Neuropharmacology 70 (2013) 35–42

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Activation of the α7 nicotinic ACh receptor induces anxiogenic effects in rats which is blocked by a 5-HT_{1a} receptor antagonist

Anshul A. Pandya, Jerrel L. Yakel*

Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, MD F2-08, P.O. Box 12233, NC 27709, USA

ARTICLE INFO

Article history:
Received 4 April 2012
Received in revised form
19 December 2012
Accepted 6 January 2013

Keywords:
5-HT1a receptor (5-HT_{1a} R)
Alpha7 (α7) receptor agonist
Anxiety
Memory and learning
Nicotinic acetylcholine receptors (nAChRs)
Positive allosteric modulators (PAMs)

ABSTRACT

The α7 nicotinic acetylcholine receptor (nAChR) is highly expressed in different regions of the brain and is associated with cognitive function as well as anxiety. Agonists and positive allosteric modulators (PAMs) of the α7 subtype of nAChRs have been shown to improve cognition. Previously nicotine, which activates both α7 and non-α7 subtypes of nAChRs, has been shown to have an anxiogenic effect in behavioral tests. In this study, we compared the effects of the α7-selective agonist (PNU-282987) and PAM (PNU-120596) in a variety of behavioral tests in Sprague Dawley rats to look at their effects on learning and memory as well as anxiety. We found that neither PNU-282987 nor PNU-120596 improved spatial-learning or episodic memory by themselves. However when cognitive impairment was induced in the rats with scopolamine (1 mg/kg), both PNU-120596 and PNU-282987 were able to reverse this memory impairment and restore it back to normal levels. While PNU-120596 reversed the scopolamine-induced cognitive impairment, it did not have any adverse effect on anxiety. PNU-282987 on the other hand displayed an increase in anxiety-like behavior at a higher dose (10 mg/kg) that was significantly reduced by the serotonin 5-HT_{1a} receptor antagonist WAY-100135. However the α7 receptor antagonist methyllycaconitine was unable to reverse these anxiety-like effects seen with PNU-282987. These results suggest that α7 nAChR PAMs are pharmacologically advantageous over agonists, and should be considered for further development as therapeutic drugs targeting the α7 receptors.

Published by Elsevier Ltd.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are cationic-conducting ion channels belonging to the cys-loop ligand-gated ion channel superfamily (Brams et al., 2011; Gay and Yakel, 2007; German et al., 2011; Pandya and Yakel, 2011b; Yakel and Shao, 2004). The neuronal nAChRs are either homomeric or heteromeric pentamers with combinations of α (α2–α10) and β (β2–β4) subunits, which have different pharmacological and biophysical properties (Arias, 2006; Gotti et al., 2007). The nAChRs are involved both in physiological functions (including cognition, reward, motor activity and analgesia) and in pathological conditions such as Alzheimer's disease, Parkinson's disease, some forms of epilepsy, depression, autism and schizophrenia (Gotti and Clementi, 2004; Pandya and Yakel, 2011b; Williams et al., 2011b). The α7 nAChR subtype is one of the most abundant nAChRs in the brain (Flores et al., 1992; Gopalakrishnan et al., 1996; Lindstrom, 1996; Lindstrom et al., 1996; Liu et al., 2009; Nashmi and Lester, 2006; Whiting et al., 1987), and are present at both presynaptic (Alkondon et al., 1997; Chang et al., 2011; McGehee et al., 1995; Mok and Kew, 2006; Radcliffe and Dani, 1998) and postsynaptic sites (Gu and Yakel, 2011; Parri et al., 2011), as well as non synaptic locations (Horiguchi et al., 2009; Koval et al., 2011).

Nicotine, an agonist for all nAChRs, is known to have pro-cognitive effects in both animals as well as humans (Levin et al., 2006; Newhouse et al., 2004; Sacco et al., 2004). The α7 receptor-selective agonists have been found to have a positive effect in a wide range of animal models for cognition (Boess et al., 2007; Feuerbach et al., 2009; Hauser et al., 2009; Meyer et al., 1997; Roncarati et al., 2009; Van Kampen et al., 2004; Wishka et al., 2006; Young et al., 2009). Some of the pro-cognitive effects of nicotine are due to its action on α7 receptors (Levin et al., 2006; Thomsen et al., 2010). PNU-282987 is a selective agonist for α7 receptors that restores auditory gating deficits (Hajos et al., 2005) and impaired sensory gating in rats (Bodnar et al., 2005). In a radial arm maze test, PNU-282987 improved memory performance when injected directly into the frontal cortex (Chan

* Corresponding author. Tel.: +1 919 541 1407; fax: +1 919 316 4653.
E-mail addresses: pandyaaa@niehs.nih.gov (A.A. Pandya), yakel@niehs.nih.gov (J.L. Yakel).

0028-3908/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.neuropharm.2013.01.004

et al., 2007), and PNU-282987 ameliorates scopolamine-induced behavioral changes in a modified continuous Y-maze task in mice (Redrobe et al., 2009). Another study found that a higher dose of PNU-282987 (5 mg/kg) diminished motor activity in the open-field following 5 and 12 days of administration (acute and sub-chronic, respectively) in mice (Vicens et al., 2011). PNU-282987 administered acutely had no effects on acquisition in the Morris water maze, however chronic administration over a period of 5 days showed beneficial effects on retention in the same test (Vicens et al., 2011). In another study which used a novel object recognition test, PNU-282987 reversed phencyclidine (PCP)-induced deficits of two cognitive paradigms relevant to schizophrenia (McLean et al., 2011a).

There is considerable evidence to suggest that nicotine can also modulate anxiety in a dose-dependent manner. In a social interaction test for anxiety, (−)-nicotine has been shown to have an anxiolytic effect at lower doses and an anxiogenic effect at high doses (File et al., 1998). A similar low dose (0.1 mg/kg) of nicotine has different effects at different times after injection, with an anxiogenic effect being observed at 5 min, followed by an anxiolytic effect at 30 min, and a later anxiogenic effect at 60 min (Irvine et al., 1999). In the elevated plus-maze test for anxiety, no effects of (−)-nicotine at lower doses (e.g. <0.1 mg/kg) were found, however an anxiogenic effect at higher doses (0.5 and 1 mg/kg) was seen (Ouagazzal et al., 1999a). An anxiogenic effect of nicotine at high doses was observed in the social interaction test of anxiety, when administered directly to the dorsal hippocampus (File et al., 1998). Nicotine, when injected into the lateral septum, had an anxiogenic effects in both the social interaction and the elevated plus-maze tests (Ouagazzal et al., 1999b), which was antagonized by concomitant administration of WAY-100635, a 5-HT_{1a} receptor inhibitor (Cheeta et al., 2000). Additionally, neuronal nAChR antagonists have been demonstrated to reduce anxiety-like behavior in mice (Roni and Rahman, 2011).

In addition to the orthosteric site ligands, selective allosteric ligands have been discovered for the α7 receptors. (Bertrand and Gopalakrishnan, 2007; Hurst et al., 2005; Thomsen et al., 2010). These ligands that increase the function of α7 receptors by acting outside of the orthosteric ACh site, are known as positive allosteric modulators (PAMs) (Bertrand and Gopalakrishnan, 2007). While these compounds do not activate the receptor by themselves, they do alter the ability of the orthosteric ligand to affect channel gating (Bertrand and Gopalakrishnan, 2007; Kim et al., 2007; Pandya and Yakel, 2011a, 2011b; Williams et al., 2011a). The behavioral effects of a few α7 receptor PAMs have been studied in rodents (Dunlop et al., 2009; Ng et al., 2007; Timmermann et al., 2007). PNU-120596 is an α7 receptor-selective PAM which ameliorates amphetamine-induced auditory gating deficits (Hurst et al., 2005), and MK-801 (a glutamate receptor antagonist) -induced impairments in pre-pulse inhibition in rats (Dunlop et al., 2009). In an attentional set-shifting task, PNU-120596 significantly improved performance of phencyclidine-treated rats, demonstrating that this drug improves cognitive dysfunction in an animal model relevant to schizophrenia (McLean et al., 2011b). However in another behavioral test for cognation, PNU-120596 failed to produce any memory-enhancing effects with either chronic or acute doses (Thomsen et al., 2011).

In this behavioral study, we initially compared the selective α7 agonist PNU-282987 and PAM PNU-120596 for their ability to improve episodic and spatial-learning memory. Furthermore since nicotine (acting through α7 receptors) has been shown to have anxiety-inducing effects (especially at high doses), we wanted to test whether PNU-282987 and PNU-120596 could also induce anxiety. Our results show that α7 agonists at high doses may have an anxiogenic effect that is not observed with α7 PAMs.

2. Material and methods

2.1. Subjects

Male Sprague-Dawley rats were obtained from Charles River Laboratories (Saint-Constant, Québec). The animals were acclimatized for a minimum of 7 days after arrival. All rats used for the behavioral experiments were over 80 days old and weighed a minimum of 300 g. The rats were housed individually in transparent plastic cages (L45 × B20 × H10 cms) with free access to water. They were maintained on a reversed 12-h light/dark cycle (lights on at 2100 h and off at 0900 h). The temperature of the room was maintained at 22 +/− 3°C with humidity at 50 +/− 10%. All the training and tests were performed when the rats were behaviorally active during the dark phase. The rats were examined regularly by a veterinarian and their weights recorded once every week. All procedures were approved and performed in compliance with NIEHS/NIH Humane Care and Use of Animals in Research protocols.

2.2. Drug treatment

The rats were divided into 5 cohorts of 10–12 animals each. Each cohort was trained and used for one of the five different behavioral tests that were conducted. PNU-282987, PNU-120596 and WAY-100135 were purchased from Tocris Inc. (Ellisville, MO, USA). Methyllycaconitine (MLA) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Scopolamine was purchased from Calbiochem-Novachem Corp (La Jolla, CA, USA). All solutions were made fresh in 0.9% sterile saline on the day of the injection. PNU-282987, scopolamine, MLA and WAY-100135 were dissolved in 0.9% saline (sterile), while solutions of PNU-120596 were made in 5% DMSO + 5% Solutol dissolved in 0.9% saline (sterile). A stock solution of 2 mg/ml was prepared and the volume of drug solution injected according to the weight of each individual rat. All drugs were administered via intraperetonial (i.p) injections 40 min before the behavioral test. The route of administration and time between injection and the behavioral test was based on the mean plasma concentration for PNU-282987 (McLean et al., 2011a) and PNU-120596 (McLean et al., 2011b) that were previously reported. The rats were dosed twice a week. Each rat received vehicle treatment followed by different doses of agonist, PAM and scopolamine and PAM or agonist + scopolamine or agonist + MLA. All the tests were done within 5 weeks for all the behavioral paradigms.

2.3. Behavioral tests

2.3.1. 12-Arm radial maze test

All rats used for this test were maintained on a restricted died. The rats were offered 80% of their regular food intake adjusted for body weight. The procedure of conducting this test was similar to that described previously (Timofeeva et al., 2010) with modification. The radial arm maze consisting of 12 arms was located in a room with a variety of environmental cues and an experimenter who always sat in the same position. The maze consisted of a central area 50 cm in diameter and 12 arms which were 10 cm wide and 75 cm long. The end of each arm was closed to ensure that the rats returned to the center between arm choices. Nine out of 12 arms were baited with reward consisting of a piece of Fruity Bites™ Bio-Serv (Frenchtown, NJ, USA) which were placed at the end of each arm. Each rat had a unique pattern of baited and unbaited arms that remained constant throughout the training and testing periods. The rats underwent a minimum of 15 training sessions, in order to achieve a level of performance so that memory function could be assessed reliably.

The rats were tested in the maze for a period of 10 min. Each test session began by placing the rat in the center of the maze where they were free to choose any arm for entry. The rats were then allowed to navigate through the maze where each arm entered was recorded. The parameters scored during the 10 min test sessions were similar to those defined previously for a 16–arm radial maze test (Timofeeva et al., 2010). Working memory, which is memory with changing contents, was differentiated from reference memory, which is memory with constant contents. Working memory errors were defined as the number of times the rat re-entered the initially baited arms. Reference memory errors were recorded each time the rat entered an initially unbaited arm. The session was completed either when the rat had entered all 9 baited arms, or when 10 min had expired.

2.3.2. Novel object recognition test

Episodic memory was evaluated by the novel object recognition test which was conducted in a transparent plexiglas box (L75 × W55 × H40 cm) without bedding. The test objects were made of plastic. The familiar object was translucent plastic containers and novel objects were pipette tip boxes. The objects had no natural significance for the rats. Before being placed in the arena, each object was wiped with 70% ethanol. The objects were placed 10 cm from the back in opposing corners of the arena, and the rats were always placed equidistant and facing both the objects. One day before testing, animals were habituated to the experimental arena with no objects present for 6 min. On the test day 40 min after the injections, the rats were placed in the arena for acquisition phase with two identical objects for a period of 3 min. Each rat was allowed to explore the arena and the objects. After a delay of 5 min (inter-trial time) in the home cage, the rat was returned to the same arena and

presented with two different objects for the retention phase with one familiar object and one novel object for a period of another 3 min. An area of ≤1 cm around each object was defined as a zone of interaction. Exploration time of an object was defined as directing the nose to the object at a distance of ≤1 cm or touching the object with the nose or tongue.

2.3.3. Open field exploration test

Anxiety behavior was measured by the open-field exploration test in a transparent plexiglas box (L75 × W55 × H40 cm). During the test, rats were allowed to move freely for a period of 5 min. In order to evaluate anxiety-like behavior, central and peripheral areas of the apparatus were differentiated. An area of L30 × W20 cm in the center was designated as the center zone. The rat was considered to be in the center zone when any 2 or all 4 of its paws were in it. Two parameters were counted during this test; time spent in the center zone and number on rearing (standing up on hind limbs) during the 5 min period. Only those rats that spent a minimum of 15 s (5% of total time) in the center zone were included in the test.

2.3.4. Novelty suppressed feeding test

The novelty suppressed feeding test was carried out as described by Furmaga et al. (2011) with minor modifications. Rats were food deprived for 24 h before testing. The test was carried out in a transparent plexiglas open field (L75 × W55 × H40 cm). The food pellets were placed in the center of the plexiglas field. The test began when the rat was placed in left front corner of the open field, facing the center. The rats were allowed to be in the field for a period of 10 min. Latency to feed was measured for each rat, which was defined as the time from when the rats were placed in the open field until they ate the pellets. Food consumption during the test was monitored. After the 10 min, the rats were returned to the home cage where they had access to food ad libitum.

2.3.5. Rotarod test

The rotarod test was carried out on Rotamex-5 (Rotarod apparatus from Columbus instruments, Columbus, OH, USA). The rotarod was set at a rotating speed of 1 RPM (revolutions per minute) with an incremental acceleration of 1 RPM every 5 s up to a maximum speed of 40 RPM reached in 195 s. A day before each test, the rats were trained on the rotarod instruments for 3 training session of 5 min each. During the test, the motor response in the rats was observed for a period of 5 min after placing them on the rotating bar. The time required for the rat to fall (latency to fall) from the rotating bar was recorded during the testing session.

2.4. Statistics

Data from the behavioral tests (except the rotarod test) were analyzed using Ordinary one-way ANOVA and Dunnett's multiple comparison post hoc test where all the variables obtained were compared with vehicle injection (control). Additionally, two tailed Student's *t*-test was also used to measure differences between variables for rotarod and other behavioral tests. All statistical differences were deemed significant at the level of $p < 0.05$. Statistical analysis was carried out using GraphPad Prism 6.00 Software (San Diego, CA, USA).

3. Results

3.1. Memory

3.1.1. Actions of α7 ligands on spatial-learning memory

Spatial-learning memory was assessed by the 12-arm radial maze test. PNU-120596, the α7 PAM, was administered at doses of 1, 3 or 10 mg/kg to the rats in order to assess its effects on spatial-learning memory (Fig. 1). By itself, PNU-120596 had no effect on the number of reference (Fig. 1A) or working memory errors (Fig. 1B) at any of the doses that were tested. Next we tested whether PNU-120596 had any effect on memory when it was impaired by scopolamine. Scopolamine, a muscarinic acetylcholine receptor antagonist, is known to induce memory impairment in the rats (Falsafi et al., 2012; Redrobe et al., 2009). Scopolamine (1 mg/kg) induced significant increases in the number of working ($F_{5, 60} = 4.482$, $p = 0.0015$) and reference memory errors ($F_{5, 60} = 5.835$, $p = 0.0002$) in the rats. PNU-120596 (3 mg/kg), which was then co-administered with scopolamine (1 mg/kg), was able to significantly reduce the working ($p = 0.0004$) and reference ($p = 0.0004$) memory errors as compared to dosing with scopolamine (1 mg/kg) alone. The working and reference memory errors in the rats returned to baseline levels as seen with the dosing of vehicle alone.

The α7 agonist PNU-282987, tested at doses of 1 or 10 mg/kg, also did not show any significant effect on reference (Fig. 2A) or working (Fig. 2B) memory errors, similar to what was observed with PNU-120596. We tested whether PNU-282987 had any effect on spatial learning memory when impaired by scopolamine. PNU-282987 (3 mg/kg) co-administered with scopolamine (1 mg/kg) was able to significantly reduce the working ($p = 0.023$) and reference ($p = 0.0063$) memory errors as compared to dosing with scopolamine (1 mg/kg) alone. The working and reference memory errors returned to baseline levels as seen with the dosing of vehicle alone.

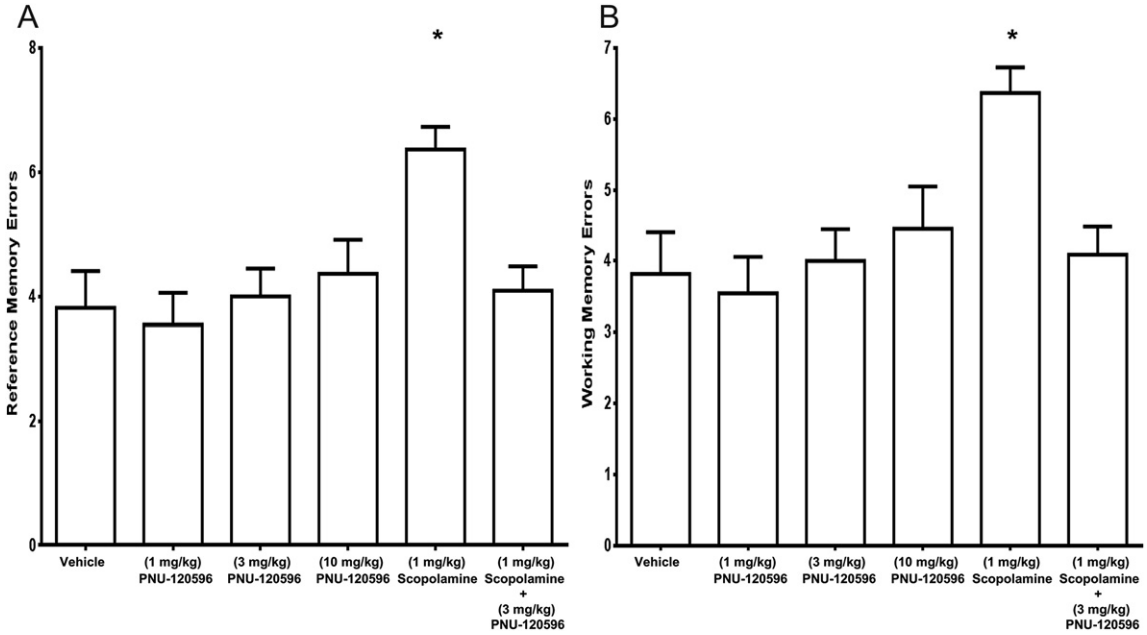

Fig. 1. Effects of PNU-120596 and scopolamine on working (A) and reference (B) memory errors. The data represent mean +/− SEM in 11 rats. * indicates significant difference of $p < 0.05$.

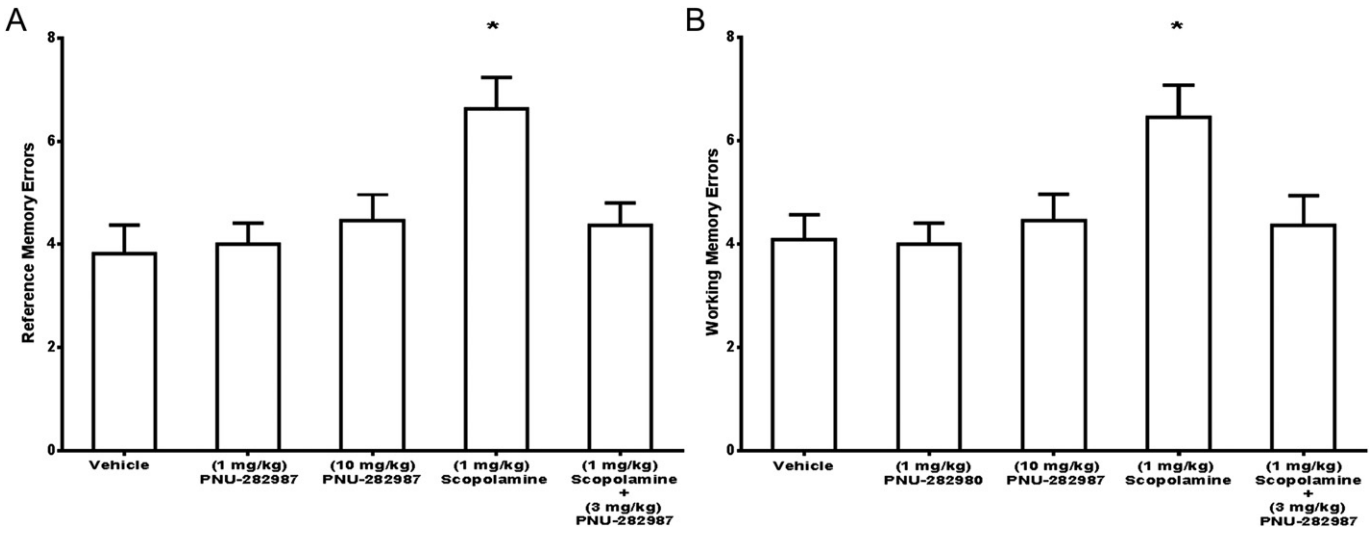

Fig. 2. Effects of PNU-282987 on working (A) and reference (B) memory errors. The data represent mean +/− SEM in 11 rats. * indicates significant difference of $p < 0.05$.

3.1.2. *Actions of α7 ligands on episodic memory*

Episodic memory was evaluated by the novel object recognition test. The exploration time of the novel object was measured during the retention phase of the test (Fig. 3). Similar to its effect on spatial-learning memory, PNU-120596 (Fig. 3) alone did not affect episodic memory in the rats when tested at doses of 1, 3 or 10 mg/kg, however scopolamine (1 mg/kg) caused a significant reduction in the exploration time ($F_{5,\ 54} = 2.844$, $p = 0.0237$). When PNU-120596 (3 mg/kg) was co-administered with scopolamine (1 mg/kg), there was a significant increase in exploration time ($p = 0.0063$) as compared to the dosing with scopolamine (1 mg/kg) alone. The exploration time of the novel object by the rats returned to baseline levels equivalent to that seen with the dosing of the vehicle alone.

Previously, PNU-282987 had been shown to significantly improve the PCP-induced deficit in exploration of a novel object in the novel object recognition test (McLean et al., 2011a). In this study, PNU-282987 (Fig. 4) administered at doses of 1 or 10 mg/kg to the rats, did not show any significant effect on the exploration time. Scopolamine (1 mg/kg) caused a significant reduction in the exploration time ($F_{4,\ 45} = 5.478$, $p = 0.0011$) of the novel object by the rats in the retention phase. Co-administration of PNU-282987 (3 mg/kg) with scopolamine (1 mg/kg) caused a significant increase in exploration time ($p = 0.0007$) of the novel object as compared to the dosing with scopolamine (1 mg/kg) alone. The exploration time of the novel object by the rats returned to baseline levels equivalent to that seen with the dosing of the vehicle alone.

The results from the novel object recognition test performed to assess episodic memory are similar to that obtained from the radial arm maze test for spatial learning memory. While the α7 PAM PNU-120596 and agonist PNU-282987 could not improve episodic and spatial-learning memory by themselves, both were able to reverse the memory impairment induced by scopolamine.

3.2. *Anxiety*

3.2.1. *Actions of α7 ligands in open field exploration*

Open field exploration is a method for studying anxiety-related behavior in rodents (Prut and Belzung, 2003). Here the amount of time spent in the center (Fig. 5) and the number of rearings (Fig. 6)

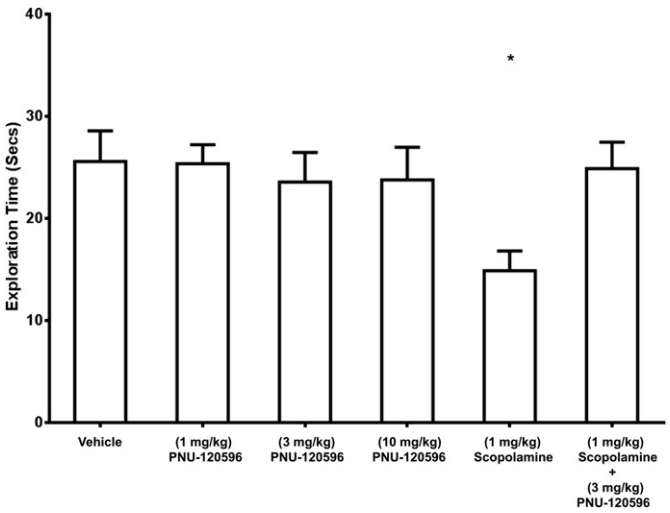

Fig. 3. Effects of PNU-120596 on exploration time in novel object recognition test. The data represent mean +/− SEM in 10 rats. * indicates significant difference of $p < 0.05$.

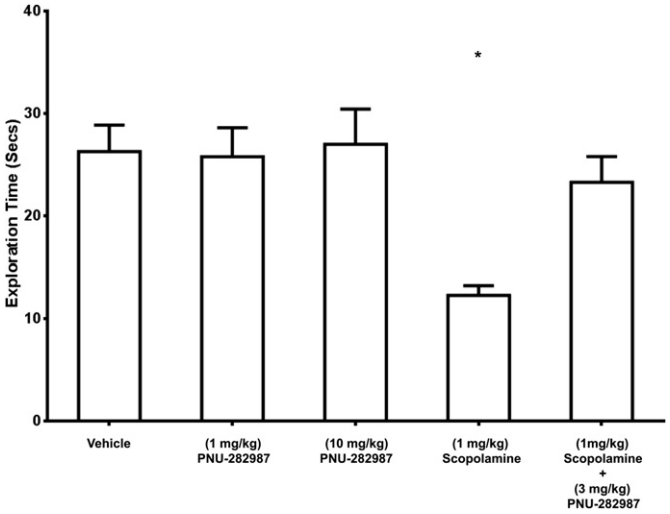

Fig. 4. Effects of PNU-282987 on exploration time in novel object recognition test. The data represent mean +/− SEM in 10 rats. * indicates significant difference of $p < 0.05$.

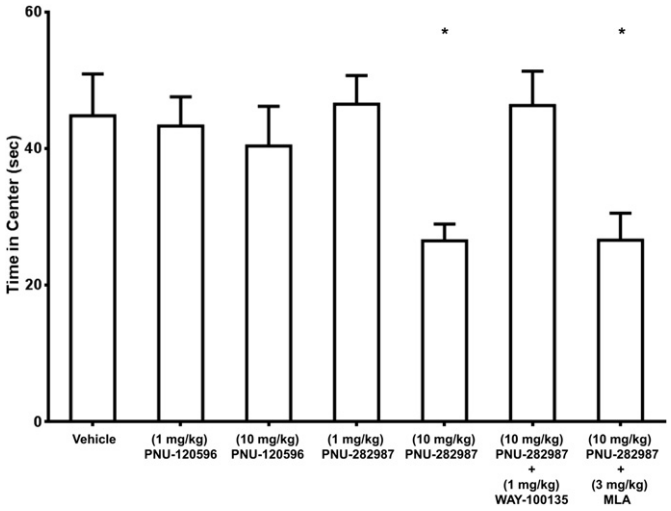

Fig. 5. Effects of PNU-120596 and PNU-282987 on time spent in center zone in open field exploration test. The data represent mean +/− SEM in 10 rats. * indicates significant difference of $p < 0.05$.

was used as a measure of anxiety in Sprague-Dawley rats. PNU-120596 (1 mg/kg or 10 mg/kg) and PNU-282987 (1 mg/kg) did not significantly change the amount of time spent in the center zone or the number of rearings (Figs. 5 and 6). However a higher dose of PNU-282987 (10 mg/kg) induced anxiety, since a significant reduction in the number of rearings ($F_{6, 63} = 4.792$, $p = 0.0004$) and the time spent in the center zone ($F_{6, 63} = 3.599$, $p = 0.0039$) was observed at this dose.

Previously, it had been shown that the anxiety induced by nicotine was blocked by the serotonin 5-HT_{1a} receptor antagonist WAY-100635 (Cheeta et al., 2000). In order to test if the anxiety induced by the 10 mg/kg dose of PNU-282987 that we had observed was due to some involvement of the 5-HT_{1a} receptors, PNU-282987 was co-administered with WAY-100135 (1 mg/kg). The co-administration of these two compounds significantly increased the number of rearings ($p = 0.0118$) and time spent in the center zone significantly ($p = 0.0023$) compared to the treatment with PNU-282987 (10 mg/kg) alone. Therefore WAY-100135 was able to reverse the anxiety induced by the higher dose of PNU-282987. Interestingly, co-administration of MLA (3 mg/kg; the selective α7 receptor antagonist) with PNU-282987 (10 mg/kg) did not show a significant increase in either the number of rearings ($p = 0.6297$) or in the time spent in the center zone ($p = 0.9831$) compared to the treatment with PNU-282987 (10 mg/kg) alone.

3.2.2. Actions of α7 ligands in novelty suppressed feeding test

Latency to feed was used as a measure of anxiety in the novelty suppressed feeding test (Fig. 7). PNU-120596 (1 mg/kg or 10 mg/kg) and PNU-282987 (1 mg/kg) had no significant effect on the time it took for the rats to start feeding. However at a dose of 10 mg/kg, PNU-282987 induced anxiety in the rats; the time it took for the rats to start feeding was significantly increased (Fig. 7; $F_{6, 63} = 10.80$, $p < 0.0001$). As seen with the open field exploration test above, administration of WAY-100135 (1 mg/kg) with PNU-282987 (10 mg/kg) was able to cause significant reduction in the latency to feed ($p < 0.0001$) compared to the treatment with PNU-282987 (10 mg/kg) alone. Co-administration of MLA (3 mg/kg) with PNU-282987 (10 mg/kg) did not cause a significant decrease in the latency to feed ($p = 0.6297$) compared to the treatment with PNU-282987 (10 mg/kg). The results from these anxiety tests suggests that blocking the 5-HT_{1a} receptor was able to reverse the anxiety induced by the10 mg/kg dose of PNU-282987, however the α7 receptor antagonist MLA was not able to prevent these anxiogenic effects.

3.3. Motor coordination

3.3.1. Actions of α7 ligands on motor coordination in rats

Since the 10 mg/kg dose of PNU-282987 was anxiogenic in both tests, we investigated whether it had any effect on locomotor coordination using the rotarod test; the time taken by the rats to fall (latency to fall) from the rotating rod was measured (Fig. 8). The mean latency to fall was similar when the rats were treated with PNU-282987 (10 mg/kg) or the vehicle (0.9% saline) alone ($p = 0.4652$, $n = 12$), suggesting that the dose of PNU-282987 that induced anxiety did not produce any effect on motor coordination in the rats.

4. Discussion

Agonists and positive allosteric modulators (PAMs) selective for the α7 nAChR have been reported to improve cognitive function in

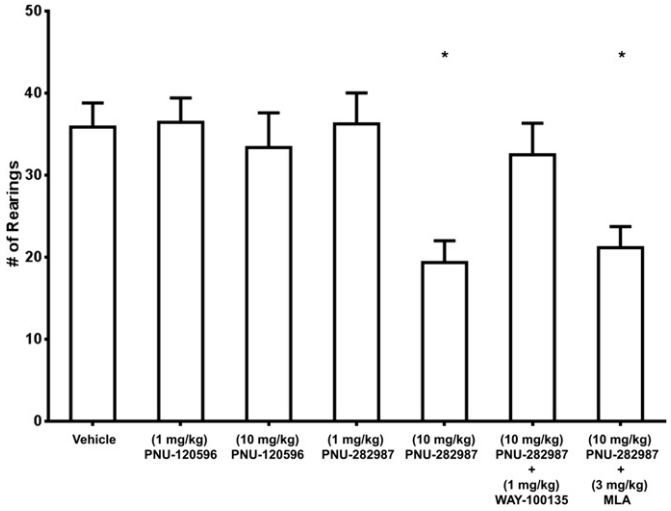

Fig. 6. Effects of PNU-120596 and PNU-282987 on number of rearings in open field exploration test. The data represent mean +/− SEM in 10 rats. * indicates significant difference of $p < 0.05$.

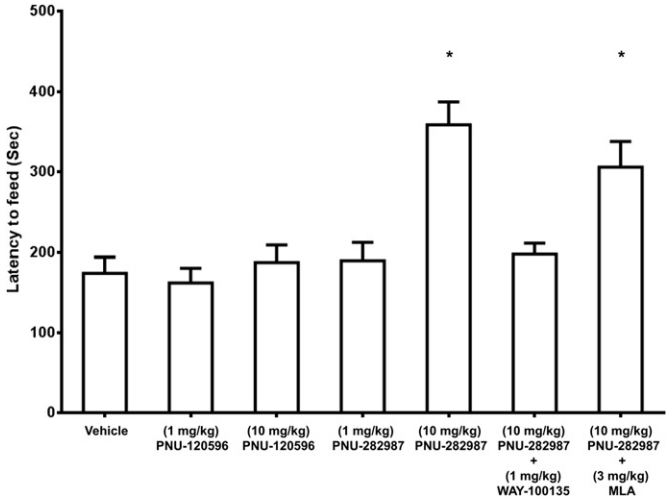

Fig. 7. Effects of PNU-120596 and PNU-282987 on latency to feed in novelty suppressed feeding test. The data represent mean +/− SEM in 10 rats. * indicates significant difference of $p < 0.05$.

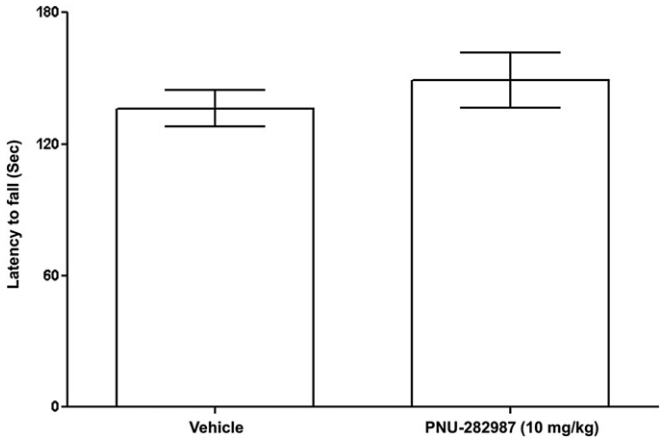

Fig. 8. Effects of PNU-282987 on motor coordination in rotarod test. The data represent mean +/− SEM in 12 rats. * indicates significant difference of $p < 0.05$.

various animal models (Redrobe et al., 2009; Thomsen et al., 2010). PNU-120596 (an α7 selective PAM) has been shown to significantly improve cognitive performance in phencyclidine-treated rats in the attentional set-shifting task (McLean et al., 2011b), and ameliorates amphetamine-induced auditory gating deficits (Hurst et al., 2005), and MK-801 (a glutamate receptor antagonist)- induced impairments in pre-pulse inhibition in rats (Dunlop et al., 2009). Chronic doses of the α7 receptor agonist PNU-282987 have been shown to improve both working memory and reference memory in rats when tested in a radial arm maze (Chan et al., 2007). In addition, PNU-282987 was able to reverse learning deficits and improve episodic memory in rats (McLean et al., 2011a). Here we compared and contrasted the effects of PNU-120596 and PNU-282987 in a series of behavioral tests to look at their effects on cognition as well as anxiety. This was done to assess whether the selective agonist or PAM of the α7 nAChR had any advantage over the other. In the spatial-learning and the episodic memory test for cognition, acute administration of either PNU-120596 or PNU-282987 did not produce any cognitive improvements by itself. This may be due to a ceiling effect where the adult rats may already be performing at an optimal level during the testing. However, both PNU-120596 and PNU-282987 in this study were able to restore memory impairment induced by scopolamine in the behavioral tests used.

The two predominant neuronal subtypes of nAChRs which are widely expressed in the brain (and in particular the hippocampus) are the α7 and the $(\alpha4)_2(\beta2)_3$ receptors (Gopalakrishnan et al., 1996; Lindstrom, 1996; Lindstrom et al., 1996; Liu et al., 2009; Whiting et al., 1987). While agonists as well as partial agonists for α4β2 receptors are known to have antidepressant effects in rodents (Mineur et al., 2009, 2011; Rollema et al., 2009), the actions of selective α7 receptor agonists or partial agonists on anxiety and depression have not been well studied. As seen from the results presented here, the α7 receptor agonist PNU-282987, but not the PAM PNU-120596, is anxiogenic at the higher dose of 10 mg/kg. This effect was similar to that seen with nicotine at higher doses (0.5 and 1 mg/kg) in the elevated plus-maze test (Ouagazzal et al., 1999a). Previously, it had been argued that the anxiety-inducing effect of nicotine, due to the activation of α7 receptors, is mediated by an increased release of 5-HT in the dorsal hippocampus (Tucci et al., 2003). In our study, the anxiety induced by PNU-282987 (10 mg/kg) was reversed by co-administration with the 5-HT_{1a} receptor antagonist WAY-100135 (1 mg/kg). Serotonergic neurons of the dorsal raphe nucleus (DRN) that project to the nucleus accumbens (NAc) show increased firing upon application of nicotine (Chang et al., 2011), inducing the release of serotonin from the terminals. This action is presumed to be due to the activation of nAChRs present on the serotonergic neurons (Chang et al., 2011). Presynaptic 5-HT_{1a} receptors in the basolateral amygdala as well as the raphe nucleus and postsynaptic 5-HT_{1a} receptors in the dorsal hippocampus are known to modulate anxiety in different behavioral tests (File and Gonzalez, 1996; File et al., 1996; Gonzalez et al., 1996). Therefore the activation of presynaptic α7 receptors by PNU-282987 (10 mg/kg), present on the serotonergic neurons in the raphe nucleus that project to the dorsal hippocampus, may cause the release of 5-HT in this region, thereby activating postsynaptic $5HT_{1a}$ receptors in the dorsal hippocampus and inducing anxiety. However we found that MLA, the α7 receptor antagonist, was unable to prevent the anxiety induced by PNU-282987. This may suggest that these anxiogenic effects are not due to the activation of the α7 receptor, but instead interactions with other receptors or transporter proteins at higher concentrations in the rats.

While it has been suggested that PNU-282987 (5 mg/kg) diminishes motor activity (Vicens et al., 2011), our results do not show any effect of this drug on motor coordination, negating the possibility that the anxiety seen with PNU-282987 may be due to its effects on motor coordination in the rats. Interestingly the α7 receptor PAM (PNU-120596) did not cause anxiety in either dose that was tested in the rats. This may be due to different mechanisms of action since α7 receptor PAMs are known to increase receptor function only in the presence of agonists; they cannot activate the receptors by themselves in contrast to agonists (Hajos et al., 2005; Hurst et al., 2005; Pandya and Yakel, 2011b).

5. Conclusions

While α7 receptor agonists have the potential as therapeutic drugs in many neurological diseases and conditions, they may also produce unwanted side effects (e.g. anxiety). Therefore PAMs for α7 receptors may be a better alternative at the present time as they may not produce such side effects, however this has to be explored further in more detail.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Acknowledgments

We would like to thank the NIEHS animal facility and specifically Hanna Harris for taking care of the rats and for performing injections for this study. We would also like to thank Pattie Lamb for her involvement in the process of writing the animal safety protocols for behavior tests described here. We extend our appreciation to C. Erxleben and R. Saha for advice in preparing the manuscript. This research was supported by the Intramural Research Program of National Institute of Environmental Health Sciences, NIH.

References

Alkondon, M., Pereira, E.F., Barbosa, C.T., Albuquerque, E.X., 1997. Neuronal nicotinic acetylcholine receptor activation modulates gamma-aminobutyric acid release from CA1 neurons of rat hippocampal slices. J. Pharmacol. Exp. Ther. 283, 1396–1411.

Arias, H.R., 2006. Biological and Biophysical Aspects of Ligand-gated Ion Channel Receptor Superfamilies, 2006. Research Signpost, Kerala, India.

Bertrand, D., Gopalakrishnan, M., 2007. Allosteric modulation of nicotinic acetylcholine receptors. Biochem. Pharmacol. 74, 1155–1163.

Bodnar, A.L., Cortes-Burgos, L.A., Cook, K.K., Dinh, D.M., Groppi, V.E., Hajos, M., Higdon, N.R., Hoffmann, W.E., Hurst, R.S., Myers, J.K., Rogers, B.N., Wall, T.M., Wolfe, M.L., Wong, E., 2005. Discovery and structure-activity relationship of quinuclidine benzamides as agonists of alpha7 nicotinic acetylcholine receptors. J. Med. Chem. 48, 905–908.

Boess, F.G., De Vry, J., Erb, C., Flessner, T., Hendrix, M., Luithle, J., Methfessel, C., Riedl, B., Schnizler, K., van der Staay, F.J., van Kampen, M., Wiese, W.B., Koenig, G., 2007. The novel alpha7 nicotinic acetylcholine receptor agonist N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide improves working and recognition memory in rodents. J. Pharmacol. Exp. Ther. 321, 716–725.

Brams, M., Pandya, A., Kuzmin, D., van Elk, R., Krijnen, L., Yakel, J.L., Tsetlin, V., Smit, A.B., Ulens, C., 2011. A structural and mutagenic blueprint for molecular recognition of strychnine and d-tubocurarine by different cys-loop receptors. PLoS Biol. 9, e1001034.

Chan, W.K., Wong, P.T., Sheu, F.S., 2007. Frontal cortical alpha7 and alpha4beta2 nicotinic acetylcholine receptors in working and reference memory. Neuropharmacology 52, 1641–1649.

Chang, B., Daniele, C.A., Gallagher, K., Madonia, M., Mitchum, R.D., Barrett, L., Vezina, P., McGehee, D.S., 2011. Nicotinic excitation of serotonergic projections from dorsal raphe to the nucleus accumbens. J. Neurophysiol. 106, 801–808.

Cheeta, S., Kenny, P.J., File, S.E., 2000. The role of 5-HT1A receptors in mediating the anxiogenic effects of nicotine following lateral septal administration. Eur. J. Neurosci. 12, 3797–3802.

Dunlop, J., Lock, T., Jow, B., Sitzia, F., Grauer, S., Jow, F., Kramer, A., Bowlby, M.R., Randall, A., Kowal, D., Gilbert, A., Comery, T.A., Larocque, J., Soloveva, V., Brown, J., Roncarati, R., 2009. Old and new pharmacology: positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine(2B/C) receptor antagonist SB-206553 (3,5-dihydro-5-methyl-N-3-pyridinylbenzo[1,2-b:4,5-b']di pyrrole-1(2H)-carboxamide). J. Pharmacol. Exp. Ther. 328, 766–776.

Falsafi, S.K., Deli, A., Hoger, H., Pollak, A., Lubec, G., 2012. Scopolamine administration modulates muscarinic, nicotinic and NMDA receptor Systems. PLoS One 7, e32082.

Feuerbach, D., Lingenhoehl, K., Olpe, H.R., Vassout, A., Gentsch, C., Chaperon, F., Nozulak, J., Enz, A., Bilbe, G., McAllister, K., Hoyer, D., 2009. The selective nicotinic acetylcholine receptor alpha7 agonist JN403 is active in animal models of cognition, sensory gating, epilepsy and pain. Neuropharmacology 56, 254–263.

File, S.E., Gonzalez, L.E., 1996. Anxiolytic effects in the plus-maze of 5-HT1A-receptor ligands in dorsal raphe and ventral hippocampus. Pharmacol. Biochem. Behav. 54, 123–128.

File, S.E., Gonzalez, L.E., Andrews, N., 1996. Comparative study of pre- and post-synaptic 5-HT1A receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16, 4810–4815.

File, S.E., Kenny, P.J., Ouagazzal, A.M., 1998. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav. Neurosci. 112, 1423–1429.

Flores, C.M., Rogers, S.W., Pabreza, L.A., Wolfe, B.B., Kellar, K.J., 1992. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. Mol. Pharmacol. 41, 31–37.

Furmaga, H., Shah, A., Frazer, A., 2011. Serotonergic and noradrenergic pathways are required for the anxiolytic-like and antidepressant-like behavioral effects of repeated vagal nerve stimulation in rats. Biol. Psychiatry 70, 937–945.

Gay, E.A., Yakel, J.L., 2007. Gating of nicotinic ACh receptors; new insights into structural transitions triggered by agonist binding that induce channel opening. J. Physiol. 584, 727–733.

German, N., Kim, J.S., Jain, A., Dukat, M., Pandya, A., Ma, Y., Weltzin, M., Schulte, M.K., Glennon, R.A., 2011. Deconstruction of the alpha4beta2 nicotinic acetylcholine receptor positive allosteric modulator desformylflustrabromine. J. Med. Chem. 54, 7259–7267.

Gonzalez, L.E., Andrews, N., File, S.E., 1996. 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Res. 732, 145–153.

Gopalakrishnan, M., Monteggia, L.M., Anderson, D.J., Molinari, E.J., Piattoni-Kaplan, M., Donnelly-Roberts, D., Arneric, S.P., Sullivan, J.P., 1996. Stable expression, pharmacologic properties and regulation of the human neuronal nicotinic acetylcholine alpha 4 beta 2 receptor. J. Pharmacol. Exp. Ther. 276, 289–297.

Gotti, C., Clementi, F., 2004. Neuronal nicotinic receptors: from structure to pathology. Prog. Neurobiol. 74, 363–396.

Gotti, C., Moretti, M., Gaimarri, A., Zanardi, A., Clementi, F., Zoli, M., 2007. Heterogeneity and complexity of native brain nicotinic receptors. Biochem. Pharmacol. 74, 1102–1111.

Gu, Z., Yakel, J.L., 2011. Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. Neuron 71, 155–165.

Hajos, M., Hurst, R.S., Hoffmann, W.E., Krause, M., Wall, T.M., Higdon, N.R., Groppi, V.E., 2005. The selective alpha7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. J. Pharmacol. Exp. Ther. 312, 1213–1222.

Hauser, T.A., Kucinski, A., Jordan, K.G., Gatto, G.J., Wersinger, S.R., Hesse, R.A., Stachowiak, E.K., Stachowiak, M.K., Papke, R.L., Lippiello, P.M., Bencherif, M., 2009. TC-5619: an alpha7 neuronal nicotinic receptor-selective agonist that demonstrates efficacy in animal models of the positive and negative symptoms and cognitive dysfunction of schizophrenia. Biochem. Pharmacol. 78, 803–812.

Horiguchi, K., Horiguchi, S., Yamashita, N., Irie, K., Masuda, J., Takano-Ohmuro, H., Himi, T., Miyazawa, M., Moriwaki, Y., Okuda, T., Misawa, H., Ozaki, H., Kawashima, K., 2009. Expression of SLURP-1, an endogenous alpha7 nicotinic acetylcholine receptor allosteric ligand, in murine bronchial epithelial cells. J. Neurosci. Res. 87, 2740–2747.

Hurst, R.S., Hajos, M., Raggenbass, M., Wall, T.M., Higdon, N.R., Lawson, J.A., Rutherford-Root, K.L., Berkenpas, M.B., Hoffmann, W.E., Piotrowski, D.W., Groppi, V.E., Allaman, G., Ogier, R., Bertrand, S., Bertrand, D., Arneric, S.P., 2005. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J. Neurosci. 25, 4396–4405.

Irvine, E.E., Cheeta, S., File, S.E., 1999. Time-course of changes in the social interaction test of anxiety following acute and chronic administration of nicotine. Behav. Pharmacol. 10, 691–697.

Kim, J.S., Padnya, A., Weltzin, M., Edmonds, B.W., Schulte, M.K., Glennon, R.A., 2007. Synthesis of desformylflustrabromine and its evaluation as an alpha4beta2 and alpha7 nACh receptor modulator. Bioorg. Med. Chem. Lett. 17, 4855–4860.

Koval, L., Lykhmus, O., Kalashnyk, O., Bachinskaya, N., Kravtsova, G., Soldatkina, M., Zouridakis, M., Stergiou, C., Tzartos, S., Tsetlin, V., Komisarenko, S., Skok, M., 2011. The presence and origin of autoantibodies against alpha4 and alpha7 nicotinic acetylcholine receptors in the human blood: possible relevance to Alzheimer's pathology. J. Alzheimers Dis. 25, 747–761.

Levin, E.D., McClernon, F.J., Rezvani, A.H., 2006. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. Psychopharmacology (Berl) 184, 523–539.

Lindstrom, J., 1996. Neuronal nicotinic acetylcholine receptors. Ion Channels 4, 377–450.

Lindstrom, J., Anand, R., Gerzanich, V., Peng, X., Wang, F., Wells, G., 1996. Structure and function of neuronal nicotinic acetylcholine receptors. Prog. Brain Res. 109, 125–137.

Liu, Q., Huang, Y., Xue, F., Simard, A., DeChon, J., Li, G., Zhang, J., Lucero, L., Wang, M., Sierks, M., Hu, G., Chang, Y., Lukas, R.J., Wu, J., 2009. A novel nicotinic acetylcholine receptor subtype in basal forebrain cholinergic neurons with high sensitivity to amyloid peptides. J. Neurosci. 29, 918–929.

McGehee, D.S., Heath, M.J., Gelber, S., Devay, P., Role, L.W., 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269, 1692–1696.

McLean, S.L., Grayson, B., Idris, N.F., Lesage, A.S., Pemberton, D.J., Mackie, C., Neill, J.C., 2011a. Activation of alpha7 nicotinic receptors improves phencyclidine-induced deficits in cognitive tasks in rats: implications for therapy of cognitive dysfunction in schizophrenia. Eur. Neuropsychopharmacol. 21, 333–343.

McLean, S.L., Idris, N., Grayson, B., Gendle, D., Mackie, C., Lesage, A., Pemberton, D.J., Neill, J., 2011b. PNU-120596, a positive allosteric modulator of alpha7 nicotinic acetylcholine receptors, reverses a sub-chronic phencyclidine-induced cognitive deficit in the attentional set-shifting task in female rats. J. Psychopharmacol., 333–343.

Meyer, E.M., Tay, E.T., Papke, R.L., Meyers, C., Huang, G.L., de Fiebre, C.M., 1997. 3-[2,4-Dimethoxybenzylidene]anabaseine (DMXB) selectively activates rat alpha7 receptors and improves memory-related behaviors in a mecamylamine-sensitive manner. Brain Res. 768, 49–56.

Mineur, Y.S., Eibl, C., Young, G., Kochevar, C., Papke, R.L., Gundisch, D., Picciotto, M.R., 2009. Cytisine-based nicotinic partial agonists as novel antidepressant compounds. J. Pharmacol. Exp. Ther. 329, 377–386.

Mineur, Y.S., Einstein, E.B., Seymour, P.A., Coe, J.W., O'Neill, B.T., Rollema, H., Picciotto, M.R., 2011. alpha4beta2 nicotinic acetylcholine receptor partial agonists with low intrinsic efficacy have antidepressant-like properties. Behav. Pharmacol. 22, 291–299.

Mok, M.H.S., Kew, J.N., 2006. Excitation of rat hippocampal interneurons via modulation of endogenous agonist activity at the alpha7 nicotinic ACh receptor. J. Physiol. 574, 699–710.

Nashmi, R., Lester, H.A., 2006. CNS localization of neuronal nicotinic receptors. J. Mol. Neurosci. 30, 181–184.

Newhouse, P.A., Potter, A., Singh, A., 2004. Effects of nicotinic stimulation on cognitive performance. Curr. Opin. Pharmacol. 4, 36–46.

Ng, H.J., Whittemore, E.R., Tran, M.B., Hogenkamp, D.J., Broide, R.S., Johnstone, T.B., Zheng, L., Stevens, K.E., Gee, K.W., 2007. Nootropic alpha7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. Proc. Natl. Acad. Sci. U S A 104, 8059–8064.

Ouagazzal, A.M., Kenny, P.J., File, S.E., 1999a. Modulation of behaviour on trials 1 and 2 in the elevated plus-maze test of anxiety after systemic and hippocampal administration of nicotine. Psychopharmacology (Berl) 144, 54–60.

Ouagazzal, A.M., Kenny, P.J., File, S.E., 1999b. Stimulation of nicotinic receptors in the lateral septal nucleus increases anxiety. Eur. J. Neurosci. 11, 3957–3962.

Pandya, A., Yakel, J.L., 2011a. Allosteric modulator desformylflustrabromine relieves the inhibition of alpha2beta2 and alpha4beta2 nicotinic acetylcholine receptors by beta-Amyloid(1-42) Peptide. J. Mol. Neurosci.

Pandya, A., Yakel, J.L., 2011b. Allosteric modulators of the alpha4beta2 subtype of neuronal nicotinic acetylcholine receptors. Biochem. Pharmacol.

Parri, H.R., Hernandez, C.M., Dineley, K.T., 2011. Research update: alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochem. Pharmacol. 82, 931–942.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.

Radcliffe, K.A., Dani, J.A., 1998. Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission. J. Neurosci. 18, 7075–7083.

Redrobe, J.P., Nielsen, E.O., Christensen, J.K., Peters, D., Timmermann, D.B., Olsen, G.M., 2009. Alpha7 nicotinic acetylcholine receptor activation ameliorates scopolamine-induced behavioural changes in a modified continuous Y-maze task in mice. Eur. J. Pharmacol. 602, 58–65.

Rollema, H., Guanowsky, V., Mineur, Y.S., Shrikhande, A., Coe, J.W., Seymour, P.A., Picciotto, M.R., 2009. Varenicline has antidepressant-like activity in the forced swim test and augments sertraline's effect. Eur. J. Pharmacol. 605, 114–116.

Roncarati, R., Scali, C., Comery, T.A., Grauer, S.M., Aschmi, S., Bothmann, H., Jow, B., Kowal, D., Gianfriddo, M., Kelley, C., Zanelli, U., Ghiron, C., Haydar, S., Dunlop, J., Terstappen, G.C., 2009. Procognitive and neuroprotective activity of a novel alpha7 nicotinic acetylcholine receptor agonist for treatment of neurodegenerative and cognitive disorders. J. Pharmacol. Exp. Ther. 329, 459–468.

Roni, M.A., Rahman, S., 2011. Neuronal nicotinic receptor antagonist reduces anxiety-like behavior in mice. Neurosci. Lett. 504, 237–241.

Sacco, K.A., Bannon, K.L., George, T.P., 2004. Nicotinic receptor mechanisms and cognition in normal states and neuropsychiatric disorders. J. Psychopharmacol. 18, 457–474.

Thomsen, M.S., El-Sayed, M., Mikkelsen, J.D., 2011. Differential immediate and sustained memory enhancing effects of alpha7 nicotinic receptor agonists and allosteric modulators in rats. PLoS One 6, e27014.

Thomsen, M.S., Hansen, H.H., Timmerman, D.B., Mikkelsen, J.D., 2010. Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: from animal models to human pathophysiology. Curr. Pharm. Des. 16, 323–343.

Timmermann, D.B., Gronlien, J.H., Kohlhaas, K.L., Nielsen, E.O., Dam, E., Jorgensen, T.D., Ahring, P.K., Peters, D., Holst, D., Christensen, J.K., Malysz, J., Briggs, C.A., Gopalakrishnan, M., Olsen, G.M., 2007. An allosteric modulator of the alpha7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo. J. Pharmacol. Exp. Ther. 323, 294–307.

Timofeeva, O.A., Eddins, D., Yakel, J.L., Blackshear, P.J., Levin, E.D., 2010. Hippocampal infusions of MARCKS peptides impair memory of rats on the radial-arm maze. Brain Res. 1308, 147–152.

Tucci, S.A., Genn, R.F., File, S.E., 2003. Methyllycaconitine (MLA) blocks the nicotine evoked anxiogenic effect and 5-HT release in the dorsal hippocampus: possible role of alpha7 receptors. Neuropharmacology 44, 367–373.

Van Kampen, M., Selbach, K., Schneider, R., Schiegel, E., Boess, F., Schreiber, R., 2004. AR-R 17779 improves social recognition in rats by activation of nicotinic alpha7 receptors. Psychopharmacology (Berl) 172, 375–383.

Vicens, P., Ribes, D., Torrente, M., Domingo, J.L., 2011. Behavioral effects of PNU-282987, an alpha7 nicotinic receptor agonist, in mice. Behav. Brain Res. 216, 341–348.

Whiting, P.J., Schoepfer, R., Swanson, L.W., Simmons, D.M., Lindstrom, J.M., 1987. Functional acetylcholine receptor in PC12 cells reacts with a monoclonal antibody to brain nicotinic receptors. Nature 327, 515–518.

Williams, D.K., Wang, J., Papke, R.L., 2011a. Investigation of the molecular mechanism of the alpha7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. Mol. Pharmacol. 80, 1013–1032.

Williams, D.K., Wang, J., Papke, R.L., 2011b. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. Biochem. Pharmacol. 82, 915–930.

Wishka, D.G., Walker, D.P., Yates, K.M., Reitz, S.C., Jia, S., Myers, J.K., Olson, K.L., Jacobsen, E.J., Wolfe, M.L., Groppi, V.E., Hanchar, A.J., Thornburgh, B.A., Cortes-Burgos, L.A., Wong, E.H., Staton, B.A., Raub, T.J., Higdon, N.R., Wall, T.M., Hurst, R.S., Walters, R.R., Hoffmann, W.E., Hajos, M., Franklin, S., Carey, G., Gold, L.H., Cook, K.K., Sands, S.B., Zhao, S.X., Soglia, J.R., Kalgutkar, A.S., Arneric, S.P., Rogers, B.N., 2006. Discovery of N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide, an agonist of the alpha7 nicotinic acetylcholine receptor, for the potential treatment of cognitive deficits in schizophrenia: synthesis and structure – activity relationship. J. Med. Chem. 49, 4425–4436.

Yakel, J.L., Shao, Z., 2004. Functional and molecular characterization of neuronal nicotinic ACh receptors in rat hippocampal interneurons. Prog. Brain Res. 145, 95–107.

Young, J.W., Powell, S.B., Risbrough, V., Marston, H.M., Geyer, M.A., 2009. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacol. Ther. 122, 150–202.