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Delayed procedural learning in α7-nicotinic acetylcholine receptor knockout mice

J. W. Young[*], **J. M. Meves**, **I. S. Tarantino**, **S. Caldwell**, and **M. A. Geyer**
Department of Psychiatry, University of California San Diego, La Jolla, CA, USA

Abstract

The α7-nicotinic acetylcholine receptor (nAChR) has long been a procognitive therapeutic target to treat schizophrenia. Evidence on the role of this receptor in cognition has been lacking, however, in part due to the limited availability of suitable ligands. The behavior of α7-nAChR knockout (KO) mice has been examined previously, but cognitive assessments using tests with cross-species translatability have been limited to date. Here, we assessed the cognitive performance of α7-nAChR KO and wild-type (WT) littermate mice in the attentional set-shifting task of executive functioning, the radial arm maze test of spatial working memory span capacity and the novel object recognition test of short-term memory. The reward motivation of these mutants was assessed using the progressive ratio breakpoint test. In addition, we assessed the exploratory behavior and sensorimotor gating using the behavioral pattern monitor and prepulse inhibition, respectively. α7-nAChR KO mice exhibited normal set-shifting, but impaired procedural learning (rule acquisition) in multiple paradigms. Spatial span capacity, short-term memory, motivation for food, exploration and sensorimotor gating were all comparable to WT littermates. The data presented here support the notion that this receptor is important for such procedural learning, when patterns in the environment become clear and a rule is learned. In combination with the impaired attention observed previously in these mice, this finding suggests that agonist treatments should be examined in clinical studies of attention and procedural learning, perhaps in combination with cognitive behavioral therapy.

Keywords

α7-nicotinic acetylcholine receptor; exploration; motivation; mice; mutant; prepulse inhibition; procedural learning; schizophrenia; set-shifting; short-term memory

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Procognitive agents are needed to improve functional outcome in patients with schizophrenia. The lack of an approved drug for treating cognitive dysfunction in schizophrenia prompts basic research on specific targets. Relative to healthy comparison subjects, patients with schizophrenia are more likely to smoke cigarettes, smoke more cigarettes per day and extract more nicotine per cigarette (Kumari & Postma 2005). It is theorized that these patients are self-medicating because nicotine exerts procognitive effects across several cognitive domains (Barr *et al*. 2008; D'souza & Markou 2011; Levin *et al*. 1998; Myers *et al*. 2008; Newhouse *et al*. 2004; Poltavski & Petros 2006). Unfortunately, nicotine also possesses deleterious effects such as nausea and addiction, limiting its use as a therapeutic drug. Nicotine is the prototypical ligand for the nicotinic acetylcholine receptors

[*]Corresponding author: J. W. Young, PhD, Department of Psychiatry, University of California San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804, USA. jaredyoung@ucsd.edu.

(nAChRs), of which the α7 and α4β2 nAChRs are the most populous. Given links between the α7-nAChR and schizophrenia, this receptor has been hailed as one of the most promising targets for treating cognition dysfunction in these patients (Geyer & Tamminga 2004; Tamminga 2006).

Several lines of evidence support the α7-nAChR as a target for treating patients with schizophrenia (Levin *et al* 2006; Martin *et al* 2004). Abnormalities in the 15q13-15 region of chromosome 15, which contains the α7-nAChR subunit genotype CHRNA7, are linked to poor sensory gating in schizophrenia patients and may constitute a susceptibility genotype (Freedman *et al* 1997). Moreover, lower α7-nAChR protein levels are observed in the post-mortem brains of patients with schizophrenia and associated with cognitive dysfunction (Martin-Ruiz *et al* 2003), despite no alterations in cortical mRNA expression (De Luca *et al* 2006; Martin-Ruiz *et al* 2003). Early clinical tests of a partial α7-nAChR agonist (GTS-21, aka DMXBA) were promising with regard to improving cognition in patients with schizophrenia (Olincy *et al* 2006), but were not replicated in larger studies (Freedman *et al* 2008).

Determining α7-nAChR pharmacological effects has been problematic due to the lack of available compounds that are both selective and blood–brain barrier (BBB) permeable. The prototypical α7-nAChR antagonist, α-Bungarotoxin (α-BgT) is BBB impermeable, while the antagonist methyllycaconitine (MLA) exhibits only 5–20% permeability (Turek *et al* 1995). MLA permeability is further lowered by co-administration of nicotine (Lockman *et al* 2005). The full α7-nAChR agonist AR-R1779 also exhibits poor BBB permeability (Cilia *et al* 2005), while DMXBA is only a partial agonist (Olincy *et al* 2006). Although more selective BBB-permeable compounds are being created by companies (Thomsen *et al* 2009), the availability and thus independent assessment of these drugs remain limited.

A more precise mechanism to understand contributions of α7-nAChRs to behavior is to phenotype α7-nAChR knockout (KO) and heterozygous (HT) littermate mice (Orr-Urtreger *et al* 1997). Previously, Paylor *et al* (1998) compared α7-nAChR null mutants to wild-type (WT) littermates and found no abnormalities in a variety of behaviors, including activity levels, water maze spatial learning, prepulse inhibition (PPI) and Pavlovian fear conditioning. Nevertheless, the limited cognitive paradigms used were minimally relevant to the cognitive deficits characteristic of patients with schizophrenia (Young *et al* 2009b). When more complex food-motivated tasks are used, α7-nAChR null mutant mice exhibit impaired sustained attention as measured by the five-choice serial reaction time task (5-CSRTT) (Hoyle *et al* 2006; Young *et al* 2004, 2007a), as well as impaired operant learning (Keller *et al* 2005; Levin *et al* 2009; Young *et al* 2004). Patients with schizophrenia, however, exhibit deficits in other cognitive domains, including executive functioning, working memory (WM) and short-term memory, which can be assessed preclinically in paradigms with putative cross-species translational validity, e.g. the attentional set-shifting task (ASST), radial arm maze (RAM) and novel object recognition task (NORT), respectively (Young *et al* 2009b).

Like the 5-CSRTT, the ASST is an appetitive-rewarding task and although deficits in such tasks could mean impaired cognition, poor performance of α7-nAChR KOs in these paradigms may reflect reduced motivation (Hoyle *et al* 2006), given the interaction between nicotine and food motivation (Perkins *et al* 1991).While α7-nAChR null mutant mice did not differ in their speed to collect food rewards in the 5-CSRTT (Hoyle *et al* 2006; Young *et al* 2004, 2007a), a putative measure of motivation (Robbins 2002), there are more sensitive measures of reward motivation such as the progressive ratio breakpoint paradigm (PRBP) (Bensadoun *et al* 2004; Young & Geyer 2010).

A greater understanding is required on the cognitive domains affected by null mutation of the α7-nAChR using cross-species relevant tasks. Moreover, cross-species paradigms are available to assess exploratory behavior using multivariate analysis (Perry *et al* 2009; Young *et al* 2007c) and there are further aspects of PPI to assess (Ouagazzal *et al* 2006; Young *et al* 2010c). Finally, the motivation levels of α7-nAChR mutant mice requires clarification to interpret apparent deficits in attention, as do other aspects of cognition that are relevant to schizophrenia (Marder 2006; Nuechterlein *et al* 2004). Here, we describe the behavioral characterization of α7-nAChR WT, HT and KO mice in the ASST, NORT, RAM, BPM (behavioral pattern monitor), PPI and PRBP.

Methods

Animals

The α7-nAChR mutant and WT mice were derived from HT breeding pairs. The mice were backcrossed for at least 11 generations onto a C57B/6J background. Male WT, HT and KO mice were used in the present studies. Genotype was confirmed by tail tipping mice at around 28 days under isoflurane anesthesia, DNA obtained by proteinase K digestion of tail, with PCR conducted as described (Marks *et al* 1999; Orr-Urtreger *et al* 1997) (www.jax.org). The mice had unlimited access to water and food (Harlan, Madison, WI, USA), except during treatment and testing where stated below. Age, sex, sample sizes, weights and test naivety are provided for each experiment below. During training and testing periods that used food rewards, mice were maintained at 85% of free-feeding weight, with water available *ad libitum*. Mice were housed separately in groups of maximum four per cage in a vivarium on a reversed day–night cycle (lights on at 2000 h, off at 0800 h). Mice were brought to the laboratory 60 min before testing between 0900 h and 1800 h. All behavioral testing procedures were approved by the UCSD Institutional Animal Care and Use Committee. All mice were maintained in an animal facility that meets all federal and state requirements for animal care and was approved by the American Association for Accreditation of Laboratory Animal Care.

Attentional set-shifting task

Fourteen WT (10 ♀ and 4 ♂) and 14 KO mice (11 ♀ and 3 ♂), naive to behavioral testing, were assessed using the ASST when they were approximately 5 months old and weighed between 20 and 40 g. The ASST apparatus and procedure were performed as previously described (Young *et al* 2010b). Ceramic pots (4.5 × 2.5 cm) were used as digging bowls, given that mice readily dig in bedding placed in such bowls to retrieve a food reward using olfactory cues to solve tasks (Young *et al* 2007a, b, 2008). These bowls were located on platforms (11 × 5 cm). Specific odors and platform textures were utilized as cues to guide the selection of a bowl. Odors were derived from commercially available powdered spices including nutmeg, ground ginger, coriander, garlic, thyme and cinnamon (Albertsons®, San Diego, CA, USA). Platforms included tile, sandpaper, neoprene, metal wire, wood and a scrubber (Homebase®, San Diego, CA, USA). We have previously demonstrated that mice readily use platforms to identify reward location (Young *et al* 2010b). The food reward was a single 25 mg food pellet (Noyes Precision, TestDiet, Richmond, IN, USA). The test apparatus was an adapted perspex home cage (30 × 18 × 12 cm). Clear plastic panels were used to separate half of the cage into two equal sections. Two digging bowls were placed in each quarter section with access limited by removable dividers, used to deny the mice access to the other bowl after they had made a selection.

Shaping—Day 1: Mice were brought into the testing room and chamber and trained to dig in unscented bedding for food reward. Day 2: Mice were required to dig in bowls containing each of the odors mounted on each of the platforms that they would encounter in the main

task. The criterion for a dig was defined as when the nose or paws of the mouse broke the surface of the digging medium and was used throughout testing.

ASST paradigm—The ASST procedure utilized was consistent with previous studies (Young *et al* 2010b). Briefly, mice were required to perform a series of discriminations selecting bowls dependent on the stimulus in a particular dimension, either odor or platform. These discrimination stages included a simple discrimination (SD) stage (only one relevant dimension presented), a compound discrimination (CD) stage (introducing the second dimension), CD reversal (CDR) stage (requiring mice to respond to the bowl associated with the previously irrelevant stimulus), an intradimensional (ID) shift (novel stimuli presented), an ID reversal (IDR) stage (reversal of ID rule), an extradimensional (ED) shift (a stimulus from the previously irrelevant dimension would identify the baited bowl) and finally ED reversal (EDR) stage (reversal of the ED rule). In trials 1–4 of SD and CD, mice were permitted to dig in the unbaited bowl without consequence, although an error was recorded, so that the mouse could move to the baited bowl and learn the cue contingency. Subsequent errors in trials resulted in the mouse being denied entry to the other area, an error was recorded and the trial restarted. The mice were required to make six consecutive correct responses at each stage prior to moving to the next. Mice were counterbalanced so that the initial SD would be either odor or platform. Stimuli combinations and locations were selected in a varied order. *Trials to criterion, errors and mean correct latencies* (cumulative correct latency/total correct responses) were recorded for each stage. Latencies were measured using stopwatches; initiated as the doors were raised and stopped when the mouse dug in one of the choices available. Despite all stages requiring some form of learning, it has been demonstrated that different stages are mediated by different neuroanatomical substrates, in rats andmice, e.g. ED shifts mediated by the medial prefrontal cortex (Birrell & Brown 2000; Bissonette *et al* 2008), while reversal learning is mediated by the orbitofrontal cortex (Bissonette *et al* 2008; McAlonan & Brown 2003). Given the different neuroanatomical contributions to stages, performance in the task is often analyzed by conceptual grouping of ED shifting (the ‘set-shifting’ measure), reversal learning and SDs.

Radial arm maze

Apparatus—Eight WT (♂) and 6 KO (♂) mice naive to behavioral testing were trained in the RAM WM span task when they were approximately 3 months old and weighed between 20 and 30 g. We used an automated 12-arm RAM (Coulbourn Instruments, Whitehall, PA, USA) interfaced with Med Associates controlling software (Med Associates Inc., St. Albans, VT, USA) as described previously (Tarantino *et al* 2011). Twelve arms radiated from a dodecahedral central hub (43 cm in diameter), with each arm (36 × 10 × 12 cm) separated by 30°. Access from the central hub to each of the 12 arms was controlled using automated guillotine doors. The floor of each arm was a metal grid and the walls and ceiling were made of acrylic glass. Arm activity was monitored using an infrared beam 5.5 cm from the entrance. An infrared beam also monitored entries into the dipper mechanism located at the distal end of each arm. Each dipper provided liquid reinforcement via computer-controlled dipping into a liquid-filled bowl below the dipper. The entire RAM was fixed to a circular acrylic table (124 cm in diameter) raised 53 cm from the floor painted black to avoid a potential confound of depth perception-induced anxiety (Komada *et al* 2008). Seven extra-maze spatial cues were created and fixed at conspicuous locations around the four walls at varying heights. Dim fluorescent lighting was provided in the room (110 lux in the dodecahedral central hub). A camera was positioned above the maze to track animal behavior. External auditory distractions were minimized by providing a constant background white noise (60 dB(A) scale) throughout training and testing.

Habituation to the testing environment—Acclimation to strawberry milkshake (Nesquik® + non-fat milk) reinforcement occurred prior to training in the home cage tominimize a neophobic response to the reinforcer during training. Mice were initially placed in the RAM with all 12 doors opened and food dippers raised providing a single reward to acclimate the mice to the RAM. Exploration of the RAM was encouraged by randomly placing strawberry milkshake throughout the apparatus. Habituation occurred for five consecutive days with exposure time initially at 10 min (day 1), then reduced to 7 min (days 2–5).

RAM protocol—WM span capacity (WMC) was assessed within a session by baiting ten arms (designated WM arms) only at the start of each session. The two remaining arms (allocated as RM arms) were never baited throughout training or testing. For each session, mice were placed in the hub with all doors closed. The session began with all 12 doors being raised allowing entry into all 12 arms. Entry into an arm resulted in the doors to the remaining 11 arms being closed, barring entry into any other arm. Upon retrieval of the food reward from the magazine (if a WM arm), returning to the hub resulted in all 11 doors being raised after a 1-second delay. The 1-second delay was instituted to interfere with the development of simple turn left/right strategies (Wenk 2004). Entry into any of the 12 arms was again possible. *WMC* was measured as the number of WM arms entered prior to a repeat entry. Repeat entries into a WM arm (recorded as a *WM error*) did not require a magazine head entry to initiate the arms to reopen. Similarly, for entries into RM arms (recorded as an *RM error*), magazine head entries were not required to reopen the remaining doors. The total number of WM errors were totaled and divided by the total number of WM arms entered (%*WM errors*). The total number of RM errors were totaled and divided by the total number of RM arms entered (%*RM errors*). These percentages were calculated to provide a measure of WM and RM errors as a function of total arms entered. Total arms entered (*Arms*) composed of total WM and RM arms entered. The development and use of a simple turn left/right strategy was assessed by totaling all the possible relative transitions from one arm to another. The degree of strategy use was then quantified by calculating the coefficient of variation (*StrategyCV*) of the total possible relative transitions for each subject. Sessions continued until 7 min had elapsed or all 10 WM arms were entered.

Novel object recognition task

The short-term memory of 11 WT (4 ♀ and 7 ♂) and 9 KO (3 ♀ and 6 ♂) mice was assessed using the NORT as described previously (Ali *et al* 2011).Mice weighed between 20 and 30 g, were approximately 3 months old and were naive to behavioral testing. Single trial NORT was performed in an open arena (60 × 60 cm) using two object types (Lego pyramid and 50 ml plastic conical tube) affixed to the floor using Velcro tape in opposing corners of the open field 10 cm away from the walls. Each mouse completed one session that comprised of three successive trials. In trial 1, habituation phase, the mouse was placed in the center of the empty open field box and allowed to freely explore. During trial 2, the sample phase, two of the same objects were placed in opposite corners of the box and the mouse was allowed to explore the objects. Half of the mice were randomly assigned to either starting with the Lego pyramid or the conical tube as the familiar object. In trial 3, one of the objects was replaced with a new object to assess novel object exploration. Each trial was 5 min with a 3-min inter-trial interval (ITI). This ITI was chosen to allow for the largest behavioral window to detect group differences. The mouse was removed from the testing box and placed in a holding cage during the ITI. The box and the objects were carefully cleaned with water between each trial and cleaned with 70% alcohol at the end of each testing session. Locomotor activity was assessed using Ethovision tracking software (Noldus Information Technology, Wageningen, Netherlands). Object exploration was defined as when the mouse's nose was within 2 cm of the object. Performance was calculated as the

percentage of novel object interaction (%NI): the length of time exploring the novel object/ the time exploring the novel object + the familiar object.

Progressive ratio breakpoint study

The motivation/value of reward for 12 WT (♂), 16 HT (♂) and 13 KO (♂) mice was trained in the PRBP as described previously (Young & Geyer 2010). Mice weighed between 20 and 30 g, were approximately 3 months old and were naive to behavioral testing. Shaping and progressive ratio testing took place in 16 five-hole operant chambers (25 × 25 × 25 cm; Med Associates Inc.). Each chamber consisted of an array of five square apertures (2.5 × 2.5 × 2.5 cm) arranged horizontally on a curved wall 2.5 cm above the grid floor opposite to a reward delivery area (Lafayette Instruments, Lafayette, IN, USA) at floor level with a house light near the ceiling. The chamber was housed in a sound-attenuating box with ventilation provided by a fan that also provided a low level of background noise. Performance was monitored during training and testing using an infrared camera installed in each chamber. Responses with a nose-poke to illuminated LEDs recessed into the apertures were detected by infrared beams mounted vertically located 3 mm from the opening of the aperture (in-house modification). Liquid reinforcement in the form of strawberry milkshake (Nesquik® + non-fat milk, 30 µl, Nestle, Glendale, CA, USA) was utilized and delivered by peristaltic pump (Lafayette Instruments) to a well located in the magazine opposite the five-hole wall. Reward delivery area entries were monitored using an infrared beam mounted horizontally, 5 mm from the floor and recessed 6 mm into the magazine. The control of stimuli and recording of responses were managed by a SmartCtrl Package 8-In/16-Out with additional interfacing by MED-PC for Windows (Med Associates Inc.) using custom in-house programming.

Progressive ratio breakpoint testing—Mice were initially shaped to nose-poke in the central aperture for a single food reward (fixed ration, FR1) as described previously (Young *et al* 2009a). Each trial was initiated by nose-poking in the reward delivery area, after which the central light was immediately illuminated. The central light remained illuminated until the mouse nose-poked that aperture. A nose-poke in the central aperture resulted in a single food reward being delivered in the reward delivery area. Each session lasted 30 min with no trial limit. Standard acquisition criterion was >70 responses for two consecutive days, although training continued until the number of responses were stable (no effect of day when assessed Tuesday through Friday).

Once nose-poking reliably, mice were challenged in the breakpoint study. The number of nose-pokes required to gain a reward increased according to the following progression: 1, 2, 4, 7, 11, 16, 22, 29, 37, 46, 56 and 67. To maintain responding, the mice could respond three times at each ratio before moving to the next, receiving one reward each time. The session continued for 60 min or until 5 min had passed without a single nose-poke. The *breakpoint* was defined as the last ratio to be completed before the session end. Mean response latency (*MRL*) was also calculated. The progressive ratio challenge was conducted on Tuesday with normal shaping on the previous day.

Behavioral pattern monitor

The exploratory behaviors of 12 WT (5 ♀ and 7 ♂) and 14 KO (8 ♀ and 6 ♂) mice were assessed using the BPM. Mice weighed between 23 and 47 g, were approximately 7 months old and a subset of which were assessed in the NORT. Nine mouse BPM chambers were used to assess the spontaneous exploratory behavior as described previously (Halberstadt *et al* 2009; Risbrough *et al* 2006). Each chamber is illuminated from a single light source above the arena (30.5 × 61 × 38 cm) area with a Plexiglas hole board floor equipped with three floor holes and eight wall holes (Young *et al* 2010a). Nose-poking behavior was

detected using an infrared photobeam. The location of the mouse was recorded every 0.1 seconds using a grid of 12× 24 infrared photobeams located 1 cm above the floor. The position of the mouse was defined across nine unequal regions (Geyer *et al* 1986; Risbrough *et al* 2006; Young *et al* 2010a). Rearing behavior was recorded using an array of 16 infrared photobeams 2.5 cm above the floor aligned with the long axis of the chamber. At the start of each test session, mice were placed in the bottom left hand corner of the chamber, facing the corner and the test session started immediately.

Three main factors were investigated based on previous analysis of multivariate factor loading (Paulus & Geyer 1993): locomotor activity as measured by *transitions* (calculated as a movement across a defined region); exploratory behavior as measured by *holepoking*, *varied holepoking* (total holepokes minus repetitious poking) and *rearing*; and locomotor pattern as assessed by the *spatial d* measure of dimensionality. Spatial d uses analyses based on fractal geometry to quantify the geometrical structure or dimensionality of the locomotor path, where a value of 2 represents highly localized two-dimensional movements and 1 represents one-dimensional straight distance-covering movements (for calculations of the spatial d value, please see Paulus & Geyer 1991).

Prepulse inhibition

The sensorimotor gating of 15 WT (8 ♀ and 7 ♂), 17 HT (8 ♀ and 9 ♂) and 16 KO (9 ♀ and 7 ♂) mice was assessed using the PPI paradigm. Mice weighed between 23 and 52 g, were approximately 10 months old and a subgroup had previously been tested in the NORT and the BPM. Startle chambers (SR-LAB; San Diego Instruments, San Diego, CA, USA) consisted of nonrestrictive Plexiglas cylinders 5 cm in diameter resting on a Plexiglas platform in a ventilated chamber. High-frequency speakers mounted 33 cm above the cylinders produced all acoustic stimuli. Piezoelectric accelerometers mounted under the cylinders transduced movements of the animal, which were digitized and stored by an interface and computer assembly. Beginning at the stimulus onset, 65 consecutive 1 millisecond readings were recorded to obtain the peak amplitude of the animals' startle response to acoustic (40 milliseconds) startle stimuli. Peak responses to these stimuli are presented in arbitrary units. A dynamic calibration system was used to ensure comparable sensitivities across chambers. Sound levels were measured as described elsewhere (Mansbach & Geyer 1988) using the A weighting scale in units of dB(A) SPL. The light was delivered via a bare 15 W incandescent bulb located on the ceiling of the testing chamber. A 65 dB background noise was presented continuously throughout the session.

PPI challenge 1—The experimental session consisted of a 5-min acclimatization period to a 65-dB background noise, followed by the PPI test. Because no effect of genotype on PPI using variable prepulses was observed previously (Paylor *et al* 1998), we used a mixed modality PPI test session which we have previously demonstrated to be sensitive to aging effects (Young *et al* 2010c). During the session, four trial types that varied in modality (auditory, visual) were presented 12 times in a pseudorandom order. Trial types were 120 dB pulse (*pulse-alone*), 73 dB prepulse preceding a 120 dB pulse (*prepulse + pulse*), 100 milliseconds light prepulse preceding a 120 dB pulse (*light + pulse*). No stimulus (*nostim*) trials occurred between each trial with an average ITI of 15 seconds. In addition, six of the *pulse-alone* trials, which were not included in the calculation of PPI values, were presented at the beginning of the test session to achieve a relatively stable level of startle reactivity for the reminder of the session (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus (Geyer *et al* 1986).

PPI challenge 2—The next PPI test session consisted of three testing blocks. Block 1 (*pulse intensities*) assessed acoustic startle responding across stimulus intensities with four presentations of 80, 90, 100, 110 and 120 dB pulse-alone trials. Block 2 (*prepulse intensities*) consisted of twelve 120 dB startle pulse-alone intensities interspersed with 10 each of 3 different prepulse trials: 69, 73 and 81 dB prepulses preceding a 120 dB pulse. Prepulses preceded the pulse by 100 milliseconds [i.e. interstimulus interval (ISI), onset to onset]. Block 3 (*ISI variation*) varied the ISI. The block consisted of 7 startle pulses at 120 dB and 4 each of 73 dB prepulses preceding a 120 dB pulse by 20, 50, 100, 200, 500 and 1000 milliseconds (onset to onset).

Statistical analyses

For every study, an analysis of variance (ANOVA) was used to examine performance by genotype and sex as between-subject factors. For ASST, performance was analyzed in terms of trials to criterion using a repeated-measure ANOVA with stage (SD, CD, CDR, ID, IDR, EDS and EDR) as a within-subject factor and starting dimension as a between-subject factor. Selected stages were also analyzed separately to examine specific cognitive constructs: discrimination learning was assessed by comparing SD, CD and ID; reversal learning was evaluated by comparing CDR, IDR and EDR; attentional set formation was investigated by comparing ID and ED. The RAM was analyzed during acquisition and stability using day as a within-subject factor. Acquisition of the PRB study was measured in terms of days to criterion, while PRB was assessed in a single day. NORT performance was compared with chance (50%) using a one sample *t* test. For the BPM, time was analyzed as a within-subject factor. For PPI, varying prepulses, modalities and ISIs were analyzed as within-subject factors. Tukey *post hoc* analyses were performed on any significant main effect or interaction. Alpha level was set to 0.05. Data were analyzed using SPSS for Windows 14 (Chicago, IL, USA) and Biomedical Data Programs statistical software (Statistical Solutions Inc., Saugus, MA, USA).

Results

Attentional set-shifting task

Overview of task performance—The performance of α7-nAChR WT and KO mice was compared in the ASST. Performance was initially assessed for conceptual validity – that is, an attentional set was formed by the mice as measured by a significant difference in the total trials to criterion between ID and ED, irrespective of starting dimension. A main effect of stage was observed on trials to criterion ($F_{6,120} = 10.3$, $P < 0.0001$; Fig. 1) and mean correct latency ($F_{6,96} = 4.1$, $P < 0.05$). No stage by starting dimension interaction was observed ($F < 1.1$, ns). Moreover, no sex by stage interaction was observed ($F < 1$, ns).

To provide evidence that an attentional set had been formed and thus the mice performed set-shifting behavior, the performance of ID vs. ED stages was compared. A main effect of stage was observed in trials to criterion ($F_{1,20} = 4.7$, $P < 0.05$; Fig. 1b) with no interaction with genotype ($F < 1$, ns) or starting dimension ($F < 1$, ns). A main effect of ID/ED stage was also observed in mean correct latencies ($F_{1,20} = 5.8$, $P < 0.05$) with no interaction with genotype ($F < 1.8$, ns) or starting dimension ($F < 1.1$, ns). Thus, more trials to criterion were required and longer mean correct latencies were observed in the ED stage relative to the ID stage irrespective of genotype. No main effect of genotype was observed for trials to criterion or MCL in the ED stage ($F < 1$, ns; data not shown).

Genotypic effects on specific stages were investigated based on the underlying cognitive constructs described previously (Birrell & Brown 2000; Young *et al* 2009).

Discrimination learning—The ability of α7-nAChR WT and KO mice to perform discriminations within a dimension (set of stimuli) as measured by SD, CD and ID was compared. A significant effect of discrimination stage on trials to criterion was observed ($F_{2,40}$ = 24.3, P < 0.0001), as was a genotype × stage interaction ($F_{2,40}$ = 3.3, P < 0.05). *Post hoc* analysis revealed that KO mice took more trials to reach criterion relative to WT mice in the SD stage ($F_{1,26}$ = 6.8, P < 0.05; Fig. 1a), but not in the CD or ID stage (F < 1, ns).

Reversal learning—The reversal learning of the mutant mice was assessed in the CDR, IDR and EDR stages. A main effect of reversal stage on trials to criterion was found ($F_{2,48}$ = 5.4, P < 0.05), with no interaction with genotype ($F_{2,48}$ = 1.2, P = 0.310). Consistent with previous reports, we observed that the number of trials taken to obtain criterion at these stages reduced with increasing reversal stages. Thus, fewer trials were required for the EDR when compared to the CDR. Upon closer examination it was observed that this learning to reverse was unequal between genotypes with a trend toward a genotype effect on IDR ($F_{1,27}$ = 3.3, P = 0.076). When comparing CDR to IDR, WT mice took fewer trials to complete the latter ($F_{1,10}$ = 18.2, P < 0.005) unlike KO mice (F < 1, ns). In the last two reversals (IDR and EDR), however, KO mice exhibited fewer trials to criterion in the latter stage ($F_{1,10}$ = 6.7, P < 0.05; Fig. 1c), an effect not observed in WT mice (F < 1, ns).

Extradimensional shifting—To assess executive functioning specifically, the ED shifting of α7-nAChR WT and KO mice was compared using a *t* test. No effect of genotype on trials to criterion or mean correct latency was observed (t< 1, ns).

Radial arm maze

Acquisition of RAM performance was assessed over 12 days. A significant main effect of days was observed ($F_{11,132}$ = 3.0, P < 0.005; Fig. 2), while a trend toward a genotype × day interaction was observed ($F_{11,132}$ = 1.9, P = 0.052). *Post hoc* analyses revealed that while the performance of WT and KO mice did not differ on days 1 and 2 (P > 0.2), WT mice exhibited significantly greater performance on days 3, 4, 5 and 6 (P < 0.05). *Post hoc* analyses across days are depicted in Fig. 2a. Performance did not differ between the two groups thereafter (days 7–12; P > 0.2). In terms of strategy development, a trend toward reduced strategy use over continuing sessions was observed ($F_{11,132}$ = 1.8, P = 0.06; Fig. 2b), with no interaction with genotype (F < 1, ns).

Performance was assessed over 3 days once stable (Table 1). Performance did not vary over the 3 days in terms of WM span, RM errors or WM omissions (F < 1.1, ns), nor was there an effect of genotype (F < 1, ns) or a genotype × day interaction for any of these measures (F < 1.5, ns).

Novel object recognition memory

The mutants did not differ in any aspect of NORT performance. No main effect of genotype, sex or a sex × genotype interaction (F < 0.3, ns) was observed for %NI (WT = 71.6% ± 5.7, KO ♀ = 71.1% ± 6.6). Each group interacted with the novel object at rates higher than chance (P < 0.05).

Progressive ratio breakpoint study

Mice were trained to nose-poke in a single lit hole on an FR1 schedule. When compared directly, α7-nAChR KO required significantly more training sessions to attain criterion (>70 responses on two consecutive days) than WT mice (t = 2.3, P < 0.05), while HT mice did not differ from either group (P > 0.1). Once trained to a stable performance, however, the

number of responses made during the FR1 schedule did not differ between the three genotypes ($F < 1$, ns; Fig. 3a). When challenged with the PRB reinforcement schedule, there was no difference between genotypes on the breakpoint, total trials completed or MRL ($F < 1.2$, ns; Fig. 3b–d).

Behavioral pattern monitor

The exploratory profile of α7-nAChR null mutants was compared to WT littermate mice using the cross-species exploratory BPM paradigm over 60 min binned into 20 min intervals. No effect of genotype was observed on activity (transitions and center entries; Table 2), specific exploration (total holepoking, varied holepoking, rearing and center duration) or the predictability of locomotor pattern (temporal and spatial CV) (all $F < 1$, ns). Moreover, no genotype × time bin interaction was observed for any of these measures ($F < 1.3$, ns), except varied holepoking. A genotype × time bin interaction was observed for varied holepoking ($F_{2,48} = 4.0$, $P < 0.05$) with *post hoc* analyses revealing that KO mice produced more holepokes in the first 20 min compared to WT mice ($P < 0.05$), but this effect was not observed for subsequent time bins ($P > 0.1$). The linearity of activity (spatial d) was similarly unaffected ($F_{1,24} = 1.8$, $P = 0.18$), with a trend toward a genotype × time bin interaction observed ($F_{2,48} = 2.8$, $P < 0.08$). Both groups reduced the linearity of their locomotor pattern over time (increased spatial d; $F_{2,48} = 28.7$, $P < 0.001$), with no effect of genotype observed at any time bin ($P > 0.1$).

Prepulse inhibition

PPI challenge 1—The sensorimotor gating of the mutant mice as measured by PPI was intact, irrespective of the stimulus modality used (Table 3). When using light as the prepulse stimulus, no effect of genotype, sex or a sex × genotype interaction was observed ($F < 1.2$, ns). For prepulse + pulse trials, while there was no effect of genotype ($F < 1.4$), a main effect of sex ($F_{1,42} = 12.6$, $P < 0.005$) and a trend toward a sex × genotype interaction ($F_{2,42} = 3.1$, $P = 0.0554$) were observed. Tukey's *post hoc* analysis revealed that male KO and WT mice exhibited higher PPI than female KO and WT mice ($P < 0.05$), with no sex effect in HT mice ($P > 0.1$). A main effect of genotype was observed for P120 ($F_{2,42} = 7.7$, $P < 0.005$), but no effects of sex or genotype × sex interaction were observed ($F < 1$, ns). Tukey's *post hoc* analyses revealed that KO mice exhibited higher startle values than WT or HT mice ($P < 0.05$).

PPI challenge 2—The sensorimotor gating of the mice was challenged across multiple parameters in separate blocks within the same session. These parameters included varying (1) the prepulse intensities (67, 69, 73 and 81 dB), (2) the ISIs (25, 50, 100, 200, 500 and 1000 milliseconds between a 73 dB prepulse and 120 dB pulse) and (3) the pulse intensities. No effect of sex or genotype, nor a sex × genotype interaction was observed for varying prepulse intensities ($F < 1.2$, ns; Table 4). A main effect of prepulse intensity was observed ($F_{3,126} = 126.3$, $P < 0.0001$) with a trend toward a sex × prepulse intensity interaction ($F_{3,126} = 2.2$, $P < 0.1$). No interaction of genotype, or sex × genotype with prepulse intensity was observed ($F < 1.3$, ns). No effects of sex ($F_{1,42} = 2.2$, $P = 0.112$), genotype or a sex × genotype interaction ($F < 1$, ns) were observed on PPI across ISIs (Table 4). A main effect of ISI was observed ($F_{5,210} = 19.8$, $P < 0.0001$), but no ISI interaction with sex, genotype or sex × genotype was observed ($F < 1$, ns). A main effect of pulse intensity was observed ($F_{6,252} = 38.7$, $P < 0.0001$), with trends toward an effect of genotype ($F_{2,42} = 3.1$, $P < 0.06$) and sex ($F_{1,42} = 3.8$, $P < 0.06$), with males exhibiting higher startle values than females. A pulse intensity × genotype interaction was observed ($F_{12,252} = 2.5$, $P < 0.005$; Fig. 4), but no sex × pulse intensity or sex × genotype × pulse intensity ($F < 1$, ns). *Post hoc* analyses revealed that while startle reactivity increased with increasing pulse intensity for all three genotypes, KO mice exhibited higher startle reactivity when compared with WT and HT

mice ($P < 0.05$) at pulse intensities of 110 and 120 dB but not at smaller intensities ($P > 0.1$).

Discussion

We assessed the effects of null mutation of the α7-nAChR on mice in a variety of cognitive and behavioral paradigms. Null mutation of the α7-nAChR impaired procedural learning irrespective of the rule being acquired or the stimuli employed. The α7-nAChR KO mice took longer to learn to associate obtaining a food reward with: (1) digging in a bowl of specific odor or platform, (2) nose-poking in a lit hole and (3) entering previously unvisited arms. While reduced motivation for food rewards could impact learning for a reward, these mice exhibited normal breakpoint in a progressive ratio study, indicative of normal motivation for a single food reward in an operant task. Despite impaired learning of the initial rule and previous reports of impaired sustained attention in these mice, null mutation of the α7-nAChR did not affect attentional set-shifting, reversal learning, WM span capacity or short-term memory. Exploratory behavior and sensorimotor gating were unaffected, although KO mice exhibited increased startle reactivity when compared to WT mice. The data presented here support a role for the α7-nAChR in procedural learning.

Null mutation of the α7-nAChR appears to affect specific cognitive domains. Sustained attention is impaired (Hoyle *et al* 2006; Young *et al* 2004, 2007a) as well as learning as measured by learning to lever press (Keller *et al* 2005), to nose-poke in a lit hole (Young *et al* 2004) or to enter previously unvisited arms (Levin *et al* 2009) for food rewards. Interestingly, however, α7-nAChR KO mice learned (i.e. performance improved with training) and remembered (during probe challenges) the location of the escape platform as readily as WT mice when aversively motivated in the water maze (Paylor *et al* 1998). The differentiation of performance in these cognitive domains could reflect reduced motivation for food rewards. In the 5-CSRTT, α7-nAChR mutant mice exhibit genotype dose-dependent increases in omissions (Hoyle *et al* 2006; Young *et al* 2004, 2007a), which could reflect reduced motivation for reward in the task (Hoyle *et al* 2006). In the present studies, we specifically assessed the motivation of α7-nAChR KO mice for a food reward using the PRBP (Bensadoun *et al* 2004; Young & Geyer 2010). While the KO mice took more sessions to attain criterion on an FR1 schedule (nose-poke in a single lit hole), once trained to a stable level of performance, there was no genotype effect on the number of nose-pokes performed. When challenged, WT, HT and KO mice all attained the same breakpoint, nose-poking 22 times for a single reward. Previously, we reported that dopamine D1 receptor WT and HT mice also made approximately 22 responses for a single reward while D1 KO mice did not (Young & Geyer 2010). Thus, the breakpoint of these mice appears consistent both within and between mutant lines. These data support the interpretation that: (1) increased omissions of α7-nAChR KO mice are attributable to impaired attention and (2) differences in performance in various paradigms reflect differentially affected cognitive domains in these mice.

Evidence for normal motivation for food rewards assists in the interpretation of the present data. Spatial location learning and long-term memory appear normal in α7-nAChR KO mice (Paylor *et al* 1998). In the present studies, attentional set-shifting and reversal learning was also unimpaired in α7-nAChR KO mice as measured by the ASST. Surprisingly, however, α7-nAChR KO mice took longer to acquire SD learning (stage 1) of the ASST, despite normal learning in nearly every other ASST stage. Ordinarily, if these mice exhibited a generalized learning deficit, one might expect that performance would be impaired at every stage of the ASST (Birrell & Brown 2000). The KO mice, however, exhibited impaired learning at the SD stage and a trend toward impaired learning at the IDR stage. The SD stage requires not only learning the appropriate stimulus that predicts the reward, but also

the premise that a stimulus could predict a reward. While CDR was the first reversal stage, IDR represents the first stage to which the knowledge can be applied that rule reversals occur. Subjecting rodents or primates to repeated reversal challenges, i.e. serial reversal learning, commonly results in the rodents appearing to learn to rule reverse faster (Castane *et al* 2010; Dickson *et al* 2010; Leary 1962; Rygula *et al* 2010; Seu & Jentsch 2009), due to increasing awareness that such events occur. Here, improved reversal learning was evident in the WT mice by the second reversal stage but only by the third reversal stage in KO mice. Improved reversal learning has also been observed within previous ASST studies in rats (Izquierdo *et al* 2010; Tait *et al* 2009) andmice (Young *et al* 2010b). Therefore, the SD ASST data provide support for the notion that α7-nAChR KO mice exhibit impaired procedural learning (rule acquisition). While only a trend toward a genotype effect was observed in the IDR stage, the faster learning exhibited by the WT from the first to second reversal – not seen in the KO mice – supports this hypothesis of impaired procedural learning in the KO mice. By the third stage of reversal (EDR), the KO mice took fewer trials to criterion resulting in similar performance to WT mice. Given the unimpaired performance of α7-nAChR KO mice in CD, CDR, ID, ED and EDR stages, the data support the hypothesis that once acquired, these mice can apply a rule and are motivated to obtain food rewards.

The present RAM study provided further evidence of procedural learning deficits in α7-nAChR KO mice. The KO mice took longer to learn or apply the rule of not reentering arms. This observation is consistent with earlier reports, although previous reports interpreted these findings of the mice exhibiting poorer choice accuracy and not necessarily impaired acquisition (Levin *et al* 2009). Fully trained stable performance was not presented in the previous studies, however, and therefore it is unclear whether performance would have improved to WT levels with more training sessions, as the present findings suggest. When stable, RAM performance did not differ between the two genotypes. These data support the hypothesis presented here that these mice exhibit impaired procedural learning, but normal choice accuracy once the task is acquired. This RAM procedure was developed to assess spatial WM span capacity, controlling for simple turn left strategies by briefly closing doors for 1 second between choices (Wenk 2004). The StrategyCV measure was developed to quantify the degree to which repeated strategy choices are made. Consistent with previous reports (Tarantino *et al* 2011), as performance in this RAM procedure improved, the use of a simple strategy decreased. No difference in strategy use was observed between α7-nAChR WT and KO mice. Thus, impaired procedural learning of the KO mice was unlikely to be due to utilizing a different strategy.

When using non-spatial cues requiring digging, α7-nAChR KO exhibited impaired learning of the odor span task (Young *et al* 2007a). These mice also exhibited poorer performance on this non-spatial WM span task, suggesting that spatial and non-spatial WM span capacity are dissociable in mice as in humans (Buchanan *et al* 2005; Gruzelier *et al* 1988; Milner 1971). Another possibility is that because the testing environment for the odor span task was a large open space, the α7-nAChR KO were more distracted, thus taking longer than the 10 min allotted to complete the task (Young *et al* 2007a). Thus, inattention may mediate the odor span and procedural learning deficits of α7-nAChR KO mice (Young *et al* 2007a).

In humans, attentional aspects of cognitive abilities play a role in learning and WM (Vanderploeg *et al* 1994). Specifically, attention can impact procedural/perceptual learning (Feldman 2004; Tsushima & Watanabe 2009). Procedural/perceptual learning, observing patterns in the environment to understand an action, utilizes distinct hippocampofronto-parietal networks (Feldman 2004; Wimber *et al* 2010). Once the pattern is observed and the rule learned, it can be applied to other aspects of behavior. This rule realization has been referred to as the ‘eureka’ effect (Giovannelli *et al* 2010). It could be hypothesized that these

studies provide some support that removal of the α7-nAChR delays this 'eureka effect' in mice, while leaving rule application, reversal learning and other aspects of behavior intact. The precise mechanism of action by which the α7-nAChR contributes toward procedural learning remains unclear, however. Cholinergic projections from the basal forebrain innervate a number of structures including the hippocampus and frontal cortices (Miwa *et al* 2011) where α7-nAChRs are located (Whiteaker *et al* 1999). It has been hypothesized that α7-nAChR located on inhibitory neurons in the hippocampus participate in controlling sensory responses indirectly resulting in pyramidal neurons decreasing their response to repeated stimuli (Miwa *et al* 2011; Poorthuis *et al* 2009). Given that the hippocampus contributes toward relational learning between sensory stimuli (DeVito *et al* 2010a; Eichenbaum & Fortin 2009), the loss of α7-nAChRs may limit the ability of the hippocampus to integrate incoming sensory stimuli, inhibiting sensory input to the cortex. While speculative, the primary location of causal procedural deficits in these mice could be investigated using the transitive inference task. This task requires animals to make relational inferences between stimuli that had not previously been associated, based on the knowledge previously gained about the stimuli (DeVito *et al* 2010a, b). Because hippocampal and prefrontal lesions differentially affect performance in this task, one could predict that if hippocampal α7-nAChR mediate this procedural learning and integration of stimuli, their deficits in the task would resemble hippocampal lesioned mice as opposed to prefrontal lesioned mice (DeVito *et al* 2010a, b). Future studies are warranted to test this hypothesis.

The studies presented here extend the behavioral phenotype of α7-nAChR mutant mice. While normal PPI has been observed previously in male and female α7-nAChRmice at younger ages (3–4 months; Paylor *et al* 1998) the present study extends these findings using a multivariate/multimodal approach (Barr *et al* 2006; Csomor *et al* 2008; Young *et al* 2010c). Thus, it is clear α7-nAChR KO exhibit normal auditory, tactile and light-induced PPI. Moreover, these mice exhibit normal PPI across multiple inter-stimulus intervals. Increased startle amplitude was observed in KO mice compared to WT and HT mice although only at higher pulse intensities (110 and 120 dB). The present studies also extend the exploratory behavioral profile of these α7-nAChR mutants at an older age (10 months) using the cross-species test of exploration, the BPM (Henry *et al* 2010; Perry *et al* 2009; Young *et al* 2007c). Null mutation of the α7-nAChR mice had no effect on multivariate measures of activity, specific exploratory responses or locomotor path patterns, consistent with activity measures of younger (3–4 months) mice (Paylor *et al* 2008). Finally, the lack of difference between α7-nAChR KO mice and WT mice in the NORT suggests that these mice do not exhibit impaired short-term memory (Ali *et al* 2011; Young *et al* 2009b). No sex by genotype effects were observed in any of the current studies. The potential for such interactions cannot be readily discounted, however, given the limited sample sizes utilized in the current studies. Moreover, the female estrus cycle was not taken into account in these studies, which may have increased the variance observed. Thus, while certain aspects of behavior are affected by null mutation of the α7-nAChR, numerous behaviors remain unchanged, but to fully investigate sex × genotype interactions, greater sample sizes will be required with estrus cycles being examined concurrently.

Previous studies have assessed the efficacy of the partial α7-nAChR agonist GTS-21 for improving cognitive dysfunction in schizophrenia. Although early studies were promising (Olincy *et al* 2006), larger multisite studies failed to support the efficacy of this drug (Freedman *et al* 2008). GTS-21 is one of a long list of pharmacological treatments that have so far failed to gain approval for improving cognition in schizophrenia. There is evidence that cognitive behavioral (remediation) therapy, where patients learn to perform cognitive tasks (Twamley *et al* 2008), may be a viable treatment option (Adcock *et al* 2009; Genevsky *et al* 2010; Rathod *et al* 2010; Tarrier 2010) and such therapy can even produce fronto-parietal structural changes (Eack *et al* 2010; Haut *et al* 2010; Premkumar *et al* 2010). It is a

common complaint that while patients take longer to acquire the rule governing the task, they can perform adequately once acquired (Twamley *et al* 2008). In the future, cognitive behavioral therapy may and perhaps should be commonly used to assist in the treatment of cognitive dysfunction in schizophrenia (Swerdlow 2010). Add-on pharmacotherapy may assist cognitive behavioral therapy and, based on the data presented here, perhaps future studies could examine the effects of an α7-nAChR agonist on assisting learning during cognitive behavioral therapy in patients with schizophrenia.

The present studies provide a more in-depth behavioral and cognitive characterization of α7-nAChRmutantmice than previously described. These studies, using tasks with varying degrees of cross-species translational validity (Young *et al* 2009b), support the notion that the α7-nAChR contributes toward normal procedural learning because of impaired learning in these mice observed across several testing paradigms. While the internal representation of learning is difficult to assess in animals given the requirement of behavioral output to interpret the animal's knowledge, we feel these data may reflect at least in part a delayed 'eureka' of α7-nAChR KO mice. These deficits are unlikely to be attributable to reduced motivation for reward because these mice exhibited normal breakpoint behavior, albeit in an operant chamber. The poor attention of these mice may contribute to this delayed procedural learning and requires further investigation. Future studies evaluating α7-nAChR agonists for the treatment of schizophrenia should examine the effects of specific α7-nAChR ligands on procedural learning both in preclinical and in clinical settings.

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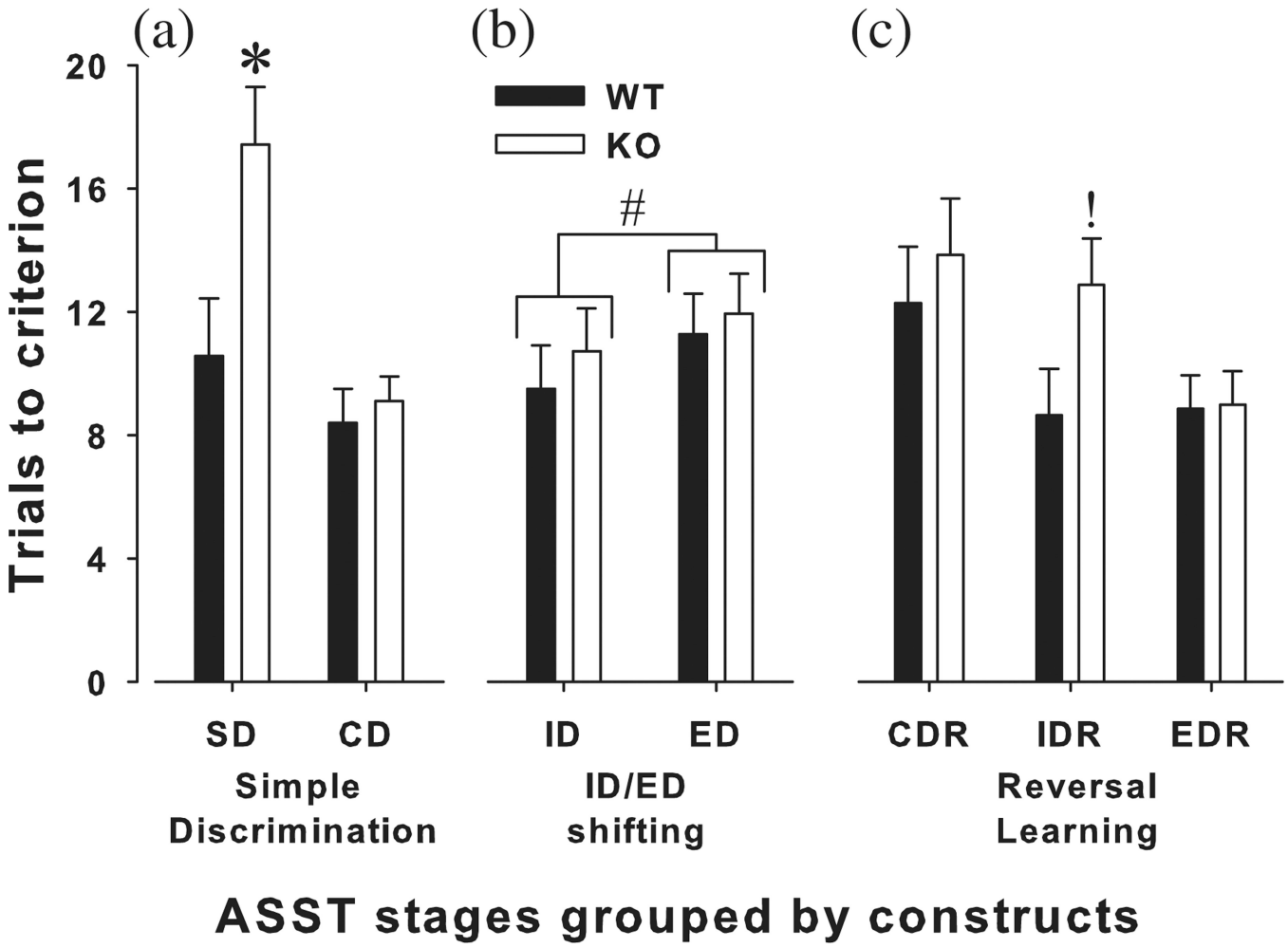

Figure 1. Performance of α7-nAChR mutant mice in the ASST

The performance of α7-nAChR KO mice in the ASST was compared with their WT littermates. KO mice exhibited impaired acquisition of the rule to associate a specific stimulus with reward location, taking longer to acquire the SD but not CD stage as compared to WT mice (a). Set-shifting was normal in KO mice, with both KO and WT mice demonstrating the formation of an attentional set given that it took both groups more trials to complete the ED stage compared with the ID stage (b). KO mice exhibited (1) normal reversal learning at the first reversal stage (CDR stage), (2) took more trials to complete the second reversal stage (IDR stage) due to WT mice taking fewer trials compared to CDR while KO did not and (3) exhibited comparable reversal learning to WT mice at the third stage (EDR) because KO mice improved by this stage when compared to the IDR stage (c). Data are presented as mean + SEM, grouped by construct as opposed to the order of testing (SD, CD, CDR, ID, IDR, ED, EDR), **P* < 0.05 when compared to WT mice, *P* < 0.1 when compared to WT mice, #*P* < 0.05 when ID is compared to ED shifting.

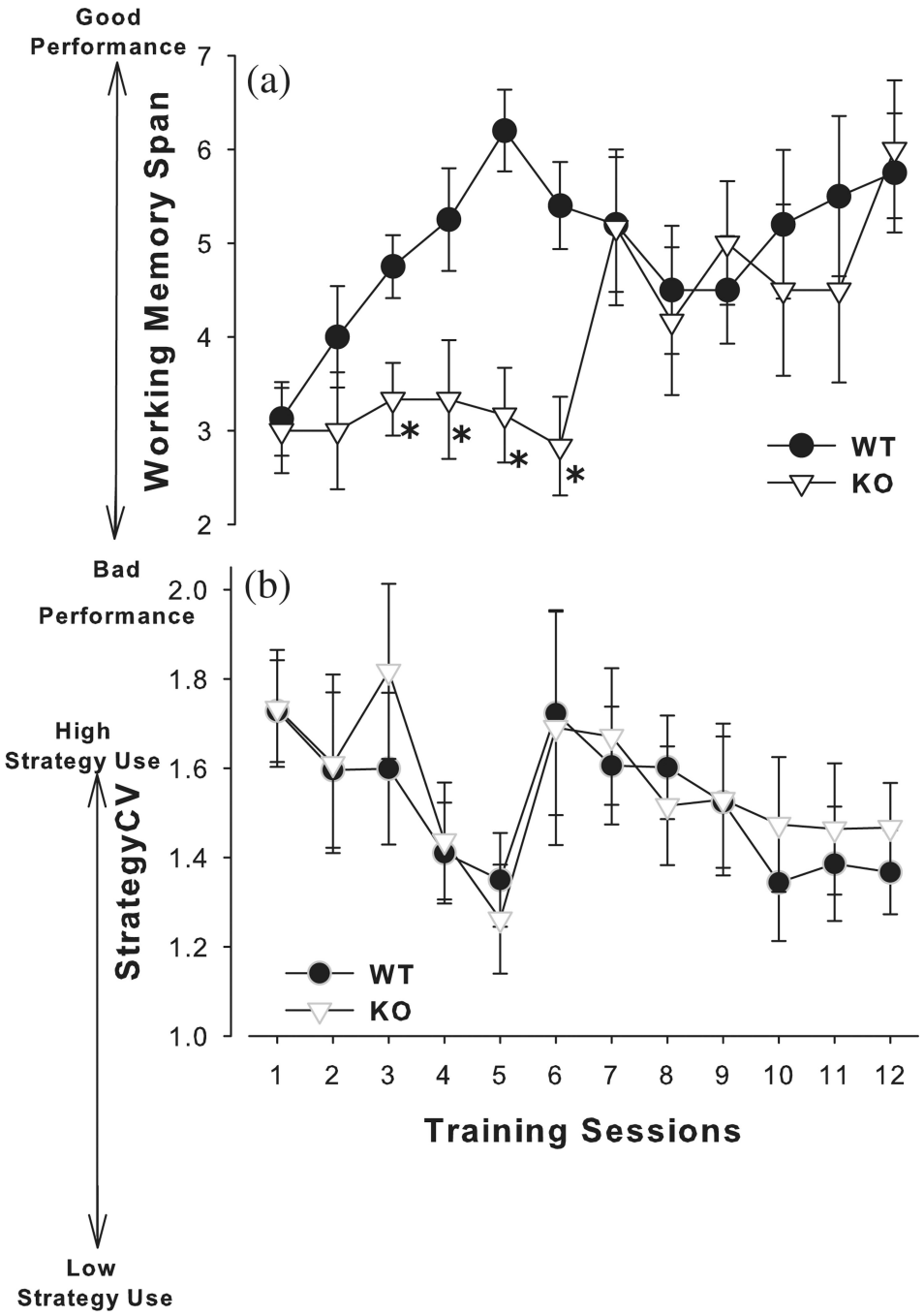

Figure 2. Acquisition of α7-nAChR mutant mice in the RAM

α7-nAChR KO and WT littermate mice were trained to perform a win-shift search strategy using the RAM. Doors to the arms closed between arm choices for 1 second to limit simple turnleft/right strategies. KO mice took more sessions to acquire the win-shift rule compared to WT mice, as measured by the number of arm entries prior to repeat [WM span (a)]. The strategy used by these mice also changed with increasing sessions, indicative of a simple strategy being used less as time went on, as measured by strategyCV (b). Data are presented as mean ± SEM, **P* < 0.05 when compared to WT mice on that session day.

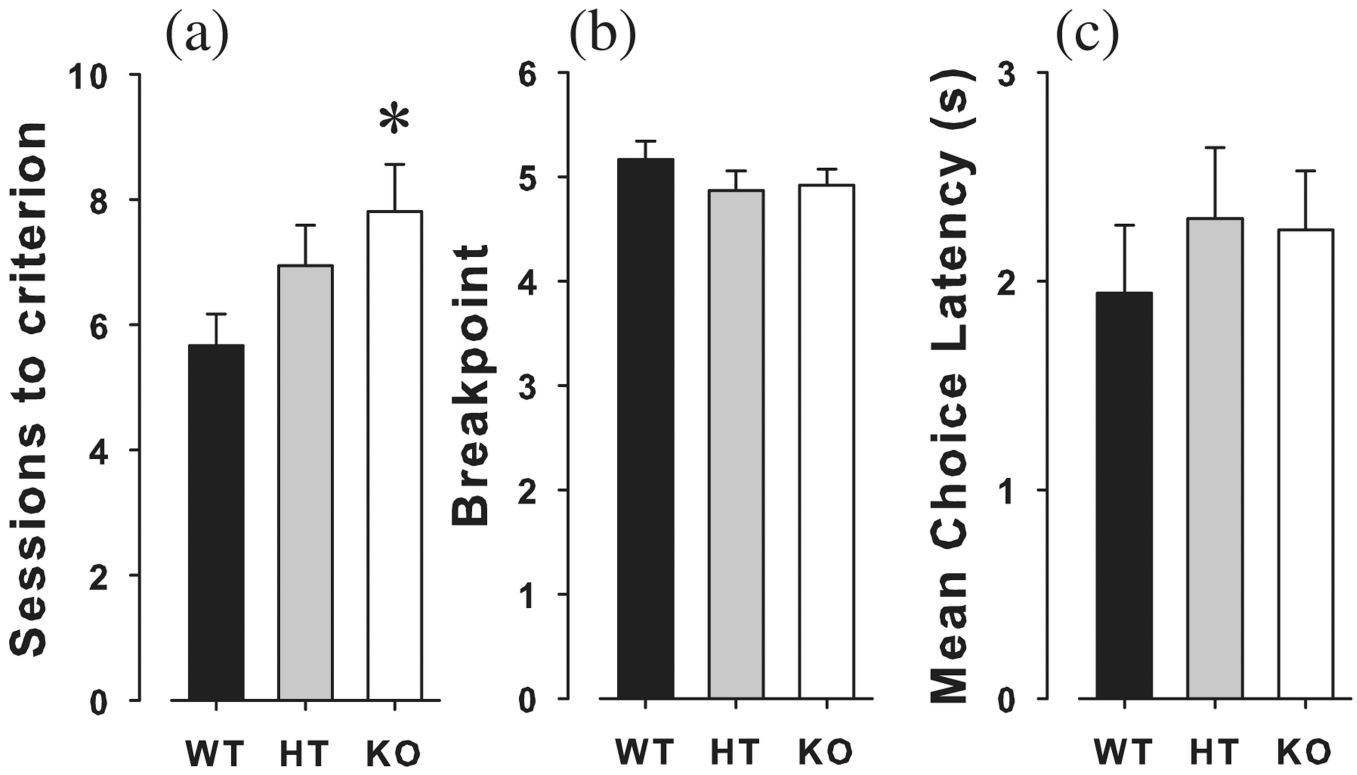

Figure 3. Acquisition and performance of α7-nAChR mutant mice in the progressive ratio breakpoint study

α7-nAChR KO, HT and WT littermate mice were trained to nose-poke in a single lit hole. KO mice took significantly more sessions than WT mice to attain criterion of >70 responses for two consecutive days with HT mice exhibiting a numerically intermediate learning speed (a). Once trained, however, the number of nose-pokes did not differ between the groups on a fixed ratio 1 schedule. When challenged with a progressive ratio breakpoint schedule, the KO, HT and WT littermates were willing to nose-poke the same number of times for one reward (b), and did not differ in response latency (c). Data are presented as mean + SEM, **P* < 0.05 when compared to WT littermate mice.

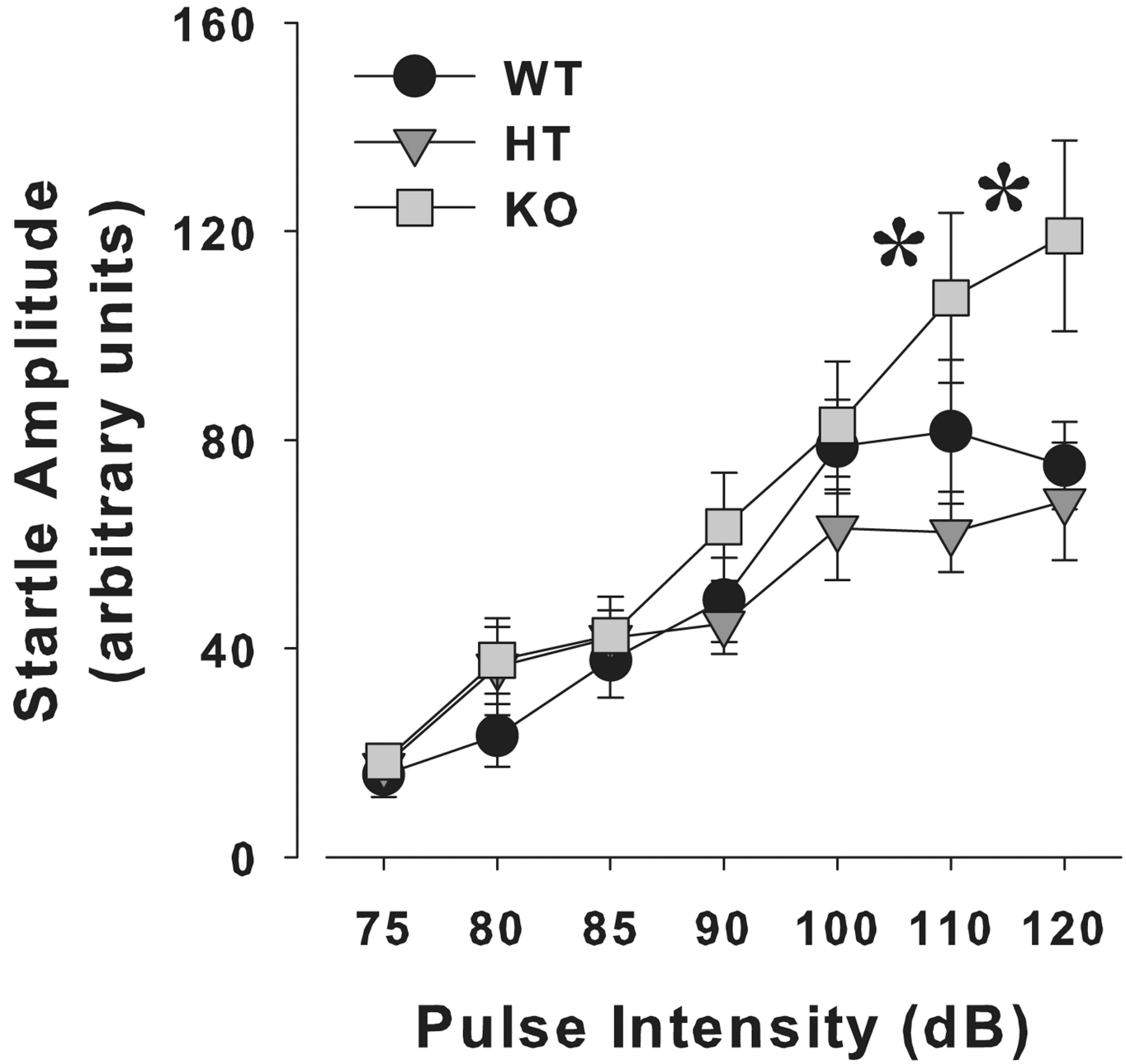

Figure 4. Startle reactivity of α7-nAChR mutant mice across pulse intensities

The startle reactivity of α7-nAChR KO, HT and WT littermate mice to various pulse intensities was assessed. Startle reactivity increased with increasing pulse intensity. The startle amplitude of HT and WT mice reached plateau at 110 and 120 dB, while KO mice continued to increase in amplitude. Data are presented as mean + SEM, **P* < 0.05 when compared to WT and HT mice.

Table 1

Stable RAM performance in α7-nAChR WT and KO mice

Measure	WT	KO
WM span	5.575 ± 0.46	5.72 ± 0.55
RM errors	2.54 ± 0.46	2.22 ± 0.53
WM omissions	1.83 ± 0.33	1.89 ± 0.38
StrategyCV	1.39 ± 0.12	1.45 ± 0.13

Table 2

Measures of exploration of α 7- nAChRWT and KO mice as assessed in the BPM

Factor	Measure	Time Bin (min)	Mean
Activity	Transitions	0–20	WT = 506.3 ± 9.1
			KO = 486.4 ± 9.3
		20–40	WT = 366.9 ± 7.5
			KO = 374.1 ± 10
		40–60	WT = 307.3 ± 8.1
			KO = 277,5 ± 6.5
	Center entries	0–20	WT = 59.17 ± 1.7
			KO = 55.57 ± 1.6
		20–40	WT = 38.83 ± 1.2
			KO = 44.14 ± 1.6
		40–60	WT = 32.75 ± 1.4
			KO = 34.00 ± 1.2
Specific exploration	Total holepoking	0–20	WT = 65.33 ± 1.2
			KO = 65.86 ± 1.8
		20–40	WT = 67.00 ± 1.7
			KO = 69.50 ± 1.7
		40–60	WT = 74.42 ± 2.7
			KO = 59.93 ± 1.6
	Varied holekeeping	0–20	WT = 7.8 ± 0.6
			KO = 11.8 ± 0.9*
		20–40	WT = 9.4 ± 0.8
			KO = 11.1 ± 1.1
		40–60	WT = 11.1 ± 1.1
			KO = 9.1 ± 0.8
	Rearing	0–20	WT = 87.92 ± 2.9
			KO = 84.42 ± 1.7
		20–40	WT = 97.75 ± 3.8
			KO = 81.50 ± 2.1
		40–60	WT = 79.17 ± 3.0
			KO = 71.07 ± 2.1
Locomotor patterns	Temporal CV	0–20	WT = 1.166 ± 0.012
			KO = 1.245 ± 0.023
		20–40	WT = 1.377 ± 0.017
			KO = 1.372 ± 0.024
		40–60	WT = 1.496 ± 0.035
			KO = 1.687 ± 0.044
	Spatial CV	0–20	WT =1.024 ± 0.007
			KO = 1.017 ± 0.007
		20–40	WT = 1.106 ± 0.010

Factor	Measure	Time Bin (min)	Mean
			KO = 1.087 ± 0.008
		40–60	WT = 1.094 ± 0.010
			KO = 1.089 ± 0.011

* $P < 0.05$ when compared to WT mice.

Table 3

Multivariate assessment of sensorimotor gating of α7-nAChR WT and KO mice using PPI

Study	Measure	Genotype	Mean startle
PPI challenge 1: multiple modalities	120 dB startle pulse alone	WT	♀ = 90.4 ± 10.7
			♂ = 92.9 ± 16.3
		HT	♀ = 78.7 ± 20.7
			♂ = 78.4 ± 11.9
		KO	♀ = 125.6 ± 19.2*
			♂ = 160.7 ± 23.5*
	73 dB (65 dB background) preceding 120 dB startle	WT	♀ = 45.0 ± 3.3
			♂ = 67.6 ± 6.2#
		HT	♀ = 49.0 ± 3.0
			♂ = 49.3 ± 6.2
		KO	♀ = 45.6 ± 5.2
			♂ = 68.0 ± 4.6#
	Light preceding 120 dB pulse	WT	♀ = 33.0 ± 7.7
			♂ = 12.0 ± 10.9
		HT	♀ = 10.8 ± 10.0
			♂ = 14.6 ± 8.0
		KO	♀ = 27.5 ± 3.0
			♂ = 26.2 ± 5.8

*$P < 0.05$ when compared to WT mice;

#$P < 0.05$ when compared to male HT mice.

Table 4

Effects of varying the (a) prepulse dB level and (b) interstimulus interval duration on PPI in female and male α 7-nAChR mutant mice

Study	Measure	dB	Genotype	Mean startle
(a) PPI challenge 2 part A: varying prepulse intensities on PPI	Prepulse intensities (65 dB background)	67 dB	WT	♀ = 8.3 ± 7.3
				♂ = 12.7 ± 9.1
			HT	♀ = 22.2 ± 5.0
				♂ = 5.9 ± 8.8
			KO	♀ = 19.3 ± 6.3
				♂ = 18.1 ± 5.4
		69 dB	WT	♀ = 20.4 ± 5.3
				♂ = 30.6 ±8.8
			HT	♀ = 31.5 ±9.1
				♂ = 27.3 ±9.3
			KO	♀ = 30.0 ±9.1
				♂ = 25.9 ± 9.3
		73 dB	WT	♀ = 43.7 ± 9.1
				♂ = 53.0 ± 9.3
			HT	♀ = 49.6 ± 9.1
				♂ = 55.9 ± 9.3
			KO	♀ = 46.9 ± 9.1
				♂ = 48.3 ± 9.3
		81 dB	WT	♀ = 61.1 ± 9.1
				♂ = 72.6 ± 9.3
			HT	♀ = 59.2 ± 9.1
				♂ = 59.6 ± 9.3
			KO	♀ = 68.3 ± 9.1
				♂ = 67.8 ± 9.3
(b) PPI challenge 2 part A: varying ISI duration PPI	ISI	Isi1000	WT	♀ = 3.4 ± 10.6
				♂ = 22.5 ± 7.3
			HT	♀ = 10.4 ± 15.4
				♂ = 20.9 ± 9.4
			KO	♀ = 19.9 ± 9.2
				♂ = 16.5 ± 14.8
		Isi500	WT	♀ = 22.8 ± 11.8
				♂ = 39.1 ± 12.3
			HT	♀ = 33.9 ± 11.7
				♂ = 41.6 ± 7.9
			KO	♀ = 3.4 ± 13.1
				♂ = 25.1 ± 7.9
		Isi200	WT	♀ = 27.8 ± 16.9
				♂ = 55.7 ± 7.5

Study	Measure	dB	Genotype	Mean startle
			HT	♀ = 40.8 ± 8.7
				♂ = 46.7 ± 8.0
			KO	♀ = 39.8 ± 8.1
				♂ = 43.0 ± 6.3
		Isi100	W	♀ = 51.4 ± 7.9
				♂ = 57.3 ± 9.3
			HT	♀ = 45.7 ± 10.6
				♂ = 51.7 ± 7.1
			KO	♀ = 40.6 ± 8.1
				♂ = 41.8 ± 4.2
		Isi50	WT	♀ = 29.7 ± 9.9
				♂ = 51.7 ± 7.2
			HT	♀ = 46.8 ± 5.9
				♂ = 48.0 ± 10.6
			KO	♀ = 32.5 ± 15.2
				♂ = 41.8 ± 4.2
		Isi25	WT	♀ = 12.2 ± 23.4
				♂ = 25.1 ± 18.1
			HT	♀ = 3.6 ± 17.8
				♂ = 30.6 ± 11.9
			KO	♀ = 12.0 ± 12.0
				♂ = 15.0 ± 6.5