410$; no interaction, $F_{(3,36)} = 0.2954$, $P = 0.8284$; Figure 2D] when compared with WT controls. Furthermore, we assessed reward latency across blocks of trials, and neither the WT [RM-ANOVA: no effect of block, $F_{(1,6)} = 2.345$, $P = 0.1766$; no effect of stimulus duration, $F_{(3,18)} = 2.176$, $P = 0.1262$; and no interaction effect, $F_{(3,18)} = 0.5404$, $P = 0.6677$; Figure 2E) nor CHRNA7$^{-/-}$ mice [RM-ANOVA, no effect of block, $F_{(1,6)} = 0.02162$, $P = 0.8879$; no effect of stimulus duration, $F_{(3,18)} = 0.6190$, $P = 0.6617$; and no interaction effect, $F_{(3,18)} = 0.3581$, $P = 0.7839$; Figure 2F) showed alteration in reward collection latency. To test whether CHRNA7$^{-/-}$ mice differ from WT controls in satiety, we measured food intake following food restriction in a group of naive mice. Compared with controls, CHRNA7$^{-/-}$ mice did not differ in food consumption over the course of the test [RM-ANOVA: no effect of genotype, $F_{(1,48)} = 1.280$, $P = 0.2800$; main effect of time, $F_{(4,48)} = 73.88$, $P < 0.001$; and no interaction effect, $F_{(4,48)} = 1.296$, $P = 0.2849$; Figure 2G]. This is in line with previous work showing that these mice have normal motivation (Hoyle *et al.*, 2011) and suggests that CHRNA7$^{-/-}$ mice have specific deficits in sustained attention.

Impulsivity and compulsivity were also assessed in CHRNA7$^{-/-}$ mice during the probe trial experiment. Compared with controls, CHRNA7$^{-/-}$ mice were no different in terms of premature responses, a measure of impulsivity [RM-ANOVA: no effect of genotype, $F_{(1,36)} = 0.9222$, $P = 0.3575$; no effect of stimulus duration, $F_{(3,36)} = 0.4541$, $P = 0.7161$; no interaction effect, $F_{(3,36)} = 0.09521$, $P = 0.9621$; Supporting Information Fig. 2C, D], or perseverative responses, a measure of compulsive behaviour [RM-ANOVA: no effect of genotype, $F_{(1,36)} = 0.04477$, $P = 0.8363$, main effect of stimulus duration, $F_{(3,36)} = 4.105$, $P = 0.0140$; no interaction effect, $F_{(3,36)} = 0.8660$, $P = 0.4685$, Supporting Information Fig. 2C, D].

The ability to release normal levels of ACh is critical to attention (Kolisnyk *et al.*, 2013a,b); therefore, we investigated expression levels of the cholinergic machinery in the PFC of CHRNA7$^{-/-}$ mice. Compared with WT controls, CHRNA7$^{-/-}$ mice showed no significant change in expression of the VAChT [$t_{(4)} = 0.375$, $P = 0.7291$] or ChAT. The sustained attention deficits in CHRNA7$^{-/-}$ mice are therefore not a result of an inherent dysfunction in the machinery required for ACh release (Figure 3A).

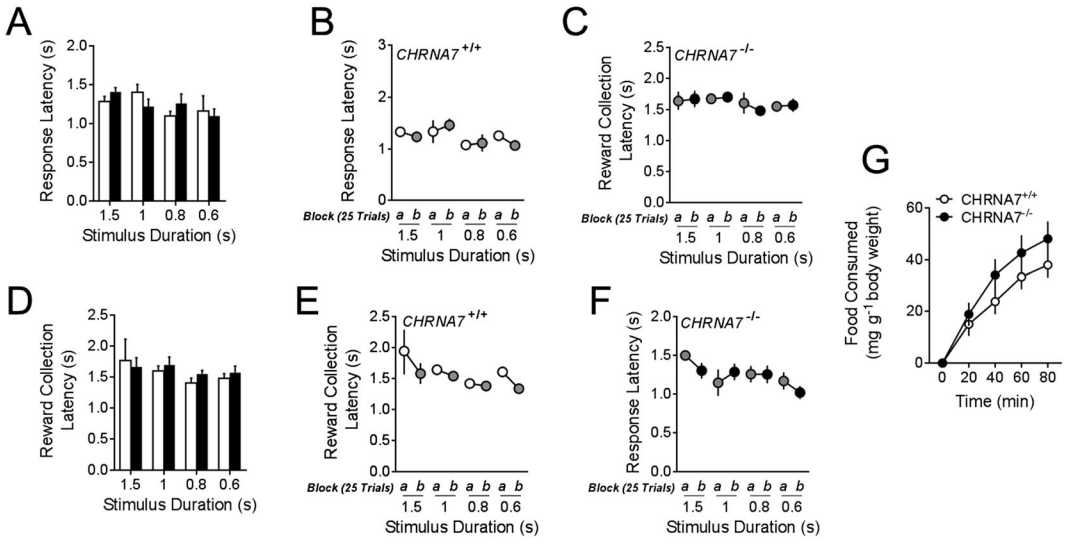

Figure 2

CHRNA7$^{-/-}$ mice have normal motivation and motor function during the 5-CSRT task. Comparison between genotypes of response latencies (A). Response latencies across bins of 25 trials in wild-type (B) and CHRNA7$^{-/-}$ mice (C). Comparison between genotypes of reward collection latencies (D). Reward collection latencies across bins of 25 trials in wild-type (E) and CHRNA7$^{-/-}$ mice (F). (G) Food consumption following food deprivation as a measure of motivation (data are presented as mean ± SEM).

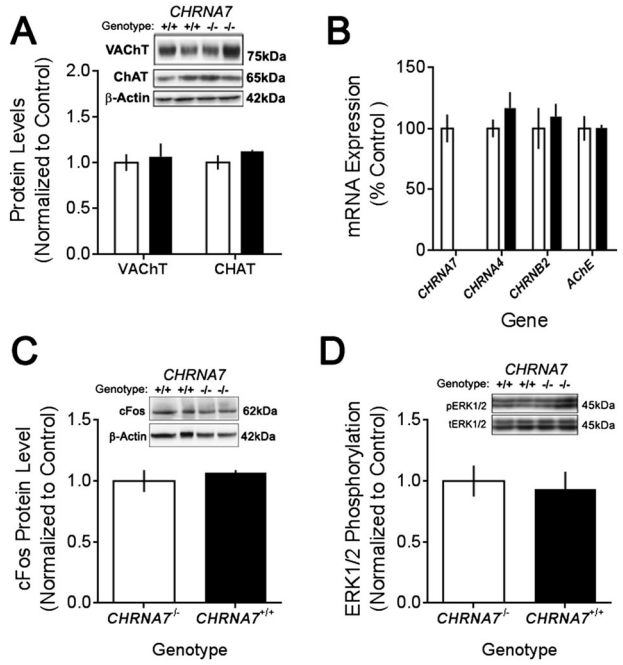

Figure 3

Evaluation of the expression of cholinergic markers and relevant signalling pathways in the PFC of CHRNA7$^{-/-}$ mice. (A) Immunoblot of VAChT and ChAT expression in the PFC. (B) qPCR expression of nicotinic receptors and AChE in the PFC. (C) cFos protein levels and (D) ERK1/2 phosphorylation in the PFC (data are presented as mean ± SEM).

α7 nicotinic ACh receptor deletion has been suggested to cause compensatory changes in other nicotinic receptors during development (Yu *et al.*, 2007). To determine if the PFC of adult CHRNA7$^{-/-}$ mice displayed altered expression of nicotinic receptors, we examined their expression by qPCR analysis. The PFC of CHRNA7$^{-/-}$ mice showed no significant change in the expression of *CHRNA4* [$t_{(8)} = 1.104$, $P = 0.3016$] or of *CHRNB2* expression [$t_{(8)} = 0.4893$, $P = 0.6378$]. In addition, we evaluated the expression of the enzyme AChE and observed no significant difference between genotypes [$t_{(8)} = 0.0409$, $P = 0.9684$]. As expected, we did not detect *CHRNA7* expression in CHRNA7$^{-/-}$ animals (Figure 3B).

To evaluate the biochemical correlates of neuronal activity in the PFC of CHRNA7$^{-/-}$ mice, we determined protein levels of the immediate-early gene cFos, a known marker of activated neurons. Compared with the WT control, CHRNA7$^{-/-}$ mice showed no significant change in cFos protein levels [$t_{(4)} = 0.779$, $P = 0.4792$, Figure 3C]. Moreover, to test if the CHRNA7$^{-/-}$ had impaired activation of relevant second messenger signalling cascades involved with nicotinic response in attention (Wallace and Porter, 2011b), we evaluated the phosphorylation status of ERK1/2 and observed no significant difference between genotypes [$t_{(4)} = 0.331$, $P = 0.7575$, Figure 3D].

Effect of α7nAChR agonists on attention

To evaluate acute roles of α7nAChR in regulating sustained attention behaviour, we investigated two selective α7nAChR agonists, PHA-543,613 (Acker *et al.*, 2008) and PNU-282,987 (Hajos *et al.*, 2005), in WT mice using the 5-CSRT task. Mice were tested at a 0.6 s stimulus duration, which represents a cognitively demanding version of the task (Romberg *et al.*, 2011; Kolisnyk *et al.*, 2013a). PHA-543,613 significantly improved rate of omissions [RM-ANOVA: main effect of dose, $F_{(3,18)} = 12.52$, $P < 0.001$; Figure 4A], with *post hoc* analysis confirming that the 1 mg kg^{-1} dose significantly improved performance over saline. Conversely, PHA-543,613 significantly altered response accuracy in higher doses [RM-ANOVA: main effect of dose, $F_{(3,18)} = 12.55$, $P < 0.001$; Figure 4B]. *Post hoc* analysis revealed that at the highest dose tested (3 mg kg^{-1}), PHA-543,613-injected mice performed significantly worse than mice injected with saline. PHA-543,613 did not significantly alter response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.568$, $P = 0.2318$, Supporting Information Fig. 3A] or reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.7517$, $P = 0.5382$; Supporting Information Fig. 3B]. In addition, PHA-543,613 did not alter premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.7599$, $P = 0.4930$) nor perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1404$, $P = 0.8821$, Supporting Information Fig. 3C, D]. To address the effects of PHA-543,613 on sustained attention, we analysed performance of mice over blocks of 25 trials and observed that PHA-543,613 did not significantly alter sustained omissions between the two blocks of testing [RM-ANOVA: main effect of dose, $F_{(3,36)} = 13.20$, $P < 0.0001$; no effect of block, $F_{(1,12)} = 0.7069$, $P = 0.4327$; and no interaction, $F_{(3,36)} = 1.288$, $P = 0.3088$; Figure 4C], nor did it alter accuracy in WT mice [RM-ANOVA: main effect of dose, $F_{(3,36)} = 14.63$, $P < 0.0001$; no effect of block, $F_{(1,12)} = 1.729$, $P = 0.101$; and no interaction effect, $F_{(3,18)} = 0.0713$, $P = 0.9749$; Figure 4D). At 1 mg kg^{-1}, the percentage of omissions seemed to be slightly reduced in the second block, suggesting modest improvement in the performance.

The second α7nAChR agonist tested, PNU-282,987, also significantly improved rate of omissions [RM-ANOVA: main effect of dose, $F_{(3,18)} = 2.767$, $P = 0.0437$, Figure 4E], with *post hoc* analysis confirming that both the 1 and 3 mg kg^{-1} doses significantly improved performance over saline. Conversely, PNU-282,978 significantly altered response accuracy [RM-ANOVA: main effect of dose, $F_{(3,18)} = 5.637$, $P = 0.0066$; Figure 4F). *Post hoc* analysis revealed that at the highest dose tested (5 mg kg^{-1}) mice injected with PNU-282,978 performed significantly worse than mice injected with saline. PNU-282,978 did not significantly alter response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.9985$, $P = 0.4018$; Supporting Information Fig. 3E] nor reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.131$, $P = 0.3375$; Supporting Information Fig. 3F]. PNU-282,978 did not alter the number of premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 3.015$, $P = 0.1157$] or perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.4522$, $P = 0.6707$, Supporting Information Fig. 3G, H]. In terms of the effects of PNU-282,978 on sustained attention, analysis of injected mice over two blocks of 25 trials showed that PNU-282,978 did alter the rate of omissions, with 3 mg kg^{-1} improving omission rates over the two blocks [RM-ANOVA: main effect of dose, $F_{(3,36)} = 6.095$, $P = 0.0031$; no effect of block, $F_{(1,12)} = 0.5240$, $P = 0.4761$; and main interaction effect, $F_{(3,36)} = 3.218$, $P = 0.0407$; Figure 4G], but did not alter sustained accuracy [RM-ANOVA: main effect of

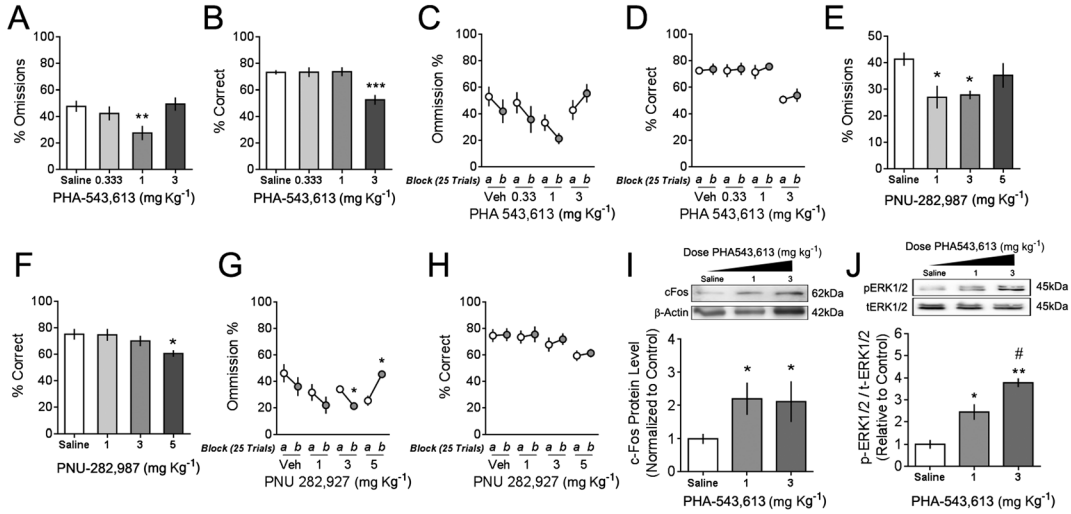

Figure 4

α7nAChR agonists improve attention in wild-type mice. (A) Omission and (B) accuracy following injections of PHA-543,613 in WT mice. (C)Omissions and (D) accuracy over bins of 25 trials following administration of PHA-543,613. (E) Omission and (F) accuracy following injections of PNU-282,987 in WT mice. (G) Omissions and (H) accuracy over bins of 25 trials following administration of PNU-282,987. (I) cFos protein levels and (J) ERK1/2 phosphorylation following injection of PHA-543,613 (data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

dose, $F_{(3,36)} = 6.044$, $P = 0.0019$; no effect of block, $F_{(1,12)} = 0.222$, $P = 0.6458$; and no interaction effect, $F_{(3,18)} = 0.0701$, $P = 0.9755$; Figure 4H].

To evaluate the biochemical correlates of acute α7nAChR agonist activation in the PFC, we injected PHA-543,613 on WT mice and determined protein levels of cFos and the phosphorylation status of ERK1/2. These experiments used a separate cohort of mice, which were injected with drug or saline and then killed 30 min later and had their PFC dissected to obtain protein extracts. Compared with saline, PHA-543,613 injected in mice led to a significant increase in the levels of cFos protein in their PFC [one-way ANOVA: main effect of dose, $F_{(2,6)} = 7.404$, $P = 0.0240$; Figure 4I], with *post hoc* analysis showing that cFos levels were increased at both doses of PHA-543,613. Similarly, injections of PHA-543,613 significantly increased ERK1/2 phosphorylation levels in a dose-dependent way [one-way ANOVA: main effect of dose, $F_{(2,6)} = 28.80$, $P < 0.001$; Figure 4J].

Positive and negative effects of α7nAChR agonists are abolished in CHRNA7−/− mice

To confirm the specificity of both PHA-543,613 and PNU-282,987 for α7nAChRs, we administered both compounds to CHRNA7$^{-/-}$ mice prior to testing them on the 5-CSRT task with a 0.6 s stimulus duration. Compared with saline, PHA-543,613 had no effect on the performance of the mice at any dose tested. PHA-543,613 did not alter rate of omissions [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.528$, $P = 0.2515$; Figure 5A] or response accuracy [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1121$, $P = 0.8733$; Figure 5B], response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.2490$, $P = 0.7348$; Supporting Information Fig. 4A] or reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.3018$, $P = 0.6621$; Supporting Information Fig. 4B]. PHA-543,613 did not alter the number of premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.104$, $P = 0.3579$] or perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 2.101$, $P = 0.1738$, Supporting Information Fig. 4C, D]. Furthermore, when we analysed performance over blocks of 25 trials, we observed that PHA-543,613 did not alter impaired omission deficit of CHRNA7$^{-/-}$ mice at 0.6 s, which remained significantly higher in block B across all doses [RM-ANOVA: no effect of dose, $F_{(3,36)} = 1.528$, $P = 0.2414$; main effect of block, $F_{(1,12)} = 14.89$, $P = 0.0084$; and no interaction $F_{(3,12)} = 0.2209$, $P = 0.8806$; Figure 5C]. PHA-543,613 did not alter sustained accuracy in CHRNA7$^{-/-}$ mice either [RM-ANOVA: no effect of dose, $F_{(3,36)} = 0.2017$, $P = 0.8945$; no effect of block, $F_{(1,12)} = 0.00701$, $P = 0.9343$; and no interaction, $F_{(3,36)} = 0.02177$, $P = 0.9956$; Figure 5D].

As with the PHA-543,613, PNU-282,987 had no effect on the performance of CHRNA7$^{-/-}$ mice at any of the tested doses. The drug did not alter rate of omissions [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1515$, $P = 0.9277$; Figure 5E], response accuracy [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1458$, $P = 0.9310$; Figure 5F], response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.0586$, $P = 2.808$, Supporting Information Fig. 4E] or reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.8089$, $P = 0.4603$, Supporting Information Fig. 4F]. PNU-282,978 did not alter the number of premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.2767$, $P = 0.8316$] or perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.2218$, $P = 0.8036$, Supporting Information Fig. 4G, H]. Also, analysis of performance of injected mice over blocks of 25 trials showed that the α7nAChR agonist had no effect on the impaired omission deficit that we consistently observed on CHRNA7$^{-/-}$ mice [RM-ANOVA: main effect of block, $F_{(1,6)} = 9.112$, $P = 0.0234$; no effect of dose, $F_{(3,18)} = 0.2542$, $P = 0.8573$; and no interaction, $F_{(3,18)} = 0.2546$, $P = 0.8570$; Figure 5G] nor on sustained accuracy [RM-ANOVA: no effect of blocks,

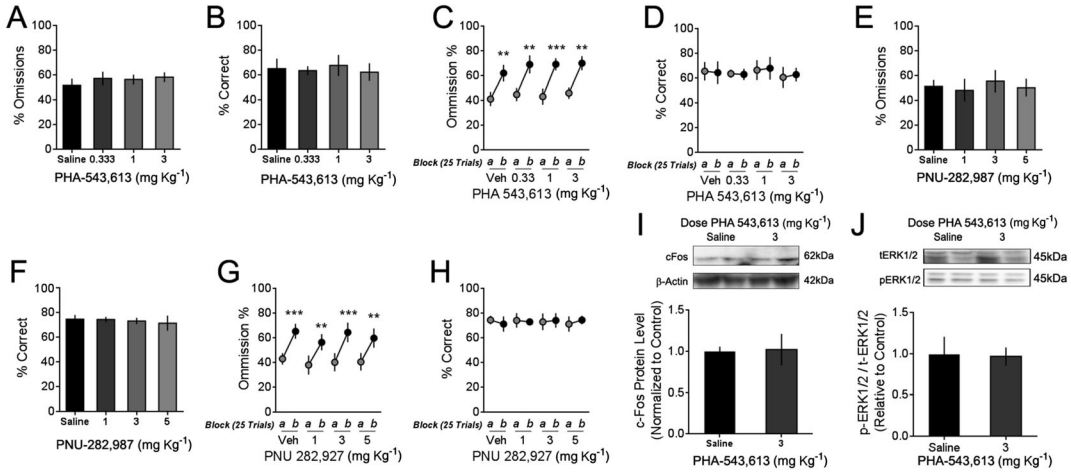

Figure 5

α7nAChR agonists do not alter attention in mice lacking α7nAChR. (A) Omission and (B) accuracy following injections of PHA-543,613 in CHRNA7$^{-/-}$ mice. (C) Omissions and (D) accuracy over bins of 25 trials following administration of PHA-543,613 in CHRNA7$^{-/-}$ mice. (E) Omission and (F) accuracy following injections of PNU-282,987 in CHRNA7$^{-/-}$ mice. (G) Omissions and (H) accuracy over bins of 25 trials following administration of PNU-282,987. (I) cFos protein levels and (J) ERK1/2 phosphorylation following injection of PHA-543,613 (data are presented as mean ± SEM; *$P < 0.05$,**$P < 0.01$, ***$P < 0.001$).

$F_{(1,6)} = 0.0258$, $P = 0.9945$; no effect of dose, $F_{(3,18)} = 1.123$, $P = 0.3729$; and no interaction, $F_{(3,18)} = 0.2665$, $P = 0.8491$; Figure 5H]. Taken together, these results demonstrate that modulation of attention performance on the 5-CSRT task by both PHA-543,613 and PNU-282,987 depends on their activity on α7nAChR.

Additionally, to confirm the selectivity of the molecular changes observed following PHA-543,613 administration in WT mice, we injected CHRNA7$^{-/-}$ mice with the highest dose of the drug (3 mg kg^{-1}) and then, 30 min later, measured the effects on cFos protein levels and ERK1/2 phosphorylation in the PFC. Unlike PHA-543,613-injected WT mice, CHRNA7$^{-/-}$ mice exhibited no change in cFos levels [$t_{(4)} = 0.387$, $P = 0.7186$; Figure 5I] or ERK1/2 phosphorylation [$t_{(4)} = 0.1029$, $P = 0.9230$; Figure 5J], suggesting that both the behaviour and molecular effects of the drug are specific to activation of α7nAChR.

The β2nAChR agonist ABT-418 improves attention

In order to explore the relationship between distinct types of nicotinic receptors on attentional performance, we used ABT-418, a β2nAChR agonist, and treated WT mice that were tested with the 0.6 s stimulus duration paradigm. Injections of ABT-418 were able to significantly improve both rate of omissions [RM-ANOVA: main effect of dose, $F_{(3,18)} = 4.544$, $P = 0.0132$; Figure 6A] and response accuracy [RM-ANOVA: main effect of dose, $F_{(3,18)} = 6.950$, $P = 0.0020$; Figure 6B] without altering response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.06377$, $P = 0.9014$; Supporting Information Fig. 5A] or reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.2936$, $P = 0.8797$; Supporting Information Fig. 5B]. ABT-418 did not alter the number of premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.228$, $P = 0.3103$] or perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.3062$, $P = 0.6764$, Supporting Information Fig. 5C, D]. To evaluate the effects of ABT-418 on sustained attention, we analysed accuracy and omissions across blocks of 25 trials. ABT-418 did not significantly alter sustained omissions across blocks for WT mice [RM-ANOVA: no effect of block, $F_{(1,6)} = 0.6582$, $P = 0.6013$; main effect of dose, $F_{(3,18)} = 0.2542$, $P = 0.8573$; and no interaction, $F_{(3,18)} = 0.6582$, $P = 0.5847$; Figure 6C].

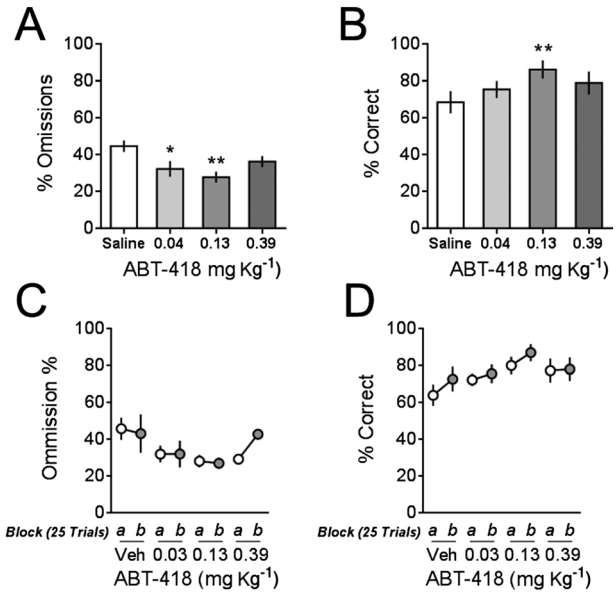

Figure 6

β2nAChR agonists improve attention in wild-type mice. (A) Omission and (B) accuracy following injections of ABT-418 in WT mice. (C) Omissions and (D) accuracy over bins of 25 trials following administration of ABT-418 in WT mice (data are presented as mean ± SEM; *$P < 0.05$,**$P < 0.01$.

ABT-418 had no effect on sustained accuracy in WT mice, with the improvements brought on by the drug spanning across both blocks of trials [RM-ANOVA: no effect of block, $F_{(1,6)} = 0.7267$, $P = 0.4083$; main effect of dose, $F_{(3,18)} = 7.744$, $P = 0.0003$; and no interaction $F_{(3,18)} = 1.084$, $P = 0.3662$; Figure 6D).

Given that nicotinic receptors may be expressed in similar populations of neurons and could crosstalk (Azam *et al.*, 2003), we evaluated whether β2nAChR receptor activation could impact attention in mice lacking α7nAChR. As with the WT mice, ABT-418 was able to significantly improve both omissions [RM-ANOVA: main effect of dose, $F_{(3,18)} = 5.466$, $P = 0.0066$; Figure 7A] and accuracy [RM-ANOVA: main effect of dose, $F_{(3,18)} = 3.383$, $P = 0.0373$; Figure 7B] in CHRNA7$^{-/-}$ mice, without altering response latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 1.622$, $P = 0.2495$; Supporting Information Fig. 6A] or reward collection latency [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.8793$, $P = 0.4359$; Supporting Information Fig. 6B]. ABT-418 did not alter the number of premature [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1450$, $P = 0.81133$] or perseverative responses [RM-ANOVA: no effect of dose, $F_{(3,18)} = 0.1254$, $P = 0.8336$; Supporting Information Fig. 6C, D]. Importantly, ABT-418 was able to reverse the sustained attention deficits observed in CHRNA7$^{-/-}$ mice (Figure 7C) and improved the sustained omission deficits in these mice [RM-ANOVA: main effect of block, $F_{(1,6)} = 11.82$, $P = 0.0138$; main effect of dose, $F_{(3,18)} = 7.640$, $P = 0.0017$; and no interaction effect, $F_{(3,18)} = 1.707$, $P = 0.2013$; Figure 7C]. *Post hoc* analysis revealed that this occurred even at the lowest dose administered. Sustained accuracy was not altered [RM-ANOVA: no effect of block, $F_{(1,6)} = 0.1284$, $P = 0.7324$; main effect of dose, $F_{(3,18)} = 5.017$, $P = 0.0106$; and no interaction, $F_{(3,18)} = 1.054$, $P = 0.3929$; Figure 7D].

To determine the biochemical correlates of treatment with ABT-418 on CHRNA7$^{-/-}$ mice, we injected a new cohort of CHRNA7$^{-/-}$ mice with 0.39 mg kg^{-1} of ABT-418 and 30 min later evaluated cFos and ERK1/2 phosphorylation levels in the PFC of the mice. Compared with saline-injected mice, CHRNA7$^{-/-}$ mice injected with ABT-418 showed a significant increase in cFos protein levels 30 min after injection [$t_{(4)} = 5.610$, $P = 0.0050$; Figure 7E]. ABT-418 was also able to significantly increase ERK1/2 phosphorylation levels in the PFC of mice lacking α7nAChR [$t_{(4)} = 5.300$, $P = 0.0061$; Figure 7F].

Importantly, given that the mice had been exposed to the task numerous times, we evaluated the performance of the mice over the course of the various injections in order to ensure that the improvements brought on by the ABT-418 were not due to the mice becoming better at the task. We compared the performance (both rates of omission and accuracy) of the mice from the vehicle injections of each drug experiment with their naive performance (the performance at a 0.6 s stimulus duration during the probe trial experiments). Both the WT {omission [one-way ANOVA: no effect of treatment, $F_{(6,18)} = 0.7467$, $P = 0.4692$; Supporting Information Fig. 7A] and accuracy [one-way ANOVA: no effect of treatment, $F_{(6,18)} = 0.6749$, $P = 0.6716$; Supporting Information Fig. 7B]} and CHRNA7$^{-/-}$ mice {omission [one-way ANOVA: no effect of treatment, $F_{(6,18)} = 2.565$, $P = 0.1154$; Supporting Information Fig. 7C] and accuracy [one-way ANOVA: no effect of treatment, $F_{(6,18)} = 1.005$, $P = 0.3876$; Supporting Information Fig. 7D]} demonstrated no significant change in performance in both omissions and accuracy from their naive performance across all injections.

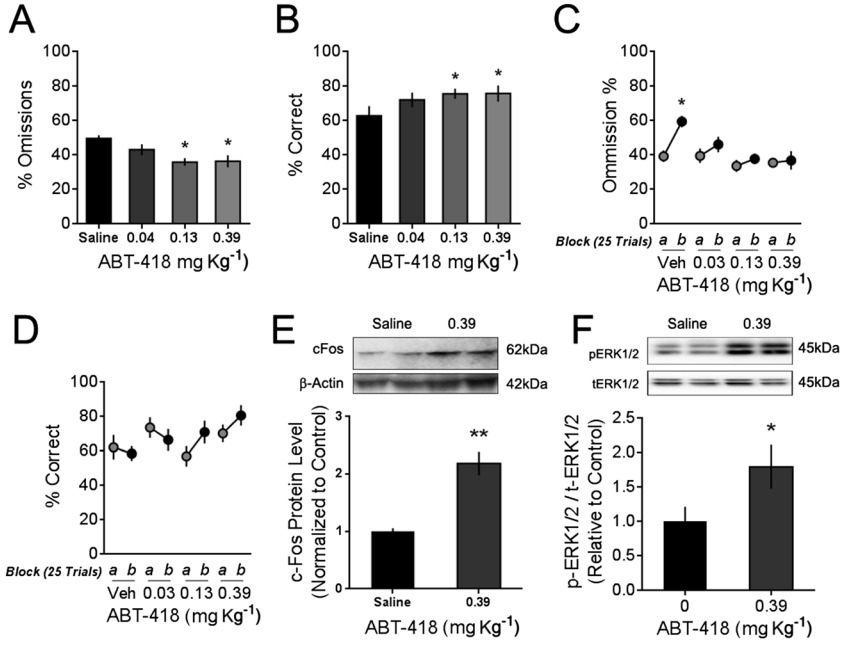

Figure 7

Sustained attention deficits of CHRNA7 null mice are reversed by β2nAChR agonists. (A) Omission and (B) accuracy following injections of ABT-418 in CHRNA7$^{-/-}$ mice. (C) Omissions and (D) accuracy over bins of 25 trials following administration of ABT-418. (E) cFos protein levels and (F) ERK1/2 phosphorylation following injection of ABT-418 (data are presented as mean ± SEM; $^{*}P < 0.05$,$^{**}P < 0.01$.

Discussion

In this study, we demonstrated that the genetic elimination of the *CHRNA7* gene disturbs sustained attentional performance, as measured by the 5-CSRT task, and that this deficit is reversed by administration of ABT-418, a β2nAChR agonist. CHRNA7$^{-/-}$ mice exhibited impaired performance (increased omission errors) during the second half of testing sessions of the 5-CSRT, suggestive of deficits in sustained attention, or vigilance. Increases in omission errors on the 5-CSRT task may reflect either decreased attentional processing or a lack of motivation (Robbins, 2002; Spinelli *et al.*, 2004). However, given the normal performance of CHRNA7$^{-/-}$ mice on the food intake test, this phenotype is unlikely to represent a motivational issue, suggesting that the lack of α7nAChRs impairs the ability to maintain performance levels during the task. Pharmacological activation of α7nAChRs in WT mice by two distinct α7nAChR agonists, PHA-543,613 and PNU-282,987, in lower doses improved attentional performance but did not change sustained attention. These effects were specific to their actions on α7nAChRs, as these compounds were ineffective on CHRNA7$^{-/-}$ mice. Pharmacological activation of the β2nAChR by ABT-418 was able to reverse the sustained attention deficit in CHRNA7$^{-/-}$ mice, suggesting that the deficits observed in these mice can be rescued by β2nAChR signalling.

Post-mortem analysis of human patient samples has shown that ERK1/2 MAP kinase signalling is reduced in brains of schizophrenic patients (Yuan *et al.*, 2010). Moreover, an inability to induce phosphorylation of ERK1/2 MAP kinases in the PFC is thought to underlie certain cognitive deficits in animal models of schizophrenia (Kamei *et al.*, 2006). α7nAChR activation has been shown to induce phosphorylation of ERK1/2 MAP kinases both *in vitro* and *in vivo* (Bitner *et al.*, 2007). Similar effects have been observed in second-generation antipsychotics (Lu *et al.*, 2004). We showed that pharmacological activation of the α7nAChR by PHA-543,613 induced a dose response increase in both ERK1/2 phosphorylation levels and cFos in WT mice. These effects were not detected in CHRNA7$^{-/-}$ mice. Whether this biochemical correlation of α7nAChR activation relates to the biochemical and attentional deficits observed in schizophrenia is unknown. Interestingly, when CHRNA7$^{-/-}$ mice were treated with the β2nAChR agonist ABT-418, both ERK1/2 phosphorylation and cFos protein levels were increased and were correlated with the reversal of the sustained attention deficits. Although it is currently unclear if the neurons that respond to ABT-418 and the α7nAChR drugs PHA-543,613 and PNU-282,987 are the same, one potential important implication of the behavioural data is that ABT-418 can reverse the sustained attention deficits due to abnormal *CHRNA7* expression.

Interestingly, in WT mice, all α7nAChR targeting drugs used presented an inverted 'U'-shaped behavioural response. This is not uncommon with nicotinic signalling with similar responses observed across cognitive domains and even species (Picciotto, 2003; Olincy *et al.*, 2006; Wallace *et al.*, 2011a; Braida *et al.*, 2013). Desensitization of the receptor is often suggested as a potential mechanism underlying this U-shaped behavioural response. Our data suggest that this may not be the case for α7nAChR activation, given that we see increases in the levels of phospho-ERK1/2 following administration of a high dose of α7nAChR agonist in WT mice, which results in poor performance on the 5-CSRT task. Interestingly, it has been proposed that overactivity of this second messenger signalling pathway can actually impair executive function and lead to distractibility (Birnbaum *et al.*, 2004). We have also recently shown that increased cholinergic tone by overexpression of VAChT and increased cholinergic signalling in BAC ChAT-Chr2-EYFP mice disturbs attentional processing (Kolisnyk *et al.*, 2013b).

The ability of nicotine to improve attention has been well documented in rodents (Young *et al.*, 2004), non-human primates (Prendergast *et al.*, 1998) and humans (Lawrence *et al.*, 2002). A common technique to evaluate the role of α7nAChR signalling in attention has been to co-treat rodents with both nicotine and the α7nAChR antagonist MLA. These studies have, however, provided mixed results. Some investigators obtained evidence for α7nAChR signalling in the pro-attentive effects of nicotine (Hahn *et al.*, 2011), and others failed to implicate α7nAChR signalling in nicotine-induced improvements in attention (Grottick and Higgins, 2000). Studies using AR-R17779, a full agonist of the α7nAChR, have failed to demonstrate pro-attentive effects of α7nAChR stimulation (Grottick and Higgins, 2000; Grottick *et al.*, 2003; Hahn *et al.*, 2003). This compound, however, has also been shown to poorly penetrate the blood brain barrier (Mullen *et al.*, 2000). On the other hand, R3487/MEM 3454, an α7nAChR agonist and 5-HT$_3$ receptor antagonist, has been shown to improve measures of sustained attention in both rats (Rezvani *et al.*, 2009) and macaque monkeys (Wallace *et al.*, 2009). Our experiments utilized both PHA-543,613 and PNU-282,987, α7nAChR agonists, which have been previously reported to easily cross the blood brain barrier (Acker *et al.*, 2008). Indeed, the biochemical activation reflected by increased cFos levels or phospho-ERK supports the contention that these drugs were able to activate the PFC in mice. Importantly, our data further support results from previous studies, suggesting that α7nAChR signalling has a role in sustained attention (Young *et al.*, 2007), specifically characterized by increased omission errors on the 5-CSRT task in α7nAChR-null mice (Young *et al.*, 2004).

Cholinergic transients in the PFC have been shown to be important for cue detection and attentional processing (Parikh *et al.*, 2007). α7nAChR activation increases the duration of these transients 10–15-fold, and interestingly, this effect is lost when dopaminergic afferents to the PFC are eliminated, suggesting a complex interplay between neurotransmitter systems (Parikh *et al.*, 2010).

An important role of nicotinic receptors in the CNS is to influence the release of other neurotransmitters. Nicotinic receptors have been shown to influence the release of glutamate (Gioanni *et al.*, 1999), dopamine (Zhou *et al.*, 2001), GABA (Alkondon *et al.*, 1999), noradrenaline (Fu *et al.*, 1998), 5-HT (Kenny *et al.*, 2000) and ACh itself (Rowell and Winkler, 1984). Efflux of all of these neurotransmitters in PFC has been associated with performance on the 5-CSRT task (reviewed in Robbins, 2002). Electron microscopy studies point to ACh release potentially being auto-regulated by presynaptic α7nAChRs in the PFC (Duffy *et al.*, 2009). On the other hand, post-synaptic β2nAChRs have been shown to be necessary and sufficient to regulate performance on the 5-CSRT task (Guillem *et al.*, 2011; Poorthuis and Mansvelder, 2013). In addition to the possibility that α7 and β2 receptors can form functional heteromeric receptors (Liu *et al.*, 2009; Moretti *et al.*, 2014), our data reveal a complex interplay between these two receptors in regulating sustained attention. Given that our results suggest that activation of

β2nAChRs can bypass α7nAChRs, it is possible that activation of α7nAChRs could induce ACh release in the PFC, which would then activate post-synaptic β2nAChRs to regulate sustained attention. If this model is correct, it may explain the inconsistency amongst studies using non-selective nicotinic agonists and antagonists. Co-treatment with nicotine and methyllycaconitine, a α7nAChR antagonist, would still activate β2nAChRs and thus improve attentive processing. Therefore, these previous experiments would not exclude a role of α7nAChRs in attentional performance.

In conclusion, our data support a role for α7nAChRs in sustained attention and reveal an intricate relationship between distinct nicotinic receptors to regulate attentional performance. Our results indicate that activation of β2nAChRs can bypass attentional deficits due to α7nAChR deficiency, suggesting that β2nAChRs may be an important pharmacological target in cognitive dysfunctions in which impaired α7nAChRs have been implicated, such as schizophrenia and Alzheimer's disease (Parri *et al*., 2011).

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Author contributions

B.K. Designed, performed experiments, analyzed data and wrote the manuscript, MAO performed experiments, VFP and MAMP conceived experiments and wrote paper.

Conflict of interest

The authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Figure S1 Pre-training on the 5-CSRT task. (a) Sessions to criteria during pretraining for the 5-CSRT task. Numbers designate phases of the pre-training (1 – 'habituation', 2 – 'initial touch', 3 – 'must touch', 4 – 'must initiate', 5 – 'punish incorrect'). (b) Sessions to criteria during training on the 5-CSRT task. (Data are presented as mean ± SEM.)

Figure S2 Response patterns did not differ in α7nAChR null mice on the 5-CSRT task probe trial. Premature (a) and perseverative (b) responses between WT (clear bars) and CHRNA7–/– mice (dark bars). (Data are presented as mean ± SEM.)

Figure S3 α7nAChR agonists did not alter response patterns in wild-type mice. Premature responses (a), perseverative responses (b), response (c) and reward collection (d) latencies following PHA-543,613 injections in WT mice. Premature responses (e), perseverative responses (f), response (g) and reward collection (h) latencies following PNU-282,927 injections in WT mice. (Data are presented as mean ± SEM.)

Figure S4 α7nAChR agonists did not alter response patterns in CHRNA7-null mice. Premature responses (a), perseverative responses (b), response (c) and reward collection (d) latencies following PHA-543,613 injections in CHRNA7–/– mice. Premature responses (e), perseverative responses (f), response (g) and reward collection (h) latencies following PNU-282,927 injections in CHRNA7–/– mice. (Data are presented as mean ± SEM.)

Figure S5 ABT-418 did not alter response patterns in wild-type mice. Premature responses (a), perseverative responses (b), response (c) and reward collection (d) latencies following ABT-418 injections in WT mice. (Data are presented as mean ± SEM.)

Figure S6 ABT-418 did not alter response patterns in CHRNA7 null mice. Premature responses (a), perseverative responses (b), response (c) and reward collection (d) latencies following ABT-418 injections in CHRNA7–/– mice. (Data are presented as mean ± SEM.)

Figure S7 Performance of mice did not differ across all drug treatments. Evaluation of accuracy (a) and omissions (b) from vehicle treatments from all drug trials in wild-type mice. Evaluation of accuracy (c) and omissions (d) from vehicle treatments from all drug trials in wild-type mice CHRNA7–/– mice. (Data are presented as mean ± SEM.)