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Maximizing the effect of an $\alpha 7$ nicotinic receptor PAM in a mouse model of schizophrenia-like sensory inhibition deficits

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Abstract

Positive allosteric modulators (PAMs) for the $\alpha 7$ nicotinic receptor hold promise for the treatment of sensory inhibition deficits observed in schizophrenia patients. Studies of these compounds in the DBA/2 mouse, which models the schizophrenia-related deficit in sensory inhibition, have shown PAMs to be effective in improving the deficit. However, the first published clinical trial of a PAM for both sensory inhibition deficits and related cognitive difficulties failed, casting a shadow on this therapeutic approach. The present study used both DBA/2 mice, and C3H *Chrna7* heterozygote mice to assess the ability of the $\alpha 7$ PAM, PNU-120596, to improve sensory inhibition. Both of these strains of mice have reduced hippocampal $\alpha 7$ nicotinic receptor numbers and deficient sensory inhibition similar to schizophrenia patients. Low doses of PNU-120596 (1 or 3.33 mg/kg) were effective in the DBA/2 mouse but not the C3H *Chrna7* heterozygote mouse. Moderate doses of the selective $\alpha 7$ nicotinic receptor agonist, choline chloride (10 or 33 mg/kg), were also ineffective in improving sensory inhibition in the C3H *Chrna7* heterozygote mouse. However, combining the lowest doses of both PNU-120596 and choline chloride in this mouse model did improve sensory inhibition. We propose here that the difference in efficacy of PNU-120596 between the 2 mouse strains is driven by differences in hippocampal $\alpha 7$ nicotinic receptor numbers, such that C3H *Chrna7* heterozygote mice require additional direct stimulation of the $\alpha 7$ receptors. These data may have implications for further clinical testing of putative $\alpha 7$ nicotinic receptor PAMs.

Keywords

schizophrenia; sensory inhibition; auditory gating; DBA/2 mice; C3H heterozygote *Chrna7* null mutant mice; positive allosteric modulator; PAM; $\alpha 7$ nicotinic receptors; PNU-120596

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1. Introduction

Deficient sensory inhibition is a hallmark of schizophrenia, which is defined as an inability to suppress responses to repeated stimuli (Adler et al 1998; 1999; Leonard et al 2001). This deficit can manifest as poor attentional control (Cullum et al 1993; Harris et al 2004; Yee et al 1998) although other studies do not find this relationship (Sánchez-Morla et al, 2013; Smith et al, 2010). Improvement in cognition is the single best predictor of improved societal functioning in schizophrenia patients (Green et al 2004). Deficient sensory inhibition is measured using a paired auditory stimulus paradigm in which 2 identical stimuli are presented 0.5 sec apart and the EEG responses to both stimuli are recorded. Taking ratio of the amplitude of the response to the second stimulus (test amplitude) over the amplitude of the response to the first stimulus (conditioning amplitude) yields the measure of the inhibition (TC ratio). TC ratios of less than 0.5 are considered normal; TC ratios equal to or greater than 0.5 are considered abnormal and indicative of deficient sensory inhibition (Adler et al 1999).

Deficient sensory inhibition has been linked to reduced numbers of hippocampal $\alpha 7$ nicotinic receptors on interneurons, limiting the efficiency of inhibitory circuits (Adler et al 1998; 1999; Freedman et al 1995; 2000a; Martin and Freedman 2007). In an attempt to ameliorate the deficiency, agonists and partial agonists for this receptor have been developed using a mouse model of the deficit (Radek et al 2012; Simosky et al 2001; Stevens et al 1998). This mouse model, the DBA/2 inbred strain of mice, has reduced numbers of hippocampal $\alpha 7$ nicotinic receptors and deficient sensory inhibition (Stevens et al 1996). While partial agonists hold promise as potential therapeutics for deficient sensory processing (Miyamoto et al 2012; Olincy and Freedman 2012; Olincy and Stevens 2007; Wallace and Bertrand 2013), full agonists, such as nicotine, are less desirable due to receptor desensitization (Stevens and Wear 1997). Thus, several partial agonists for the $\alpha 7$ nicotinic receptor have been investigated (DiPaolo et al 2014; Freedman 2014; Freedman et al 2008; Lieberman et al 2013; Olincy et al 2006) as potential therapeutics.

A more recent, potentially beneficial, approach for therapeutics is the use of positive allosteric modulators (PAMs) (Uteshev 2014). PAMs have the advantage of not inducing receptor desensitization by themselves, and have no abuse potential (Faghieh et al 2007; Williams et al 2011). The first PAM for the $\alpha 7$ nicotinic receptor was PNU-120596, published by Hurst et al in 2005. This generated significant interest in developing PAMs as therapeutics for the treatment of sensory inhibition deficits in schizophrenia patients. While several pharmaceutical companies are developing these compounds, only 2 papers have been published showing efficacy in the DBA/2 mouse model (Dinklo et al 2011; Ng et al 2007). These promising results lead to clinical trials on one compound for improvement in both sensory inhibition and cognition. Unfortunately, it failed to improve either measure (Winterer et al 2013). Clinical trials are currently underway for another compound, but results are not yet available (R Freedman, personal communication). One possible reason for the failure of the single clinical trial may be the roughly 50% reduction in hippocampal $\alpha 7$ nicotinic receptors observed in the post-mortem brains of schizophrenia patients (Freedman et al 1995). Perhaps, there are simply too few $\alpha 7$ receptors available for endogenously-released acetylcholine to produce its effects, even in the presence of a PAM.

A newer rodent model of the schizophrenia-related sensory inhibition deficit has become available in recent years. The Institute for Behavioral Genetics, University of Colorado, Boulder, bred the null mutation for the $\alpha 7$ nicotinic receptor (*Chrna7*) onto the C3H background strain of mice. The parental C3H mouse strain has adequate numbers of hippocampal $\alpha 7$ nicotinic receptors and normal sensory inhibition (Stevens et al 1996). When the *Chrna7* null mutation was introgressed onto this mouse strain, the resulting heterozygote displayed deficient sensory inhibition and a 50–60% decrease in $\alpha 7$ -selective 125I- α -bungarotoxin binding (Adams et al 2008). This phenotype more closely resembles the reduction seen in post mortem brains of schizophrenia patients (for review see Adler et al 1998; Freedman et al 2000b) than the ~30% reduction found in DBA/2 mice (Stevens et al 1996).

The present study assessed the effect of administration of PNU-120596 to both DBA/2 and C3H *Chrna7* heterozygote mice, as a potentially more relevant model of sensory processing deficits, on sensory inhibition in an effort to understand why PAMs have been found efficacious in improving sensory inhibition in DBA/2 mice but failed in clinical trials.

2. Results

Administration of PNU-120596 to DBA/2 mice at both 1 and 3.33 mg/kg, ip, induced improvement in TC ratios [$F(23,115)=2.53$, $p<0.001$; $F(23,115)=2.94$, $p<0.001$, respectively] (Figure 1A, B) produced primarily through significant increases in conditioning amplitude [$F(23,115)=3.24$, $p<0.001$; $F(23,115)=2.41$, $p=0.001$, respectively] (Figure 1A, B). Although there was a significant decrease in test amplitude at the 3.33 mg/kg dose [$F(23,115)=1.77$, $p=0.026$], no individual time points post injection were significantly different from the mean of the baseline. There was no significant change in test amplitude at the 1 mg/kg dose [$F(23,115)=1.25$, $p=0.222$].

Administration of PNU-120596 to C3H *Chrna7* heterozygote mice (1 or 3.33 mg/kg, ip) failed to alter conditioning amplitude at either dose [$F(23,161)=0.92$, $p=0.577$; $F(23,161)=1.53$, $p=0.068$, respectively; Figures 2, 3A, B]. In contrast, at the higher dose of PNU-120596, test amplitude was significantly affected [$F(23,161)=2.57$, $p<0.001$] but Fisher's LSD showed that the effect was an increase in test amplitude occurring primarily towards the end of the recording session (Figure 3B). There was no effect on test amplitude for the lower dose [$F(23,161)=0.71$, $p=0.834$, Figure 3A]. While the low dose (1 mg/kg) had no effect on TC ratio [$F(23,161)=0.92$, $p=0.575$, Figure 3A] there was a significant increase in TC ratio with the high dose (3.33 mg/kg) [$F(23,161)=1.73$, $p=0.027$], however, Fisher's LSD revealed that no single time points were significantly different from the mean baseline (Figure 3B).

Administration of choline chloride to C3H *Chrna7* heterozygote mice (10 or 33 mg/kg, ip) failed to alter conditioning amplitudes [$F(23,161)=1.22$, $p=0.232$; $F(23,161)=1.55$, $p=0.062$, respectively, Figures 2, 4A, B], however the higher dose did show a significant effect on test amplitude [$F(23,161)=1.66$, $p=0.038$] while the lower dose did not show a significant effect [$F(23,161)=1.16$, $p=0.293$] (Figures 2, 4A, B). Fisher's LSD revealed that a single time point post injection of the high dose of choline chloride (33 mg/kg) was significantly

elevated over the mean baseline (Figure 4B). The change in test amplitude was not sufficient to alter TC ratio, thus, for both doses there was no significant effect on TC ratio [$F(23,161)=0.72$, $p=0.818$ for the 10 mg/kg dose; $F(23,161)=0.92$, $p=0.577$ for the 33 mg/kg dose, Figures 2, 4A, B].

Combining the lower dose of PNU-120596 (1 mg/kg, ip) with low dose of choline chloride (10 mg/kg, ip) produced significant effects on all three parameters [Conditioning amplitude $F(23, 161)=2.20$, $p=0.002$; Test amplitude $F(23,161)=2.85$, $p<0.001$; TC ratio $F(23,161)=2.52$, $p<0.001$, Figure 5A]. Fisher's LSD for conditioning amplitude showed significantly increased amplitudes from 15 to 40 minutes post injections, while *a posteriori* analysis for test amplitude showed a significantly decreased amplitude at 10 minutes post injection and increased amplitudes at sporadic points later in the recording session (Figure 5A). Fisher's LSD for TC ratio found significantly decreased ratios from 10–20 minutes post injections (Figure 5A). Combining the low dose PNU-120596 (1 mg/kg, ip) with the high dose of choline chloride (33 mg/kg, ip) failed to significantly effect any parameter tested [Conditioning amplitude $F(23, 161)=1.31$, $p=0.169$; Test amplitude $F(23,161)=0.78$, $p=0.755$; TC ratio $F(23,161)=0.94$, $p=0.545$, Figure 5B].

3. Discussion

The present study was initiated due to the failure of the first clinical study of a PAM in schizophrenia patients to produce any improvement in either sensory inhibition or cognition (Winterer et al 2013). Previous laboratory studies in the DBA/2 mouse had suggested that PAMs would be effective in improving deficient sensory inhibition (Ng et al 2007; Dinklo et al 2011), which has been linked to improved attention in schizophrenia patients (Potter et al 2006; Freedman et al 2008; Leiser et al 2009; Smucny et al 2011). Thus, the present studies were undertaken in order to understand this disconnect between preclinical and clinical studies, since PAMs are an attractive alternative to full or partial $\alpha 7$ nicotinic agonists due to their lack of abuse potential and possibly less receptor desensitization issues (Faghih et al 2007; Williams et al 2011).

Both doses of the PAM, PNU-120596, (1 and 3.33 mg/kg, ip) produced significant improvement in sensory inhibition in the DBA/2 mouse model of the deficit observed in schizophrenia. This was evidenced by the significant decrease in TC ratios. In both cases, there were significant increases in conditioning amplitude, which primarily drove the decrease in TC ratio. The higher dose also showed a significant overall decrease in test amplitude, but no single time point post-injection was significantly lower than the mean of the baseline. Increases in conditioning amplitude are typically associated with stimulation of $\alpha 4\beta 2$ nicotinic receptors (Radek et al 2006; Wildeboer and Stevens 2008) while decreases in test amplitude are typically associated with stimulation of $\alpha 7$ nicotinic receptors (Simosky et al 2002; Stevens et al 1998). PNU-120596 is reported to be an $\alpha 7$ nicotinic receptor PAM (Hurst et al 2005). The present data would suggest greater effect on $\alpha 4\beta 2$ than $\alpha 7$ nicotinic receptors since at both doses there was a significant increase in conditioning amplitude. This may be due to stimulation of $\alpha 7$ receptors at a level too low to produce reductions in test amplitude, at least at the lower dose, but sufficient to increase the release of acetylcholine (Tani et al 1998). Since $\alpha 4\beta 2$ receptors have a higher affinity for acetylcholine than $\alpha 7$

receptors (Leonard and Bertrand 2001), the net effect could have been the observed increase in conditioning amplitude with no, or a small, observable effect on test amplitude.

Administration of either the $\alpha 7$ nicotinic receptor PAM, PNU-120596 (Hurst et al 2005), or the selective $\alpha 7$ nicotinic receptor agonist, choline chloride (Albuquerque et al 1998; Alkondon et al 1999; Fayuk and Yakel 2004), alone, failed to produce sustained changes in sensory inhibition parameters in C3H *Chrna7* heterozygote mice. Indeed, the low dose of PNU-120596 actually increased test amplitude over baseline levels. However, combining these two ineffective compounds, at the low doses, produced a significant improvement in sensory inhibition. Efficacy was seen only at the lower dose of choline chloride (10 mg/kg) and not the higher (33 mg/kg). This may have been due to the higher dose actually driving the $\alpha 7$ nicotinic receptors into desensitization (Adler et al 1998). Studies have shown that administration of PNU-120596 significantly slowed the dissociation of choline from $\alpha 7$ nicotinic receptors (Szabo et al 2014), thus increasing the probability of desensitization. As was seen with PNU-120596 alone, administered to DBA/2 mice, the decrease in TC ratio was driven primarily by an increase in conditioning amplitude, but also coupled, in this case, with a decrease in test amplitude. Again, the increase in conditioning amplitude may be due to increased acetylcholine release induced by $\alpha 7$ nicotinic receptor stimulation, but in this case, there was sufficient stimulation of those $\alpha 7$ receptors to also produce a significant reduction in test amplitude.

There is a difference in the length of the improvement in sensory inhibition between the study with the PAM alone in the DBA/2 mice and the PAM plus choline in the C3H *Chrna7* heterozygote mouse. This may be explained by the difference in the number of receptors in the hippocampus of the two strains of mice. As noted before, DBA/2 mice are reduced by ~30% while C3H *Chrna7* heterozygote mice are reduced by 50–60%. One study proposed that when an agonist is present, PNU-120596 binds to the receptor, inducing desensitization but trapping the agonist within its binding site such that when the receptor resensitizes, it is again activated (Szabo et al 2014). Thus, since DBA/2 mice have more receptors such that a greater number resensitized and were reactivated there was a prolonged effect. In the C3H *Chrna7* heterozygote mice, there were just too few receptors returning to the state which could be activated to permit a prolonged effect.

Wildtype C3H mice were not included in this study since the goal was to assess models of schizophrenia-like deficits in sensory inhibition, not mice with normal sensory inhibition. It would not be expected that either choline or PNU-120596 or the combination of these would produce improvement in sensory inhibition in normal mice. Indeed, there is no indication that lowering the TC ratio even further in either rodents or humans would produce any functional effects since they already show normal sensory inhibition. Normal humans who are smoking cigarettes or consuming chewing nicotine gum (which would increase activity at both $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors) show transient loss of sensory inhibition (Adler et al 1992; 1993). C3H mice with a subcutaneous cholesterol pellet (controls for a corticosterone experiment) did not show any change in sensory inhibition when administered nicotine (Stevens et al 2001). In a study using C57Bl/6J mice [which generally show normal sensory inhibition (Stevens et al 1996; Siegel et al 2005)] nicotine increased

the amplitude of both the conditioning and test responses approximately equally; the TC ratio was not reported but should not have changed appreciably (Metzger et al 2007).

It is plausible to attribute the improvement in sensory inhibition observed in the C3H *Chrna7* heterozygote mice to some off target effects of either, PNU-120596 or choline. However, both compounds have been shown to have their effects at the nicotinic $\alpha 7$ receptor (PNU-120596—Hurst et al 2005; choline—Albuquerque et al, 1998; Alkondon et al 1999). No studies have shown significant effects at other receptors.

Schizophrenia patients have higher hippocampal levels of choline acetyltransferase than normal individuals, which would suggest that sufficient levels of acetylcholine are being produced. They also have normal levels of acetylcholinesterase (McGeer and Mc Geer 1977), suggesting that the generated acetylcholine would be expected to be degraded at the normal rate. However, schizophrenia patients have significantly reduced numbers of hippocampal $\alpha 7$ nicotinic receptors (for review see Adler et al 1998; Freedman et al 2000a) specifically on hippocampal interneurons (Freedman et al 2000b). This could mean that as yet unbound acetylcholine, remaining in the synapse, might be degraded before binding to the remaining $\alpha 7$ nicotinic receptors. This would lead to a failure of inhibitory circuits which would manifest as poor sensory inhibition (Adler et al 1998). Agonists for these receptors have been proposed as potential therapeutics for the sensory inhibition deficits and concomitant cognitive deficits (for review see Hajos and Rogers 2010; Olincy and Stevens 2007), and indeed, several agonists and partial agonists are currently in clinical trials (for review see Wallace and Bertrand 2012). The $\alpha 7$ nicotinic receptor is a rapidly desensitizing receptor (Papke et al 2009). While partial agonists generally do not pose a problem with desensitization, full agonists do. Thus, a PAM selective for the $\alpha 7$ nicotinic receptor would seem an ideal approach to stimulation of the reduced numbers of these receptor without inducing desensitization (Jones et al 2012).

DBA/2 mice have been used extensively to predict efficacy of full and partial $\alpha 7$ nicotinic receptor agonists to improve sensory inhibition (Hashimoto et al 2005; Radek et al 2012; Stevens et al 1998; Stevens and Wear 1997; Wildeboer-Andrud and Stevens 2011). These mice not only show the sensory inhibition deficit, they also have a reduction in the number of hippocampal $\alpha 7$ nicotinic receptors (Stevens et al 1996), paralleling schizophrenia patients (deficient sensory inhibition, see Adler et al 1998 for review; reduced hippocampal $\alpha 7$ nicotinic receptors, Leonard et al 2000). However, the reduction in DBA/2 mice is only about 30% (Stevens et al 1996) while in schizophrenia patients it is closer to 50% (Leonard et al 2000).

Given the clinical failure of the one $\alpha 7$ nicotinic receptor PAM tested (Winterer et al 2013), we assessed the PAM, PNU-120596, in the alternate model, the C3H mouse heterozygote for the $\alpha 7$ null mutation and found, contrary to the positive effects of this PAM in the DBA/2 mouse, that there was no improvement in sensory inhibition in these mice, at equal doses. But coupling the lowest dose with the $\alpha 7$ nicotinic agonist, choline chloride, did produce improvement in sensory inhibition. The explanation for this may lie in the difference between these two mouse strains in the level of hippocampal $\alpha 7$ nicotinic receptors. In the DBA/2 mice, there are sufficient $\alpha 7$ receptors for the enhancing or

magnifying effect of the PAM to boost the efficacy of the endogenously released acetylcholine enough to produce the improvement in sensory inhibition. In the C3H *Chrna7* heterozygote mice, the reduction in $\alpha 7$ receptor numbers is too great for this enhancing effect to work. The released acetylcholine is degraded by esterases too quickly. But the addition of an exogenous agonist, even a dose too low to be effective in of itself, is sufficient to produce enough $\alpha 7$ stimulation to produce the improvement.

This difference between DBA/2 and C3H *Chrna7* heterozygote mice may explain why PAMs were effective in the DBA/2 mouse but failed in the schizophrenia patient clinical trials. The DBA/2 mouse may simply not be the appropriate model. These data also suggest that for clinical trials, the combination of a low dose of an agonist and a PAM may result in improvement in sensory inhibition and cognition in humans. This would suggest that administration of a PAM to concurrently smoking schizophrenia patients may be effective in improving sensory inhibition and thus, cognition. While there may initially be receptor desensitization, the additional stimulation may actually reduce the inclination of patients to smoke. This theory is supported by the spontaneous reduction in smoking behavior observed in patients taking clozapine (Nagamoto et al 1996; 1999) which is known to increase hippocampal release of acetylcholine (Shirazi-Southall et al 2002).

In summary, the present study found the PAM, PNU-120596, to be effective alone in DBA/2 mice but not in C3H *Chrna7* heterozygote mice which have a greater reduction in hippocampal $\alpha 7$ nicotinic receptors, more closely modeling the condition in schizophrenia patients. The addition of a low dose of an $\alpha 7$ nicotinic agonist (choline chloride), which alone was ineffective, produced the improvement in sensory inhibition in these mice. These data suggest that the failure of the reported clinical trial with a PAM in the schizophrenia population (Winterer et al 2013) may be due to insufficient $\alpha 7$ nicotinic receptor stimulation due to the markedly reduced numbers of these receptors in this population, and that the addition of a low dose of a selective $\alpha 7$ agonist, or perhaps a partial agonist to avoid the issue of receptor desensitization, may produce improved sensory inhibition and cognition. Thus, continued clinical evaluations of PAMs in the schizophrenia population are encouraged.

4. Experimental Methods

1.1 Animals

Male and female C3H mice carrying the null mutation for the *Chrna7* gene were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, CO. Mice were derived as previously described (Adams et al 2008; 2012). Female null mutant mice were bred to wildtype male mice to yield offspring heterozygote for the *Chrna7* gene. These mice have been shown to have significantly reduced numbers of hippocampal $\alpha 7$ nicotinic receptors (~60% dependant upon the hippocampal region) and deficient sensory inhibition (Adams et al 2008). We used this breeding pattern because of the need for heterozygote mice only, and reductions in levels of $\alpha 7$ nicotinic receptors in male mice have been shown to diminish sperm motility, thus reducing fertility (Bray et al 2005). Mice were ~22–30 gm at testing.

Male DBA/2 (20–25 gm) mice were obtained from Harlan Laboratories (Indianapolis, ID). Both strains of mice were housed in shoebox caging with aspen chip bedding, food (TekLad, Harlan Labs) and water available *ad libitum*, light on 0600 to 1800. All studies were carried out in accordance with the Guide to the Use of Laboratory Animals, and were approved by the IACUC, University of Colorado, Anchutz Medical Campus.

1.2 Sensory Inhibition

Offspring and breeder-obtained mice (22–30 gm) were anesthetized with chloral hydrate (400 mg/kg, ip) and pyrazole (400 mg/kg, ip) to retard the metabolism of the chloral hydrate. The mouse was placed into a mouse adaptor for a stereotaxic instrument and maintained at 37°C with a heating pad. The scalp was incised and retracted. A burr hole was opened over the dorsal hippocampus [1.8 mm posterior to bregma, 2.7 mm lateral to midline (Paxinos and Franklin 2004)] for the recording electrode and a second hole opened over the contralateral cortex rostral to bregma for the reference electrode. A Teflon-coated, stainless steel wire recording electrode (127 μ M diameter) was lowered to the pyramidal cell layer of hippocampal area CA3 (–1.5–1.8 mm below dura). Final recording position was determined by the presence of complex spiking patterns typical of pyramidal cells (Miller and Freedman 1995). An identical electrode was placed on the anterior cortex to act as reference. Miniature earphones attached to hollow ear bars, placed at the externalization of the aural canal, delivered the computer-generated auditory stimuli. EEG responses to paired click stimuli (3000 Hz, 10 ms, 70 dB SPL, presented 0.5 sec apart, with 9 sec between pairs) were amplified 1000 times with bandpass filtering at 1–500 Hz and led to a computer for storage and analysis. Data were collected and analyzed using SciWorks data acquisition and analysis program (DataWave, Loveland CO). The responses to 16 pairs of stimuli were collected and averaged at 5-minute intervals. The maximum negativity between 20 and 60 msec after the stimulus (N40) was selected and measured relative to the preceding positivity (P20). This composite component has been shown to be less variable than either component (P20 or N40) alone (Hashimoto et al 2005). Three parameters were measured per record: conditioning amplitude—the magnitude of the response to the first stimulus, test amplitude—the magnitude of the response to the second stimulus, and TC ratio—the ratio of the test amplitude/conditioning amplitude, which is a measure of the level of inhibition (Stevens et al 1996). A TC ratio of 0.5 or less is evidence of normal sensory inhibition (Freedman et al 1983). Six baseline records were obtained prior to administration of drug(s). Records were obtained for 90 min following drug administration. At the end of the post-drug, animals were sacrificed.

1.3 Drugs

PNU-120596 and choline chloride were obtained from Sigma (Sigma-Aldrich, St. Louis, MO). Choline chloride was dissolved in physiological saline; PNU-120596 was dissolved in 5% DMSO, 5 % Solutol and 90 % physiological saline (Hurst et al 2005). Both drugs were administered at a volume of 4 ml/kg body weight. Animals receiving both PNU-120596 and choline, received first the PNU-120596, followed immediately by the choline.

1.4 Statistics

DBA/2 mouse data were analyzed by repeated measures MANOVA with time as the within subjects factor. C3H *Chrna7* heterozygote mouse data were initially analyzed by mixed model repeated measures MANOVA with sex as the independent factor, and time as the within subjects factor. In all analyses, sex was not found to be a significant factor so data were collapsed across sex and further statistical analyses were performed as repeated measures MANOVA with time as the within subjects factor. Fisher's LSD *a posteriori* analyses were used where appropriate. An alpha of <0.05 was determined to be significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Adams CE, Yonchek JC, Schulz KM, Graw SL, Stitzel J, Teschke PU, Stevens KE. Reduced *Chrna7* expression in mice is associated with decreases in hippocampal markers of inhibitory function: implications for neuropsychiatric diseases. *Neuroscience*. 2012; 207:274–82. [PubMed: 22314319]
- Adams CE, Yonchek JC, Zheng L, Collins AC, Stevens KE. Altered hippocampal circuit function in C3H $\alpha 7$ null mutant heterozygous mice. *Brain Res*. 2008; 1194:138–45. [PubMed: 18199426]
- Adler LE, Freedman R, Ross RG, Olincy A, Waldo MC. Elementary phenotypes in the neurobiological and genetic study of schizophrenia. *Biol Psychiat*. 1999; 46:8–18. [PubMed: 10394470]
- Adler LE, Hoffer LJ, Griffith J, Waldo M, Freedman R. Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol Psychiat*. 1992; 32:607–616. [PubMed: 1450287]
- Adler LE, Hoffer LJ, Wiser A, Freedman R. Transient normalization of a defect in auditory sensory processing in schizophrenics following cigarette smoking. *Am J Psychiat*. 1993; 150:1856–1861. [PubMed: 8238642]
- Adler LE, Olincy A, Waldo M, Harri JG, Griffith J, Stevens K, Flach K, Nagamoto H, Bickford P, Leonard S, Freedman R. Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr Bull*. 1998; 24:189–202. [PubMed: 9613620]
- Albuquerque EX, Pereira EF, Braga MF, Alkondon M. Contribution of nicotinic receptors to the function of synapses in the central nervous system: the action of choline as a selective agonist of $\alpha 7$ receptors. *J Physiol*. 1998; 92:309–16.
- Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. *J Neurosci*. 1999; 19:2693–705. [PubMed: 10087082]
- Bray C, Son JH, Kumar P, Meizel S. Mice deficient in *CHRNA7*, a subunit of the nicotinic acetylcholine receptor, produce sperm with impaired motility. *Biol Reprod*. 2005; 73:807–14. [PubMed: 15944242]
- Cullum CM, Harris JG, Waldo MC, Smernoff E, Madison A, Nagamoto HT, Griffith J, Adler LE, Freedman R. Neurophysiological and neuropsychological evidence for attentional dysfunction in schizophrenia. *Schizophr Res*. 1993; 10:131–141. [PubMed: 8398945]
- Dinklo T, Shaban H, Thuring JW, Lavreysen H, Stevens KE, Zheng L, Mackie C, Grantham C, Vandenberk I, Meulders G, Peeters L, Verachtert H, De Prins E, Lesage AS. Characterization of 2-[[4-fluoro-3-(trifluoromethyl)phenyl]amino]-4-(4-pyridinyl)-5-thiazolemethanol (JNJ-1930942), a

- novel positive allosteric modulator of the $\alpha 7$ nicotinic acetylcholine receptor. *J Pharmacol Exp Ther.* 2011; 336:560–74. [PubMed: 21084390]
- Di Paolo T, Gregoire L, Feuerbach D, Elbast W, Weiss M, gomez-Mancilla T. AQW051, a novel and selective nicotinic acetylcholine receptor $\alpha 7$ partial agonist, reduces L-Dopa-induced dyskinesias and extends the duration of L-Dopa effects in parkinsonian monkeys. *Parkinsonism Related Disord.* 2014 Epub ahead of print. 10.1016/j.parkreldis.2014.05.007
- Faghih R, Gfesser GA, Gopalakrishnan M. Advances in the discovery of novel positive allosteric modulators of the $\alpha 7$ nicotinic acetylcholine receptor. *Recent Pat CNS Drug Discov.* 2007; 2:99–106. [PubMed: 18221220]
- Fayuk D, Yakel JL. Regulation of nicotinic acetylcholine receptor channel function by acetylcholinesterase inhibitors in rat hippocampal CA1 interneurons. *Mol Pharmacol.* 2004; 66:658–66. [PubMed: 15322258]
- Freedman R. $\alpha 7$ –nicotinic acetylcholine receptor agonists for cognitive enhancement in schizophrenia. *Annu Rev Med.* 2014; 65:245–61. [PubMed: 24111888]
- Freedman R, Adams CE, Adler LE, Bickford PC, Gault J, Harris JG, Nagamoto HT, Olincy A, Ross RG, Stevens KE, Waldo M, Leonard S. Inhibitory neurophysiological deficit as a phenotype for genetic investigation of schizophrenia. *Am J Med Genet.* 2000a; 97:58–64. [PubMed: 10813805]
- Freedman R, Adams CE, Leonard S. The $\alpha 7$ -nicotinic acetylcholine receptor and the pathology of hippocampal interneurons in schizophrenia. *J Chem Neuroanat.* 2000b; 20:299–306. [PubMed: 11207427]
- Freedman R, Adler LE, Waldo MC, Pachtman E, Franks RD. Neurophysiological evidence for a defect in inhibitory pathways in schizophrenia: comparison of medicated and drug-free patients. *Biol Psychiat.* 1983; 18:537–51. [PubMed: 6134559]
- Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol Psychiatry.* 1995; 38:22–33. [PubMed: 7548469]
- Freedman R, Olincy A, Buchanan RW, Harris LG, Gold JM, Johnson L, Allensworth D, Guzman-Bonilla A, Clement B, Ball MP, Kutnick J, Pender V, Martin LF, Stevens KE, Wagner BD, Zerbe GO, Sote F, Kem WR. *Am J Psychiatr.* 2008; 165:1040–7. [PubMed: 18381905]
- Green MF, Kern RS, Heaton RK. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. *Schizophr Res.* 2004; 72:41–51. [PubMed: 15531406]
- Hajós M, Rogers BN. Targeting $\alpha 7$ nicotinic acetylcholine receptors in the treatment of schizophrenia. *Curr Pharm Des.* 2010; 16:538–54. [PubMed: 19909231]
- Harris JG, Kongs S, Allensworth D, Martin L, Tregellas J, Sullivan B, Zerbe G, Freedman R. Effects of nicotine on cognitive deficits in schizophrenia. *Neuropsychopharmacol.* 2004; 29:1378–1385.
- Hashimoto K, Iyo M, Freedman R, Stevens KE. Tropisetron improves deficient inhibitory auditory processing in DBA/2 mice: role of $\alpha 7$ nicotinic acetylcholine receptors. *Psychopharmacol.* 2005; 183:13–19.
- Hurst RS, Hajós M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, Groppi VE, Allaman G, Ogier R, Bertrand S, Bertrand D, Arneric SP. A novel positive allosteric modulator of the $\alpha 7$ neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J Neurosci.* 2005; 25:4396–405. [PubMed: 15858066]
- Jones CK, Byun N, Bubser M. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacol.* 2012; 37:16–42.
- Leiberman JA, Dunbar G, Segreti AC, Grigis RR, Seoane F, Beaver JD, Duan N, Hosford DA. A randomized exploratory trial of an $\alpha 7$ receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. *Neuropsychopharmacol.* 2013; 38:968–75.
- Leiser SC, Bowlby MR, Comery TA, Dunlop J. A cog in cognition: how the $\alpha 7$ nicotinic acetylcholine receptor is geared towards improving cognitive deficits. *Pharmacol Ther.* 2009; 122:302–11. [PubMed: 19351547]
- Leonard S, Adler LE, Benhammou K, Berger R, Breese CR, Drebing C, Gault J, Lee MJ, Logel J, Olincy A, Ross RG, Stevens K, Sullivan B, Vianzon R, Virnich DE, Waldo M, Walton K,

- Freedman R. Smoking and mental illness. *Pharmacol Biochem Behav.* 2001; 70:561–70. [PubMed: 11796154]
- Leonard S, Bertrand D. Neuronal nicotinic receptors: from structure to function. *Nicotine Tob Res.* 2001; 3:203–23. [PubMed: 11506765]
- Leonard S, Breese C, Adams C, Benhammou K, Gault J, Stevens K, Lee M, Adler L, Olincy A, Ross R, Freedman R. Smoking and schizophrenia: abnormal nicotinic receptor expression. *Eur J Pharmacol.* 2000; 393:237–42. [PubMed: 10771019]
- Martin LF, Freedman R. Schizophrenia and the $\alpha 7$ nicotinic acetylcholine receptor. *Int Rev Neurobiol.* 2007; 8:225–46. [PubMed: 17349863]
- McGeer PL, McGeer EG. Possible changes in striatal and limbic cholinergic systems in schizophrenia. *Arch Gen Psychiatr.* 1977; 34:1319–23. [PubMed: 45482]
- Metzger KL, Maxwell CR, Liang Y, Seigel SJ. Effects of nicotine vary across two auditory evoked potentials in the mouse. *Biol Psychiat.* 2007; 61:23–30. [PubMed: 16497274]
- Miller CL, Freedman R. The activity of hippocampal interneurons and pyramidal cells during the response of the hippocampus to repeated auditory stimuli. *Neurosci.* 1995; 69:371–381.
- Miyamoto S, Jarskog LF, Fleischhacker WW. Alternative pharmacologic targets for the treatment of schizophrenia: results from phase I and II trials. *Curr Opin Psychiatr.* 2013; 2:158–65.
- Nagamoto HT, Adler LE, Hea RA, Griffith JM, McRae KA, Freedman R. Gating of auditory P50 in schizophrenics: unique effects of clozapine. *Biol Psychiat.* 1996; 40:181–8. [PubMed: 8830951]
- Nagamoto HT, Adler LE, McRae KA, Huettl P, Cawthra E, Gerhardt G, Hea R, Griffith J. Auditory P50 in schizophrenics on clozapine: improved gating parallels clinical improvement and changes in plasma 3-methoxy-4-hydroxyphenylglycol. *Neuropsychobiol.* 1999; 39:10–7.
- Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, Gee KW. Nootropic $\alpha 7$ nicotinic receptor allosteric modulator derived from GABAA receptormodulators. *Proc Natl Acad Sci U S A.* 2007; 104:8059–64. [PubMed: 17470817]
- Olincy A, Freedman R. Nicotinic mechanisms in the treatment of psychotic disorders: a focus on the $\alpha 7$ nicotinic receptor. *Handb Exp Pharmacol.* 2012; 213:211–32. [PubMed: 23027417]
- Olincy A, harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, Ellis J, Zerbe GO, Leonard S, Stevens KE, Stevens JO, Adler LE, Soti F, Kem WR, Freedman R. Proof-of-concept trial of an $\alpha 7$ nicotinic agonist in schizophrenia. *Arch Gen Psychiatr.* 2006; 63:630–8. [PubMed: 16754836]
- Olincy A, Stevens KE. Treating schizophrenia symptoms with an $\alpha 7$ nicotinic agonist, from mice to men. *Biochem Pharmacol.* 2007; 74:1192–201. [PubMed: 17714692]
- Papke RL, Kem WR, Soti F, López-Hernández GY, Horenstein NA. Activation and desensitization of nicotinic $\alpha 7$ -type acetylcholine receptors by benzylidene anabaseines and nicotine. *J Pharmacol Exp Ther.* 2009; 329:791–807. [PubMed: 19223664]
- Paxinos, G.; Franklin, KBJ. *The Mouse Brain in Stereotaxic Coordinates.* San Diego: Academic Press; 2004.
- Potter D, Summerfelt A, Gold J, Buchanan RW. Review of clinical correlates of P50 sensory gating abnormalities in patients with schizophrenia. *Schizophr Bull.* 2006; 32:692–700. [PubMed: 16469942]
- Radek RJ, Miner HM, Bratcher NA, Decker MW, Gopalakrishnan M, Bitner RS. $\alpha 4\beta 2$ nicotinic receptor stimulation contributes to the effects of nicotine in the DBA/2 mouse model of sensory gating. *Psychopharmacol.* 2006; 187:47–55.
- Radek RJ, Robb HM, Stevens KE, Gopalakrishnan M, Bitner RS. Effects of the novel $\alpha 7$ nicotinic acetylcholine receptor agonist ABT-107 on sensory gating in DBA/2 mice: pharmacodynamic characterization. *J Pharmacol Exp Ther.* 2012; 343:736–45. [PubMed: 22988063]
- Szabo AK, Pesti k, Mike A, Vizi ES. Node of action of the positive modulator PNU-120596 on $\alpha 7$ nicotinic acetylcholine receptors. *Neuropharmacol.* 2014; 81:42–54.
- Siegel SJ, Maxwell CR, Majumdar S, Frief DF, Lerman C, Gur RE, Kanen SJ, Liang Y. Monoamine reuptake inhibition and nicotine receptor antagonism reduce amplitude and gating of auditory evoked potentials. *Neurosci.* 2005; 133:729–738.

- Shirazi-Southall S, Rodriguez DE, Nomikos GG. Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. *Neuropsychopharmacol.* 2002; 26:583–94.
- Simosky JK, Stevens KE, Freedman R. Nicotinic agonists and psychosis. *Curr Drug Targets CNS Neurol Disord.* 2002; 1:149–62. [PubMed: 12769624]
- Simosky JK, Stevens KE, Kem WR, Freedman R. Intragastric DMXB-A, an $\alpha 7$ nicotinic agonist, improves deficient sensory inhibition in DBA/2mice. *Biol Psychiat.* 2001; 50:493–500. [PubMed: 11600102]
- Smucny J, Olincy A, Eichman LC, Lyons E, Tregellas JR. Early sensory processing deficits predict sensitivity to distraction in schizophrenia. *Schizophr Res.* 2013; 147:196–200. [PubMed: 23590872]
- Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks MJ, Rose GM. Genetic correlation of hippocampal auditory evoked response and α -bungarotoxin binding in inbred mouse strains. *Neuropsychopharmacol.* 1996; 15:152–162.
- Stevens KE, Kem WR, Mahnir V, Freedman R. Selective $\alpha 7$ nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacol.* 1998; 136:320–32.
- Stevens KE, Wear KD. Normalizing effects of nicotine and a novel nicotinic agonist on hippocampal auditory gating in two animals models. *Pharmacol Biochem Behav.* 1997; 57:869–874. [PubMed: 9259018]
- Tani Y, Saito K, Imoto M, Ohno T. Pharmacological characterization of nicotinic receptor-mediated acetylcholine release in rat brain--an in vivo microdialysis study. *Eur J Pharmacol.* 1998; 351:181–8. [PubMed: 9687001]
- Uteshev VV. The therapeutic promise of positive allosteric modulation of nicotinic receptors. *Eur J Pharmacol.* 2014; 727:181–5. [PubMed: 24530419]
- Wallace TL, Bertrand D. Neuronal nicotinic receptors as a drug target in schizophrenia. *Expert Opin Ther Targets.* 2013; 7:139–55. [PubMed: 23231385]
- Wildeboer KM, Stevens KE. Stimulation of the $\alpha 4\beta 2$ nicotinic receptor by 5-I A-85380 improves auditory gating in DBA/2 mice. *Brain Res.* 2008; 1224:29–3. [PubMed: 18582447]
- Wildeboer-Andrud KM, Stevens KE. The smoking cessation drug varenicline improves deficient P20-N40 inhibition in DBA/2 mice. *Pharmacol Biochem Behav.* 2011; 100:17–24. [PubMed: 21763340]
- Williams DK, Wang J, Papke RL. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. *Biochem Pharmacol.* 2011; 82:915–30. [PubMed: 21575610]
- Winterer G, Gallinat J, Brinkmeyer J, Musso F, Kornhuber J, Thuermer N, Rujescu D, Favis R, Sun Y, Franc MA, Ouwerkerk-Mahadevan S, Janssens L, Timmers M, Streffer JR. Allosteric $\alpha 7$ nicotinic receptor modulation and P50 sensory gating in schizophrenia: a proof-of-mechanism study. *Neuropharmacol.* 2013; 64:197–204.
- Yee CM, Neuchterlien KH, Morris SE, White PM. P50 suppression in recent-onset schizophrenia: Clinical correlates and risperidone effects. *J Abnorm Psychol.* 1998; 107:691–698. [PubMed: 9830258]

Highlights

PAMs improve sensory inhibition in DBA/2 mice but not in clinical trials

PAM PNU120596 fails to improve sensory inhibition in *Chrna7* heterozygote mice

Choline fails to improve sensory inhibition in DBA/2 or C3H *Chrna7* heterozygote mice

Combining the PAM and choline improves sensory inhibition in C3H *Chrna7* heterozygote mice

Combining a PAM and choline in clinical trials may yield beneficial effects

Figure 1.

Effects of PNU-120596 on sensory inhibition parameters in DBA/2 mice. A) PNU-120596 at a dose of 1 mg/kg produced significant increases in conditioning amplitude, which produced significant decreases in TC ratio. B) Increasing the dose of PNU-120596 to 3.33 mg/kg retained both the increases in conditioning amplitude and decreases in TC ratio, again, without affecting test amplitude. Data are mean + SEM, $n=6$ per group, * $p<0.05$, ** $p<0.01$.

Figure 2.

Individual animal waveforms for C3H *Chrna7* heterozygote mice receiving choline (10 or 33 mg/kg), PNU-120596 (1 or 3.33 mg/kg) or the combination of PNU-120596 (1 gm/kg) + choline (10 or 33 mg/kg). Neither choline nor PNU-120596, alone, were sufficient to improve sensory inhibition in this model, however, combining the low dose of PNU-120596 with either dose of choline did produce improved sensory inhibition. Although the presented wave forms for the high dose of choline + PNU-120596 suggest that it was effective, the overall analysis failed to achieve statistical significance. Arrows mark stimulus onset, ticks mark the wave of interest. Calibration: 50 μ Volts, 40 msec.

Figure 3.

Effect of PNU-120596 on sensory inhibition parameters in C3H *Chrna7* heterozygote mice.

A) PNU-120596 at a dose of 1 mg/kg, failed to alter conditioning or test amplitude, or TC ratio. B) Increasing the dose of PNU-120596 to 3.33 mg/kg, still failed to alter conditioning amplitude but produced a significant effect on test amplitude. These was a significant overall increase in TC ratio, but no individual time points post injection were significantly changed. Data are mean + SEM, $n=8$ per group, $*p<0.05$, $**p<0.01$.

Figure 4.

Effect of choline chloride on sensory inhibition parameters in C3H *Chrna7* heterozygote mice. A) At a dose of 10 mg/kg, choline chloride had no effect on any sensory inhibition parameter. B) Increasing the dose of choline chloride to 33 mg/kg produced a significant increase in test amplitude, with no changes in conditioning amplitude or TC ratio. Data are mean + SEM, $n=8$ per group.

Figure 5.

Effect of combining low dose PNU-120596 (1 mg/kg) with either dose of choline chloride on sensory inhibition parameters in C3H *Chrna7* mice. A) PNU-120596 + 10 mg/kg choline chloride produced a sustained period of increased conditioning amplitude which produced a somewhat shorter period of significantly decreased TC ratio. Test amplitude was erratic, showing a significant decrease shortly after injection, but which switched to become a significant increase later in the recording period. B) Increasing the dose of choline chloride to 33 mg/kg, coupled with the 1 mg/kg dose of PNU-120596, lost the previously observed significant changes in sensory inhibition parameters, possibly due to receptor desensitization. Data are mean + SEM, $n=8$ per group, * $p<0.05$, ** $p<0.01$.