Published in final edited form as:

Behav Genet. 2012 May ; 42(3): 423–436. doi:10.1007/s10519-011-9511-0.

Role of $\alpha 7$ - and $\beta 4$ -Containing Nicotinic Acetylcholine Receptors in the Affective and Somatic Aspects of Nicotine Withdrawal: Studies in Knockout Mice

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Abstract

To assess which nicotinic acetylcholine receptors (nAChRs) are involved in the aversive aspects of nicotine withdrawal, brain reward function and the somatic signs of nicotine withdrawal were assessed in mice that lack $\alpha 7$ and $\beta 4$ nAChR subunits. Brain reward function was assessed with the intracranial self-stimulation (ICSS) procedure, in which elevations in ICSS thresholds reflect an anhedonic mood state. At 3–6 h of spontaneous nicotine/saline withdrawal, thresholds were elevated in nicotine-withdrawing $\alpha 7^{+/+}$ and $\beta 4^{+/+}$, but not $\alpha 7^{-/-}$ or $\beta 4^{-/-}$, mice compared with saline-withdrawing mice, indicating a delay in the onset of withdrawal in the knockout mice. From 8 to 100 h of withdrawal, thresholds in $\alpha 7^{+/+}$ and $\alpha 7^{-/-}$ mice were equally elevated, whereas thresholds in $\beta 4^{+/+}$ and $\beta 4^{-/-}$ mice returned to baseline levels. Somatic signs were attenuated in nicotine-withdrawing $\beta 4^{-/-}$, but not $\alpha 7^{-/-}$, mice. Administration of a low dose of the nAChR antagonist mecamylamine induced threshold elevations in $\alpha 7^{-/-}$, but not $\alpha 7^{+/+}$, mice, whereas the highest dose tested only elevated thresholds in $\alpha 7^{+/+}$ mice. Mecamylamine-induced threshold elevations were similar in $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice. In conclusion, null mutation of the $\alpha 7$ and $\beta 4$ nAChR subunits resulted in a delayed onset of the anhedonic aspects of the spontaneous nicotine withdrawal syndrome. Previous findings of attenuated somatic signs of nicotine withdrawal in $\beta 4^{-/-}$, but not $\alpha 7^{-/-}$, mice were confirmed in the present study, indicating an important role for $\beta 4$ -containing nAChRs in the somatic signs of nicotine withdrawal. The mecamylamine-precipitated withdrawal data suggest that compensatory adaptations may occur in constitutive $\alpha 7^{-/-}$ mice or that mecamylamine may interact with other receptors besides nAChRs in these mice. In summary, the present results indicate an important role for $\alpha 7$ and $\beta 4$ -containing nAChRs in the anhedonic or somatic signs of nicotine withdrawal.

Keywords

Intracranial self-stimulation; Somatic signs; Reward deficit; Anhedonia; Mecamylamine; nAChR

Introduction

Tobacco use is a global epidemic, and urgent actions are needed to prevent the continuing detrimental effects of tobacco smoking on health and the millions of deaths that result from this harmful addiction (World Health Organization 2009). The main psychoactive ingredient in tobacco is nicotine, which is thought to contribute substantially to the maintenance of the tobacco smoking habit (Stolerman and Jarvis 1995). Tobacco smoking rapidly delivers nicotine to the brain, where nicotine binds to nicotinic acetylcholine receptors (nAChRs) that are physiologically activated by the endogenous excitatory neurotransmitter acetylcholine.

nAChRs are ligand-gated cation channels that consist of five subunits. Twelve types of subunits have been identified for neuronal nAChR, $\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$, that together form the pentameric structure of the nAChR (Picciotto et al. 2001). The present study was designed to study the role of $\alpha 7$ - and $\beta 4$ -containing nAChRs in the anhedonic and somatic aspects of nicotine withdrawal using mutant mice that lack these nAChR subunits.

$\alpha 7$ nAChR subunits predominantly form homomeric nAChR structures that consist of five $\alpha 7$ subunits. These receptors are widely and abundantly expressed throughout the brain in regions that include the hippocampus, cortex, thalamus (Quik et al. 2000), nucleus accumbens (Fu et al. 2000), and ventral tegmental area (VTA; Clarke et al. 1985; Jones and Wonnacott 2004). Homomeric $\alpha 7$ nAChR subunits typically exhibit relatively low affinity for nicotine and rapidly desensitize upon nicotine binding (Fenster et al. 1997; Gerzanich et al. 1994). Presynaptic, postsynaptic, and perisynaptic localization of $\alpha 7$ receptors has been demonstrated (Cordero-Erausquin et al. 2000). An important role has been implicated for presynaptic $\alpha 7$ nAChRs in brain reward processes by positively regulating several neurotransmitters, including glutamate (McGehee et al. 1995), dopamine (Schilstrom et al. 1998), and norepinephrine (Barik and Wonnacott 2006). Chronic nicotine exposure can upregulate $\alpha 7$ nAChRs (Marks et al. 1983), including those localized in the VTA (Pakkanen et al. 2005). The upregulation of $\alpha 7$ nAChRs, however, requires high concentrations of nicotine that exceed the nicotine levels achieved by smokers (Kawai and Berg 2001; Molinari et al. 1998).

Unlike $\alpha 7$ nAChR subunits that predominantly form homomeric receptors, $\beta 4$ subunits combine with α subunits to form heteromeric nAChR structures. The $\beta 4$ subunit is frequently found to combine with the $\alpha 3$ subunit but can also form functional nAChRs with other subunits (Gotti et al. 2009; e.g., $\alpha 4$ and $\alpha 2$ subunits). Markedly high expression of $\beta 4$ -containing nAChRs has been seen in the habenula-interpeduncular pathway and epiphysis, and moderate to high expression of $\beta 4$ -containing nAChRs has been found in the cerebellum, dentate gyrus, substantia nigra, and thalamus (Gotti et al. 2009; Grady et al. 2009; Hernandez et al. 2004; Quik et al. 2000). Specifically, codistribution of the $\beta 4$ nAChR subunit with the $\alpha 3$ nAChR subunit was shown for many of the brain regions where the $\beta 4$ receptor has been shown to be highly expressed, including the epiphysis (Hernandez et al. 2004), medial habenula, interpeduncular nucleus, hippocampus, frontal cortex, and VTA (Winzer-Serhan and Leslie 1997). Compared with $\beta 2$ -containing nAChRs, $\beta 4$ -containing nAChRs desensitized at a relatively slow rate (Cachelin and Jaggi 1991; Papke et al. 1989) and exhibited markedly less affinity for nicotine (Parker et al. 1998; Wei et al. 2005). $\beta 4$ -containing nAChRs also showed high resistance to nicotine-induced upregulation compared with $\beta 2$ -containing nAChRs. Although upregulation of $\beta 4$ -containing nAChRs has been previously demonstrated, this upregulation was quickly reversed (Meyer et al. 2001) and not consistently demonstrated (Nguyen et al. 2003; Wang et al. 1998).

Mice that carry null mutations for specific nAChR subunits provide a unique opportunity for studying the role of receptors that contain these subunits in nicotine dependence. The use of such knockout mice has significantly advanced our understanding of the involvement of nAChRs that contain different nAChR subunits in aspects of nicotine dependence and other nicotine-induced effects. Specifically, studies have demonstrated a key role for $\beta 2$ -containing nAChRs in the reinforcing effects of nicotine. $\beta 2$ knockout mice do not self-administer nicotine (Picciotto et al. 1998). In contrast, mice null for the $\alpha 5$ nAChR subunit exhibited increased nicotine self-administration behavior, suggesting that nAChRs that contain this subunit, particularly in the habenula-interpeduncular pathway, are involved in limiting nicotine intake (Fowler et al. 2011). Furthermore, somatic signs of nicotine withdrawal were absent in $\alpha 5$ and $\alpha 2$ knockout mice (Salas et al. 2009). Finally, $\alpha 3$ heterozygous and $\beta 4$ knockout mice exhibit resistance to nicotine-induced seizures (Kedmi et al. 2004; Salas et al. 2003a, 2004a).

Deficits in brain reward function, such as depressed mood or anhedonia (i.e., the inability to experience pleasure from rewarding stimuli), reflect the main symptoms of nicotine withdrawal in humans (American Psychiatric Association 2000; Hughes 2007). The involvement of nAChRs in the reward deficits associated with nicotine withdrawal has not been studied extensively. The intracranial self-stimulation (ICSS) procedure is a unique procedure that allows the quantitative assessment of brain reward function in rodents by measuring elevations in current-intensity thresholds (Stoker and Markou 2011). Chronic nicotine administration lowers brain reward thresholds in C57BL/6J mice in the ICSS procedure, reflecting reward enhancement (Stoker et al. 2008), whereas nicotine withdrawal is associated with elevations in brain reward thresholds, reflecting anhedonia in mice (Johnson et al. 2008; Stoker et al. 2008). Although other affective signs of nicotine withdrawal have been assessed in mutant mice that lack various nAChR subunits (see "Discussion" section), the anhedonic aspects of nicotine withdrawal have not been extensively investigated in nAChR subunit knockout mice.

In addition to the anhedonic aspects of nicotine withdrawal, the nicotine withdrawal syndrome in humans is also characterized by somatic signs that include gastrointestinal disturbances, weight gain, decreased heart rate (American Psychiatric Association 2000), sweating, dizziness (Hughes and Hatsukami 1986), fatigue, nausea, constipation, and diarrhea (Shiffman 1979). In mice, somatic signs observed during nicotine withdrawal include body shakes, head shakes, paw tremor, scratching, and grooming (Semenova et al. 2003; Balerio et al. 2004; Stoker et al. 2008; Salas et al. 2004b; Damaj et al. 2003; Isola et al. 1999). Somatic signs of nicotine withdrawal have been assessed previously for several nAChR subunit knockout lines. Somatic signs were attenuated during nicotine withdrawal precipitated by the broad-spectrum nAChR antagonist mecamylamine in mice that carried null mutations of the $\beta 4$ (Salas et al. 2004b), $\alpha 5$ (Jackson et al. 2008), and $\alpha 7$ (Salas et al. 2007), but not $\beta 2$ (Salas et al. 2004b), nAChR subunits.

The present study explored the somatic and anhedonic aspects of nicotine withdrawal in $\alpha 7$ knockout ($\alpha 7^{-/-}$), $\alpha 7$ wildtype ($\alpha 7^{+/+}$), $\beta 4$ knockout ($\beta 4^{-/-}$), and $\beta 4$ wildtype ($\beta 4^{+/+}$) mice using the ICSS procedure and observations of somatic signs. Nicotine was administered through subcutaneously implanted osmotic minipumps. Nicotine withdrawal was induced either spontaneously by removing the minipumps or by administering the nonselective nAChR antagonist mecamylamine.

Materials and methods

Subjects

The $\alpha 7$ (Orr-Urtreger et al. 1997) and $\beta 4$ (Xu et al. 1999) nAChR subunit null mouse lines were generated as described previously. The null lines were backcrossed to the C57BL/6J strain for 10 generations. Breeders were generously provided to our laboratory by Drs. Allan Collins and Michael Marks from the University of Colorado Boulder. Knockout and wildtype animals were bred in our laboratory from heterozygous breeding pairs. Wildtype mice were littermates of the knockout mice. Mice assessed in the present experiments were of the 5–8th generation since their transfer from the University of Colorado Boulder to the University of California San Diego, and on the 9–12th generation since the last backcross to the C57BL/6J background strain. Genotypes were determined by three-way polymerase chain reaction with the following primer sequences: $\alpha 7$ forward, 5'-CCTGGTCCTGCTGTGTAACTGCTTC-3'; $\alpha 7$ wildtype reverse, 5'-CTGCTGGGAAATCCTAGGCACACTTGAG-3'; $\alpha 7$ knockout reverse, 5'-GACAAGACCGGCTTCCATCC-3'; $\beta 4$ forward, 5'-TG TAGAGCGAGCATCCGAACA-3', $\beta 4$ wildtype reverse, 5'-TCTCTACTTAGGCTGCCTGTCT-3'; $\beta 4$ knockout reverse, 5'-AGTACCTTCTGAGGCGGAAAGA-3'. Upon weaning, the mice were maintained in groups of two to four until the beginning of the experiment, after which time the mice were housed individually. The subjects were males, 8–12 weeks of age at the beginning of the experiments. The animals were housed in a humidity- and temperature-controlled animal facility on a 12/12 h (lights off at 7:00 A.M.) reverse light/dark cycle with ad libitum access to food and water except during testing. Behavioral testing was conducted during the dark phase of the light/dark cycle, with the exception of the ICSS assessment conducted at the 12 h post-pump removal time-point that was conducted during the light phase of the cycle. During all ICSS testing and somatic signs observations, the testing room was illuminated by red light. All of the experiments were performed in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the University of California San Diego Institutional Animal Care and Use Committee.

Drugs

Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile 0.9% saline solution and infused through subcutaneous osmotic minipumps for 28 days (model 2004, Alzet, Palo Alto, CA, USA). Mecamylamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline and injected subcutaneously in a volume of 0.1 ml/10 g. Nicotine doses are reported as the base, and mecamylamine doses are reported as the salt based on conventions in the literature.

Minipump implantation and removal

The mice were anesthetized with an isoflurane (1–3%)/oxygen vapor mixture, and osmotic minipumps (model 2004, Alzet, Palo Alto, CA, USA) were inserted using aseptic surgery techniques. Nicotine minipumps were placed subcutaneously (SC) parallel to the spine at the shoulder level with the flow moderator directed away from the wound. The wound was closed with 7 mm stainless steel wound clips (Reflex, Cellpoint Scientific, Gaithersburg, MD, USA). On day 29, nicotine minipumps were surgically removed under isoflurane anesthesia using aseptic surgery techniques.

Assessment of somatic signs of nicotine withdrawal

The mice were habituated to plastic cylinders (22 cm diameter, 25 cm height) used for the observation of somatic signs prior to testing for two 20-min periods. The observation of somatic signs occurred at 24 h of nicotine withdrawal, immediately after the light–dark box test, and was 20 min in duration. The scored somatic signs included forelimb tremors, body shakes, headshakes, and abdominal constrictions. Between observations, the cylinders were cleaned by changing the bedding. Somatic signs were analyzed as the total number of somatic signs during each observation period.

Intracranial self-stimulation: surgery, apparatus, and procedure

ICSS surgery—For ICSS surgery, the mice were anesthetized by inhalation of 1–3% isoflurane in oxygen and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Stainless steel bipolar electrodes (0.20 mm diameter; 6 mm length; Plastics One, Roanoke, VA, USA) were implanted into the medial forebrain bundle at the level of the lateral hypothalamus (coordinates: anterior/posterior, 1.58 mm; medial/lateral, 1.0 mm; dorsal/ventral, 5.3 mm from flat skull; Paxinos and Franklin 2001) as described previously (Gill et al. 2004; Stoker et al. 2008). Four stainless steel screws (3.2 mm length, Plastics One, Roanoke, VA, USA) were fixed to the skull to keep the electrode in place together with the application of a resin ionomer (Den-Mat, Santa Maria, CA, USA) and dental acrylic (Ortho-Jet, Lang Dental, Wheeling, IL, USA). The mice were allowed to recover for 7 days after surgery.

ICSS apparatus—ICSS training and testing were conducted in six Plexiglas operant chambers (30.5 × 24 × 27 cm; Med Associates, St. Albans, VT, USA). Each operant chamber was enclosed within a light- and sound-attenuated chamber (40 × 60 × 63.5 cm). Intracranial stimulation was delivered by constant-current stimulators (Stimtek model 1200c, San Diego Instruments, San Diego, CA, USA). The mice were connected to the stimulation circuit through flexible bipolar leads (Plastics One, Roanoke, VA, USA) attached to goldcontact swivel commutators (model SL2C, Plastics One, Roanoke, VA, USA) mounted above the operant chamber. The operant response required by the mice was a simple one-quarter turn of a wheel manipulandum (5.5 cm diameter, 4 cm width) that extended 1.5 cm from one wall of the operant chamber. The stimulation parameters, data collection, and all test session functions were controlled by a computer.

ICSS procedure—The ICSS procedure was described previously for mice (Gill et al. 2004; Stoker et al. 2008) and adapted from a discrete-trial current-intensity threshold procedure originally used in rats (Kornetsky and Esposito 1979; Markou and Koob 1992). Initially, the mice were trained to turn the wheel manipulandum on a fixed-ratio one schedule of reinforcement. The electrical stimulus consisted of 50 pulses with a pulse duration of 100 and a 10,000 μ s interpulse interval. After successful acquisition of this schedule (i.e., two sessions in which the mouse received 200 reinforcers in less than 10 min), the mice were tested in the discrete-trial current-intensity threshold procedure. Each trial began with the delivery of a noncontingent electrical stimulus followed by a 7.5 s response window within which the subject could make a response to receive a second contingent stimulus identical in all parameters to the initial noncontingent stimulus. A response during this time window was labeled a positive response, and the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequences. The intertrial interval that followed either a positive response or the end of the response window (in the case of a negative response) had an average duration of 10 s (ranging from 7.5 to 12.5 s). Responses that occurred during the intertrial interval were labeled timeout responses and resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial

procedure, the duration of the intertrial interval and delay periods induced by timeout responses were gradually increased until both reached a duration of 10 s (ranging from 1 to 10 s during training). The animals were subsequently tested in the current-intensity threshold procedure in which stimulation current intensities were varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities, starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity changed by 5 μ A steps between blocks of trials. The initial stimulus intensity was set at ~30–40 μ A above the baseline current-threshold for each animal. The mice were originally presented with a stimulus that consisted of 50 pulses, each 100 μ s in duration, with an interpulse interval of 10,000 μ s that resulted in 99 Hz stimulation. If mice exhibited thresholds below 70 μ A during the first seven test sessions, then electrical stimuli that were half of the original frequency (25 pulses of 100 μ s in duration at an interpulse interval of 20,000 μ s) were made available to them. This procedure was implemented to ensure that baseline thresholds would not be exceedingly low and thus avoid potential floor effects. Each test session typically lasted 30–40 min and provided two dependent variables for behavioral assessment: threshold and response latency. The threshold value of each series was defined as the midpoint in microamperes between the current intensity level at which the animal made two or more positive responses out of the three stimulus presentations and the level at which the animal made less than two positive responses. The animal's estimated current-intensity threshold for each test session was the mean of the four series' thresholds. The response latency was defined as the average time in seconds that elapsed between the delivery of the electrical stimulus and the turning of the wheel manipulandum for all of the trials that led to a positive response.

Experimental design

Naive $\alpha 7^{-/-}$, $\alpha 7^{+/+}$, $\beta 4^{-/-}$, and $\beta 4^{+/+}$ mice were prepared with stimulating electrodes in the lateral hypothalamus and trained in the ICSS procedure. After the establishment of stable ICSS performance (defined as 10% variation in brain reward thresholds over three consecutive daily test sessions), the mice were prepared with 28-day subcutaneous osmotic minipumps that delivered saline or 40 mg/kg/day nicotine (base) and tested once daily. ICSS thresholds and response latencies during nicotine administration and withdrawal are expressed as a percentage of the baseline value (i.e., 5-day mean before nicotine/saline administration). The broad-spectrum nAChR antagonist mecamylamine hydrochloride was injected subcutaneously on days 9, 11, 13, and 15 of exposure to nicotine/saline using a within-subjects Latin square design for the factor *Dose* (0, 1.5, 3, and 6 mg/kg salt). The mice were tested in the ICSS procedure immediately after mecamylamine/saline injection. ICSS thresholds and response latencies after mecamylamine/saline administration are expressed as the percentage of the previous day's baseline values. The mice were habituated to the cylinders for the observation of somatic signs on days 25 and 27 of nicotine/saline exposure. On day 29, the pumps were removed, and the mice were tested in the ICSS procedure at 3, 6, 8, 12, 24, 28, 48, 52, 72, 76, 96, 100 h after pump removal. At the 24 h time-point post-pump removal, immediately after completion of the ICSS test, the mice were subjected to a 5-min light–dark box session followed immediately by a 20 min observation of somatic signs.

Because no pronounced or long-lasting withdrawal effects were observed in $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice after the termination of 40 mg/kg/day nicotine administration (see “Results” section), a separate naive cohort of $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice were treated with 80 mg/kg/day nicotine or saline for 28 days via subcutaneous osmotic minipumps. On day 29, the pumps were removed, and somatic signs were evaluated 24 h post-pump removal. Upon the successful demonstration of somatic signs of 80 mg/kg/day nicotine withdrawal in $\beta 4^{+/+}$ mice, this

nicotine dose was administered to an additional set of naive $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice that were assessed in the ICSS procedure using identical experimental procedures as those described above for mice treated with 40 mg/kg/day nicotine.

Statistical analyses

All analyses were performed using the PASW 18 Statistical Package (SPSS, Chicago, IL, USA). The data were analyzed using appropriate two- or three-way analyses of variance (ANOVAs) with *Genotype* and *Nicotine/Saline Exposure* as the between-subjects factors and *Administration Day/Withdrawal Day* and *Mecamylamine Dose* as the within-subjects factors. Newman-Keuls post hoc analyses followed statistically significant interaction effects in the ANOVAs. The level of significance was set at 0.05. To evaluate the effects of mecamylamine on ICSS performance, thresholds and response latencies are expressed as a percentage of the baseline values obtained during the last ICSS session before the mecamylamine/saline injection. To evaluate the effects of nicotine administration, thresholds and response latencies are expressed as a percentage of the baseline values obtained during the last five daily ICSS sessions before pump implantation. To evaluate ICSS performance during spontaneous nicotine/saline withdrawal, thresholds and response latencies are expressed as a percentage of the baseline values obtained during the last five ICSS daily sessions before pump removal. For ICSS withdrawal, data time-points were combined for analysis (3 + 6 h, 8 + 12 h, 24 + 28 h, 48 + 52 h, 72 + 76 h, 96 + 100 h) to provide a robust and reliable estimate of the effects of nicotine/saline withdrawal, similar to our previous work (Stoker et al. 2008). Mice that demonstrated instability in ICSS thresholds upon minipump withdrawal (three or more unstable time points [i.e., more than 10% deviation from the 3-day running average] during the 3–100 h withdrawal time window) were excluded from the analysis of the ICSS data during withdrawal. The following numbers of mice were excluded from these analyses: saline-treated $\alpha 7^{+/+}$, $n = 2$; nicotine-treated $\alpha 7^{+/+}$, $n = 1$; saline-treated $\alpha 7^{-/-}$, $n = 2$; saline-treated $\beta 4^{+/+}$, $n = 4$; nicotine-treated $\beta 4^{+/+}$, $n = 2$; saline-treated $\beta 4^{-/-}$, $n = 4$; nicotine-treated $\beta 4^{-/-}$, $n = 2$.

Results

Nicotine administration

The baseline values used to calculate all of the percentages of baseline thresholds are shown in Table 1. Chronic nicotine administration did not affect ICSS thresholds (Fig. 1) or latencies (data not shown) in $\alpha 7^{+/+}$, $\alpha 7^{-/-}$ (Fig. 1a), $\beta 4^{+/+}$, or $\beta 4^{-/-}$ (Fig. 1b) mice, reflected by a lack of significant interaction or main effects in the ANOVAs.

Mecamylamine-precipitated nicotine withdrawal

An ANOVA of the effects of mecamylamine on ICSS thresholds in $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice (Fig. 2a) revealed a significant *Mecamylamine Dose* $\times$ *Nicotine/Saline Exposure* $\times$ *Genotype* interaction ($F_{3,144} = 3.777$, $P < 0.05$) and significant main effects of *Mecamylamine* ($F_{3,144} = 7.320$, $P < 0.001$) and *Nicotine/Saline Exposure* ($F_{1,48} = 34.197$, $P < 0.001$) but not *Genotype*. Post hoc tests revealed that 1.5 mg/kg mecamylamine significantly elevated ICSS thresholds in nicotine-treated $\alpha 7^{-/-}$ mice compared with their saline-treated counterparts ($P < 0.05$), whereas thresholds in $\alpha 7^{+/+}$ mice were not significantly altered. Administration of 3 mg/kg mecamylamine significantly elevated threshold in nicotine-treated $\alpha 7^{+/+}$ ($P < 0.05$), but not $\alpha 7^{-/-}$, mice compared with their saline-treated counterparts. Administration of 6 mg/kg mecamylamine resulted in threshold elevations in nicotine-treated $\alpha 7^{+/+}$ mice compared with saline-treated $\alpha 7^{+/+}$ mice ($P < 0.01$) and nicotine-treated $\alpha 7^{-/-}$ mice ($P < 0.01$). Confirming the lack of effect of 6 mg/kg mecamylamine on thresholds in nicotine-treated $\alpha 7^{-/-}$ mice, thresholds in nicotine- and saline-treated $\alpha 7^{-/-}$ mice did not differ significantly.

Mecamylamine had similar effects on ICSS thresholds in $\beta 4^{+/+}$ and $\beta 4^{-/-}$ mice at all doses tested (Fig. 2b), reflected by the absence of a significant *Mecamylamine* $\times$ *Nicotine/Saline Exposure* $\times$ *Genotype* interaction and no main effect of *Genotype*. Significant main effects were found for *Nicotine/Saline Exposure* ($F_{1,59} = 13.984$, $P < 0.001$) and *Mecamylamine* ($F_{3,177} = 7.215$, $P < 0.001$), reflecting the fact that mecamylamine elevated thresholds in nicotine-treated mice, but this effect was not different in the mutant and wildtype mice.

The latency to turn the wheel manipulandum remained unaffected after mecamylamine administration in both $\alpha 7$ and $\beta 4$ knockout lines treated with either nicotine or saline (data not shown).

Spontaneous nicotine withdrawal

During spontaneous withdrawal from 40 mg/kg/day nicotine, $\alpha 7^{-/-}$ mice exhibited a delay in the onset of ICSS threshold elevations, reflected by a statistically significant difference between nicotine-treated $\alpha 7^{+/+}$ and $\alpha 7^{-/-}$ mice at 3–6 h of withdrawal (Fig. 3a) in a *t* test. Although an ANOVA of ICSS thresholds in $\alpha 7$ mice during spontaneous withdrawal did not show a significant *Nicotine/Saline Exposure* $\times$ *Genotype* $\times$ *Withdrawal Day* interaction or a significant main effect of *Genotype*, significant main effects were found for *Withdrawal Day* ($F_{5,215} = 3.579$, $P < 0.01$) and *Nicotine/Saline Exposure* ($F_{1,43} = 6.422$, $P < 0.05$). Pre-planned comparisons did not reveal any differences between groups at any time-points, but a *t* test revealed a significant difference between nicotine- and saline-treated $\alpha 7^{+/+}$ mice at 3–6 h of withdrawal ($t_{24} = 2.201$, $P < 0.05$). The latency to turn the wheel manipulandum was not affected by spontaneous nicotine/saline withdrawal in either $\alpha 7^{-/-}$ or $\alpha 7^{+/+}$ mice (data not shown).

Nicotine-withdrawing $\beta 4^{-/-}$ mice did not show threshold elevations at any time-point, whereas threshold elevations in nicotine-withdrawing $\beta 4^{+/+}$ mice were short-lasting and returned to baseline levels 8–12 h after the termination of chronic nicotine administration (Fig. 3b). An ANOVA of the thresholds did not yield a significant *Nicotine/Saline Exposure* $\times$ *Genotype* $\times$ *Withdrawal Day* interaction or significant main effects of *Nicotine/Saline Exposure*, *Genotype*, or *Withdrawal Day*. Pre-planned comparisons between experimental groups did not indicate any differences between groups at any time-point, but a *t* test revealed a significant difference between nicotine- and saline-treated $\beta 4^{+/+}$ mice at 3–6 h of withdrawal ($t_{21} = 2.082$, $P < 0.05$). Thresholds in nicotine-withdrawing $\beta 4^{-/-}$ mice were significantly lower than in $\beta 4^{+/+}$ mice ($t_{24} = 1.141$, $P < 0.05$). The elevation of ICSS thresholds of saline-treated mice after the pump removal was likely caused by the repeated daily assessments that took place because the mice were only tested once daily in the ICSS procedure before pump removal. The latency to turn the wheel manipulandum was not affected by spontaneous nicotine/saline withdrawal in $\beta 4^{-/-}$ or $\beta 4^{+/+}$ mice (data not shown).

The increase in somatic signs observed in $\alpha 7^{+/+}$ mice during withdrawal from 40 mg/kg/day nicotine remained intact in nicotine-withdrawing $\alpha 7^{-/-}$ mice (Fig. 4a), reflected by a significant main effect of *Nicotine/Saline Exposure* ($F_{1,43} = 17.584$, $P < 0.001$), no *Nicotine/Saline Exposure* $\times$ *Genotype* interaction, and no main effect of *Genotype*. The increase in somatic signs in mice withdrawn from nicotine, independent of genotype, was also shown by pre-planned comparisons. Nicotine-withdrawing $\alpha 7^{+/+}$ ($P < 0.05$) and $\alpha 7^{-/-}$ ($P < 0.01$) mice exhibited significantly more somatic signs of withdrawal than their saline-treated counterparts. Light–dark box data are not reported due to the absence of reliable detection of anxiety-like behavior in wildtype mice of either mouse line. Because of the non-invasive nature of the light–dark box test, it is highly unlikely that exposure to the light–dark box affected the observations of somatic signs of nicotine withdrawal that were conducted in some cases shortly after the light–dark box.

After the termination of 40 mg/kg/day nicotine administration, $\beta 4^{+/+}$ mice exhibited a tendency toward an increased number of somatic signs compared with saline-treated $\beta 4^{+/+}$ mice, whereas nicotine-withdrawing $\beta 4^{-/-}$ mice did not show increases in somatic signs compared with saline-treated $\beta 4^{-/-}$ mice (Fig. 4b). This observation was supported by a trend toward a *Nicotine/Saline Exposure* $\times$ *Genotype* interaction ($F_{1,46} = 2.903$, $P < 0.1$) in the ANOVA. However, pre-planned comparisons did not reveal any significant differences between experimental groups.

Somatic signs were significantly attenuated in $\beta 4^{-/-}$ mice compared with $\beta 4^{+/+}$ mice during withdrawal from 80 mg/kg/day nicotine (Fig. 5), reflected by a significant *Nicotine/Saline Exposure* $\times$ *Genotype* interaction ($F_{1,22} = 13.167$, $P < 0.01$) and significant main effects of *Nicotine/Saline Exposure* ($F_{1,22} = 54.450$, $P < 0.001$) and *Genotype* ($F_{1,22} = 7.601$, $P < 0.05$). Post hoc comparisons revealed significant differences between nicotine- and saline-treated $\beta 4^{+/+}$ mice ($P < 0.01$), between nicotine- and saline-treated $\beta 4^{-/-}$ mice ($P < 0.05$), and between nicotine-treated $\beta 4^{+/+}$ and $\beta 4^{-/-}$ mice ($P < 0.01$). Although withdrawal from 80 mg/kg/day nicotine induced an increase in the somatic signs of withdrawal in $\beta 4^{+/+}$ mice (Fig. 5), it did not induce long-lasting elevations in ICSS thresholds in these mice (data not shown) beyond those seen after the termination of 40 mg/kg/day nicotine administration (Fig. 3b).

Discussion

Spontaneous nicotine withdrawal elevated ICSS thresholds in $\alpha 7^{+/+}$, but not $\alpha 7^{-/-}$, mice 3–6 h post-pump removal. After this initial delay in the onset of spontaneous nicotine withdrawal in $\alpha 7^{-/-}$ mice, thresholds were gradually elevated in $\alpha 7^{-/-}$ mice until they reached levels similar to those in nicotine-withdrawing $\alpha 7^{+/+}$ mice at the 24 h and later time-points of withdrawal. Consistent with these threshold findings, $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice showed similar increases in the somatic signs of spontaneous nicotine withdrawal at 24 h of withdrawal. Interestingly, administration of the broad-spectrum nAChR antagonist mecamylamine in nicotine- and saline-treated mice induced effects in opposite directions at the low (1.5 mg/kg) and high (6 mg/kg) mecamylamine doses tested in nicotine-treated $\alpha 7^{-/-}$ mice. Specifically, the low dose of 1.5 mg/kg mecamylamine significantly elevated ICSS thresholds in $\alpha 7^{-/-}$, but not $\alpha 7^{+/+}$, mice. The intermediate and highest doses of 3 and 6 mg/kg/day mecamylamine significantly elevated thresholds only in $\alpha 7^{+/+}$, and not $\alpha 7^{-/-}$, mice.

Similar to the data in $\alpha 7$ mice, ICSS thresholds in $\beta 4^{+/+}$ mice were elevated only at the 3–6 h time-point of withdrawal from 40 mg/kg/day nicotine, whereas thresholds in $\beta 4^{-/-}$ mice remained at baseline levels. Starting at 8–12 h of withdrawal, however, ICSS thresholds in $\beta 4^{+/+}$ mice returned to baseline levels. The lack of robust spontaneous nicotine withdrawal effects, reflected by the absence of threshold elevations in $\beta 4^{+/+}$ mice, was also reflected in the lack of significant increases in somatic signs at 24 h of withdrawal from 40 mg/kg/day nicotine in these mice, although a clear trend was observed. Nevertheless, a clear increase in the somatic signs of withdrawal was observed in $\beta 4^{+/+}$ mice after the termination of 80 mg/kg/day nicotine administration, which was absent in $\beta 4$ knockout animals.

The present study was one of the first attempts to characterize the nicotine withdrawal syndrome in $\alpha 7$ and $\beta 4$ nAChR knockout mice using the ICSS procedure. Previous studies have assessed $\alpha 7$ and $\beta 4$ nAChR knockout mice in various other measures of nicotine withdrawal, including somatic signs and anxiety-related behavioral tests. Under baseline conditions, the behavior of $\alpha 7^{-/-}$ mice resembles $\alpha 7^{+/+}$ mice in various behavioral procedures, including locomotor activity, anxiety-like behavior, fear conditioning, and spatial learning (Paylor et al. 1998; Salas et al. 2007; Wehner et al. 2004). Subtle differences

between $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice include impairments in the sympathetic responses to vasodilatation, indicating autonomic dysfunction in these mutant mice (Franceschini et al. 2000). Additionally, $\alpha 7^{-/-}$ mice demonstrated a slight increase in the time spent in the center of the open field, although this anxiogenic-like effect was not seen in the light–dark box test, another procedure that assesses anxiety-like behavior (Paylor et al. 1998). The same study demonstrated a subtle improvement in spatial learning in $\alpha 7^{-/-}$ mice compared with their wildtype counterparts. $\alpha 7^{-/-}$ mice located the hidden platform in the Morris water task faster than their wildtype counterparts, but this genotype difference was not supported by the distance traveled (Paylor et al. 1998). By contrast, mild cognitive impairment was observed in $\alpha 7^{-/-}$ mice in terms of an attentional deficit under specific conditions in the five-choice serial reaction time task (Hoyle et al. 2006; Young et al. 2004), signaled nose-poke task (Keller et al. 2005), and radial arm maze (Levin et al. 2009). In the present experiments, no differences were observed in the acquisition of the discrete trial current-intensity ICSS procedure between $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice.

Nicotine administration had similar effects in $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice, including seizure sensitivity, lever-pressing behavior, locomotor activity, hyperthermia, cued and contextual fear conditioning, nicotine discrimination, and nicotine-induced conditioned place preference (Franceschini et al. 2002; Naylor et al. 2005; Salas et al. 2007; Stolerman et al. 2004; Tritto et al. 2004; Walters et al. 2006; Wehner et al. 2004). A recent study suggested that nicotine intake was similar in $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice throughout the first 21 days of administration; however, after this 21-day period, $\alpha 7^{-/-}$ mice self-administered significantly less nicotine in a two-bottle procedure (Levin et al. 2009). The present study did not find differences in ICSS thresholds between nicotine-treated $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice during the 28-day period of chronic nicotine administration via subcutaneous osmotic minipumps.

During spontaneous withdrawal from 28 days of 40 mg/kg/day nicotine, $\alpha 7^{-/-}$ mice exhibited a delay in the onset of ICSS threshold elevations. After the initial delay in the onset of withdrawal symptoms seen at the 3–6 h post-nicotine time-point, thresholds in $\alpha 7^{-/-}$ mice progressively elevated until reaching levels similar to those in $\alpha 7^{+/+}$ mice at the 48–52 h post-nicotine time-point. Furthermore, ICSS thresholds in $\alpha 7^{-/-}$ mice were differentially affected by mecamylamine-precipitated nicotine withdrawal, depending on the dose of mecamylamine. The lower dose of 1.5 mg/kg mecamylamine significantly elevated thresholds in nicotine-treated $\alpha 7^{-/-}$ mice, whereas thresholds in nicotine-treated $\alpha 7^{-/-}$ mice were not significantly different from those in saline-injected mice of the same genotype at 3 and 6 mg/kg mecamylamine. In contrast, $\alpha 7^{+/+}$ mice treated with nicotine exhibited a trend toward a linear dose-dependent elevation in thresholds with increasing doses of mecamylamine. These interesting effects seen in $\alpha 7^{-/-}$ mice may reflect compensatory mechanisms in other nAChRs that result from constitutive null mutation of the $\alpha 7$ nAChR gene. However, no significant differences were detected in the mRNA expression levels of other nicotinic receptor subunits between $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice (Franceschini et al. 2002). Thus, these differential effects of low and high mecamylamine doses in $\alpha 7^{-/-}$ mice may be explained instead by adaptations in other central nervous system receptors, besides nAChRs, in these mice. Mecamylamine is considered a broad-spectrum noncompetitive antagonist of neuronal nAChRs, although it differentially affects different nAChR subtypes. The effects of mecamylamine on β subunit-containing nAChRs are prolonged compared with the effects of mecamylamine on $\alpha 7$ receptors, on which the inhibition by mecamylamine is rapidly reversed (Frazier et al. 1998; Papke et al. 2001). The short-lasting effects that mecamylamine exerts on $\alpha 7$ nAChR subunits (Frazier et al. 1998; Papke et al. 2001) suggests that null mutation of the $\alpha 7$ receptor does not likely affect the overall effects of mecamylamine. Mecamylamine also acts as an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Chavez-Noriega et al. 1997; Frazier et al. 1998) but only at high concentrations (Papke et al. 2001). Previous work showed that chronic nicotine administration induced

changes in NMDA receptor subunit expression (Kenny et al. 2009). Moreover, the NMDA receptor antagonist dizocilpine (MK-801) showed a strong tendency to lower ICSS thresholds in nicotine-treated rats compared with saline-treated rats (Kenny et al. 2003). Considered in the context of this previous literature, the present findings in $\alpha 7$ knockout mice may be explained by antagonism of NMDA receptors at high mecamylamine doses, leading to threshold lowering that counterbalances the threshold elevations induced by mecamylamine administration in nicotine-treated $\alpha 7^{-/-}$ mice.

A recent study indicated that somatic signs were similar between nicotine-treated $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice 10 min after mecamylamine administration (Jackson et al. 2008). In an earlier study, however, Salas and colleagues observed significantly fewer somatic signs of mecamylamine-precipitated nicotine withdrawal in $\alpha 7^{-/-}$ mice compared with $\alpha 7^{+/+}$ mice when tested immediately after mecamylamine administration (Salas et al. 2007), which may indicate a delayed onset of the nicotine withdrawal symptoms in $\alpha 7^{-/-}$ mice. The present study found similar increases in somatic signs in $\alpha 7^{+/+}$ and $\alpha 7^{-/-}$ mice at 24 h of spontaneous nicotine withdrawal, consistent with results from a previous study that found similar increases in somatic signs at 48 h post-nicotine between wildtype and mutant mice (Grabus et al. 2005). Overall, this pattern of results supports the present findings of a delay in the onset of the nicotine withdrawal syndrome in $\alpha 7^{-/-}$ mice in the ICSS procedure. In the present experiments, ICSS thresholds were assessed at several time-points, whereas somatic signs were assessed only at 24 h of spontaneous nicotine withdrawal; therefore, the present data could not inform about potential alterations in the time-course of the somatic signs of nicotine withdrawal. Importantly, somatic signs and brain reward thresholds may be differentially regulated. While brain reward threshold elevations associated with nicotine withdrawal are regulated by the central nervous system (Olds and Fobes 1981; Watkins et al. 2000), somatic signs were demonstrated to be primarily mediated by the peripheral nervous systems (Watkins et al. 2000). It should be noted, however, that results from Malin and colleagues (Malin et al. 1997) indicated only central, and not peripheral, mediation of the somatic signs of nicotine withdrawal. In either case, the delay in the onset of brain reward threshold elevations and the onset of somatic signs in $\alpha 7^{-/-}$ mice may be indicative of a delay in the overall effects of the nicotine withdrawal syndrome in these mice.

Although a linkage between allelic variation in chromosome region 15q25, which holds the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nAChR subunit gene cluster, and an increased risk of tobacco addiction was recently demonstrated (Liu et al. 2010; Saccone et al. 2007), the behavioral phenotype of $\beta 4$ knockout mice has not been investigated as extensively as $\alpha 7$ knockout mice. Studies have assessed, however, the behavioral profile of these mice under various conditions, including under baseline conditions, during nicotine administration and withdrawal. Under baseline conditions, $\beta 4$ knockout mice generally resemble wildtype mice. For example, $\beta 4$ knockout mice and their wildtype counterparts exhibited similar behavior in the contextual and cued fear conditioning procedures (Wehner et al. 2004), indicating that $\beta 4$ -containing nAChRs may not be involved in these learning processes. An anxiolytic phenotype has been indicated for $\beta 4$ knockout mice in the elevated plus maze and staircase maze (Salas et al. 2003b). Interestingly, this decrease in the anxiety-like response in $\beta 4^{-/-}$ mice was not detected in other tests of anxiety, including the light-dark box, open field, and mirrored chamber (Salas et al. 2003b). Under baseline conditions, core body temperature is lower in $\beta 4^{-/-}$ than in $\beta 4^{+/+}$ mice (Sack et al. 2005), consistent with the observation that the $\beta 4$ nAChR subunit is an important component of nAChRs in the autonomic nervous system (Xu et al. 1999). Furthermore, social isolation of $\beta 4^{-/-}$ mice induced an augmented increase in heart rate above that seen in $\beta 4^{+/+}$ mice during social isolation (Salas et al. 2003b). Nevertheless, social isolation unlikely influenced the results of the present studies. Social isolation per se has not been known to alter ICSS thresholds under baseline conditions. The administration of nicotine had similar effects in $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice during contextual and cued fear

conditioning (Wehner et al. 2004). $\beta 4^{-/-}$ mice, however, were less sensitive to the effects of nicotine than $\beta 4^{+/+}$ mice in the open field test. Specifically, the administration of low nicotine doses in $\beta 4^{-/-}$ mice did not decrease the open field exploration behavior that was observed in $\beta 4^{+/+}$ mice (Salas et al. 2004a). Moreover, seizures induced by high nicotine doses in $\beta 4^{+/+}$ mice were absent in $\beta 4^{-/-}$ mice (Kedmi et al. 2004; Salas et al. 2004a).

Mecamylamine administration in nicotine-treated $\beta 4^{-/-}$ mice resulted in an attenuated increase in somatic signs compared with $\beta 4^{+/+}$ mice (Salas et al. 2004b). Furthermore, null mutation of the $\beta 4$ receptor reduced hyperalgesia induced by spontaneous nicotine withdrawal (Salas et al. 2004b). In the present study, $\beta 4^{-/-}$ mice withdrawn from 40 mg/kg/day chronic nicotine administration did not show ICSS threshold elevations throughout the 5-day assessment period or an increase in somatic signs at 24 h of withdrawal. $\beta 4^{+/+}$ mice showed a clear trend toward an increase in somatic signs induced by the cessation of 40 mg/kg/day nicotine administration and displayed a short-lasting elevation in ICSS thresholds at 3–6 h postpump removal, in contrast to the longer-lasting affective and somatic symptoms of spontaneous nicotine withdrawal exhibited by their C57BL/6J background strain (Stoker et al. 2008) and $\alpha 7^{+/+}$ mice in the present study. The cessation of 28 days of 80 mg/kg/day nicotine administration significantly increased the somatic signs of withdrawal in $\beta 4^{+/+}$ mice and significantly attenuated these increases in $\beta 4^{-/-}$ mice. However, the same high dose of nicotine did not prolong the elevations of ICSS thresholds in $\beta 4^{+/+}$ mice in a separate pilot experiment (data not shown). The lack of pronounced and long-lasting threshold elevations in $\beta 4^{+/+}$ mice leads to somewhat inconclusive findings about the role of $\beta 4$ -containing nAChRs in the anhedonic aspects of nicotine withdrawal, although a delay or rather lack of onset of the anhedonic aspects of nicotine withdrawal may occur in these mice.

Summary of results and conclusions

$\alpha 7^{-/-}$ mice showed a delay in the onset of ICSS threshold elevations during spontaneous nicotine withdrawal. The administration of a low mecamylamine dose induced significant elevations in ICSS thresholds in nicotine-treated $\alpha 7^{-/-}$ mice that were absent in $\alpha 7^{+/+}$ mice, whereas a high mecamylamine dose had no effect in nicotine-treated $\alpha 7^{-/-}$ mice and elevated thresholds of nicotine-treated $\alpha 7^{+/+}$ mice. The somatic signs of spontaneous nicotine withdrawal were similar in $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice. These results indicate that the $\alpha 7$ nAChR is involved in the initiation of the anhedonic aspects of nicotine withdrawal, but these nAChRs are not involved in the expression of the somatic signs of nicotine withdrawal. Furthermore, the effects of mecamylamine in nicotine-treated $\alpha 7$ knockout and wild-type mice suggests that compensatory adaptations may occur in constitutive $\alpha 7^{-/-}$ mice or that mecamylamine may interact with other receptors besides nAChRs in these mice.

$\beta 4^{-/-}$ mice withdrawn from nicotine did not exhibit threshold elevations at 3–100 h of withdrawal when testing occurred, whereas $\beta 4^{+/+}$ mice showed short-lasting threshold elevations that were observed only at the 3–6 h time-point of withdrawal. Therefore, the results of the present study can only lead to the conclusion of an apparent delay or lack of onset of the anhedonic aspects of the nicotine withdrawal syndrome in $\beta 4$ knockout mice. Mecamylamine-induced threshold elevations were similar in $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice. Increases in the somatic signs of spontaneous nicotine withdrawal were only seen in $\beta 4^{+/+}$ mice after the cessation of 80 mg/kg/day, but not 40 mg/kg/day, nicotine administration for 28 days. Thus, these findings indicate that $\beta 4$ -containing receptors are involved in both the anhedonic and somatic signs of nicotine withdrawal.

The aversive aspects of the early withdrawal syndrome have been implicated as powerful motivators in the continuation of the tobacco smoking habit (Baker et al. 2004a; 2004b;

Piasecki et al. 2002). In addition, depressive symptoms associated with tobacco withdrawal were found to be especially important in predicting relapse (Hughes 2007). Pharmacological treatment of the anhedonic symptoms of tobacco withdrawal may thus help prevent relapse (Leventhal et al. 2008). The present studies in $\alpha 7$ and $\beta 4$ knockout mice may suggest that blockade of $\alpha 7$ - or $\beta 4$ -containing nAChRs may alleviate the aversive anhedonic signs of the early nicotine withdrawal syndrome, thereby potentially decreasing the risk for relapse and thus increasing the chance of successful discontinuation of the tobacco smoking habit.

In summary, the results of the present study confirm previous findings of attenuated somatic signs of nicotine withdrawal in $\beta 4$, but not $\alpha 7$, knockout mice. A delay in the onset of the anhedonic aspects of spontaneous nicotine withdrawal, measured with the ICSS procedure, was demonstrated in mice null for the $\alpha 7$ and $\beta 4$ nAChR subunits.

Acknowledgments

This work was supported by NIH grant R01DA023209 to AM. Breeders for the $\alpha 7$ and $\beta 4$ nAChR subunit knockout lines were provided to our laboratory with the support of grant P30 DA015663 to Dr. Michael J. Marks at the University of Colorado, Boulder. The authors would like to thank Mr. Edwin Obaña, Ms. Holly Jarrell, and Mrs. Kimberly Edwards for their help with the mouse colony maintenance and genotyping of the knockout lines. Finally, the authors would like to thank Mr. Michael Arends for editorial assistance.

References

- American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 4th edn, text revision. American Psychiatric Press; Washington DC: 2000.
- Baker TB, Brandon TH, Chassin L. Motivational influences on cigarette smoking. *Annu Rev Psychol.* 2004a; 55:463–491. [PubMed: 14744223]
- Baker TB, Piper ME, McCarthy DE, Majeskie MR, Fiore MC. Addiction motivation reformulated: an affective processing model of negative reinforcement. *Psychol Rev.* 2004b; 111:33–51. [PubMed: 14756584]
- Balerio GN, Aso E, Berrendero F, Murtra P, Maldonado R. $\Delta 9$ -tetrahydrocannabinol decreases somatic and motivational manifestations of nicotine withdrawal in mice. *Eur J Neurosci.* 2004; 20:2737–2748. [PubMed: 15548217]
- Barik J, Wonnacott S. Indirect modulation by $\alpha 7$ nicotinic acetylcholine receptors of noradrenaline release in rat hippocampal slices: interaction with glutamate and GABA systems and effect of nicotine withdrawal. *Mol Pharmacol.* 2006; 69:618–628. [PubMed: 16269536]
- Cachelin AB, Jaggi R. β subunits determine the time course of desensitization in rat $\alpha 3$ neuronal nicotinic acetylcholine receptors. *Pflugers Arch.* 1991; 419:579–582. [PubMed: 1788053]
- Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ, Johnson EC. Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors $ha2\beta 2$, $ha2\beta 4$, $ha3\beta 2$, $ha3\beta 4$, $ha4\beta 2$, $ha4\beta 4$ and $ha7$ expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther.* 1997; 280:346–356. [PubMed: 8996215]
- Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [3H]acetylcholine, [3H]nicotine, and [^{125}I]- α -bungarotoxin. *J Neurosci.* 1985; 5:1307–1315. [PubMed: 3998824]
- Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: new perspectives from knockout mice. *Trends Pharmacol Sci.* 2000; 21:211–217. [PubMed: 10838608]
- Damaj MI, Kao W, Martin BR. Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. *J Pharmacol Exp Ther.* 2003; 307:526–534. [PubMed: 12970387]
- Fenster CP, Rains MF, Noerager B, Quick MW, Lester RA. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J Neurosci.* 1997; 17:5747–5759. [PubMed: 9221773]
- Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. Habenular $\alpha 5$ nicotinic receptor subunit signalling controls nicotine intake. *Nature.* 2011; 471:597–601. [PubMed: 21278726]

- Franceschini D, Orr-Urtreger A, Yu W, Mackey LY, Bond RA, Armstrong D, Patrick JW, Beaudet AL, De Biasi M. Altered baroreflex responses in $\alpha 7$ deficient mice. *Behav Brain Res.* 2000; 113:3–10. [PubMed: 10942027]
- Franceschini D, Paylor R, Broide R, Salas R, Bassetto L, Gotti C, De Biasi M. Absence of $\alpha 7$ -containing neuronal nicotinic acetylcholine receptors does not prevent nicotine-induced seizures. *Brain Res Mol Brain Res.* 2002; 98:29–40. [PubMed: 11834293]
- Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV. Acetylcholine activates an α -bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *J Neurosci.* 1998; 18:1187–1195. [PubMed: 9454829]
- Fu Y, Matta SG, Gao W, Sharp BM. Local α -bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion in vivo. *Neuroscience.* 2000; 101:369–375. [PubMed: 11074160]
- Gerzanich V, Anand R, Lindstrom J. Homomers of $\alpha 8$ and $\alpha 7$ subunits of nicotinic receptors exhibit similar channel but contrasting binding site properties. *Mol Pharmacol.* 1994; 45:212–220. [PubMed: 7509438]
- Gill BM, Knapp CM, Kornetsky C. The effects of cocaine on the rate independent brain stimulation reward threshold in the mouse. *Pharmacol Biochem Behav.* 2004; 79:165–170. [PubMed: 15388296]
- Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I, Moretti M, Pedrazzi P, Pucci L, Zoli M. Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochem Pharmacol.* 2009; 78:703–711. [PubMed: 19481063]
- Grabus SD, Martin BR, Imad Damaj M. Nicotine physical dependence in the mouse: involvement of the $\alpha 7$ nicotinic receptor subtype. *Eur J Pharmacol.* 2005; 515:90–93. [PubMed: 15896732]
- Grady SR, Moretti M, Zoli M, Marks MJ, Zanardi A, Pucci L, Clementi F, Gotti C. Rodent habenulo-interpeduncular pathway expresses a large variety of uncommon nAChR subtypes, but only the $\alpha 3\beta 4^*$ and $\alpha 3\beta 3\beta 4^*$ subtypes mediate acetylcholine release. *J Neurosci.* 2009; 29:2272–2282. [PubMed: 19228980]
- Hernandez SC, Vicini S, Xiao Y, Davila-Garcia MI, Yasuda RP, Wolfe BB, Kellar KJ. The nicotinic receptor in the rat pineal gland is an $\alpha 3\beta 4$ subtype. *Mol Pharmacol.* 2004; 66:978–987. [PubMed: 15247319]
- Hoyle E, Genn RF, Fernandes C, Stolerman IP. Impaired performance of $\alpha 7$ nicotinic receptor knockout mice in the five-choice serial reaction time task. *Psychopharmacology (Berl).* 2006; 189:211–223. [PubMed: 17019565]
- Hughes JR. Effects of abstinence from tobacco: etiology, animal models, epidemiology, and significance: a subjective review. *Nicotine Tob Res.* 2007; 9:329–339. [PubMed: 17365765]
- Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. *Arch Gen Psychiatry.* 1986; 43:289–294. [PubMed: 3954551]
- Isola R, Vogelsberg V, Wemlinger TA, Neff NH, Hadjiconstantinou M. Nicotine abstinence in the mouse. *Brain Res.* 1999; 850:189–196. [PubMed: 10629764]
- Jackson KJ, Martin BR, Changeux JP, Damaj MI. Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *J Pharmacol Exp Ther.* 2008; 325:302–312. [PubMed: 18184829]
- Johnson PM, Hollander JA, Kenny PJ. Decreased brain reward function during nicotine withdrawal in C57BL6 mice: evidence from intracranial self-stimulation (ICSS) studies. *Pharmacol Biochem Behav.* 2008; 90:409–415. [PubMed: 18466962]
- Jones IW, Wonnacott S. Precise localization of $\alpha 7$ nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. *J Neurosci.* 2004; 24:11244–11252. [PubMed: 15601930]
- Kawai H, Berg DK. Nicotinic acetylcholine receptors containing $\alpha 7$ subunits on rat cortical neurons do not undergo long-lasting inactivation even when up-regulated by chronic nicotine exposure. *J Neurochem.* 2001; 78:1367–1378. [PubMed: 11579145]
- Kedmi M, Beaudet AL, Orr-Urtreger A. Mice lacking neuronal nicotinic acetylcholine receptor $\beta 4$ -subunit and mice lacking both $\alpha 5$ - and $\beta 4$ -subunits are highly resistant to nicotine-induced seizures. *Physiol Genomics.* 2004; 17:221–229. [PubMed: 14996991]

- Keller JJ, Keller AB, Bowers BJ, Wehner JM. Performance of $\alpha 7$ nicotinic receptor null mutants is impaired in appetitive learning measured in a signaled nose poke task. *Behav Brain Res.* 2005; 162:143–152. [PubMed: 15922075]
- Kenny PJ, Gasparini F, Markou A. Group II metabotropic and α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate glutamate receptors regulate the deficit in brain reward function associated with nicotine withdrawal in rats. *J Pharmacol Exp Ther.* 2003; 306:1068–1076. [PubMed: 12805481]
- Kenny PJ, Chartoff E, Roberto M, Carlezon WA Jr, Markou A. NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of the ventral tegmental area and central nucleus of the amygdala. *Neuropsychopharmacology.* 2009; 34:266–281. [PubMed: 18418357]
- Kornetsky C, Esposito RU. Euphorigenic drugs: effects on the reward pathways of the brain. *Fed Proc.* 1979; 38:2473–2476. [PubMed: 488370]
- Leventhal AM, Ramsey SF, Brown RA, LaChance HR, Kahler CW. Dimensions of depressive symptoms and smoking cessation. *Nicotine Tob Res.* 2008; 10:507–517. [PubMed: 18324570]
- Levin ED, Petro A, Rezvani AH, Pollard N, Christopher NC, Strauss M, Avery J, Nicholson J, Rose JE. Nicotinic $\alpha 7$ - or $\beta 2$ -containing receptor knockout: effects on radial-arm maze learning and long-term nicotine consumption in mice. *Behav Brain Res.* 2009; 196:207–213. [PubMed: 18831991]
- Liu JZ, Tozzi F, Waterworth DM, Pillai SG, Muglia P, Middleton L, Berrettini W, Knouff CW, Yuan X, Waeber G, Vollenweider P, Preisig M, Wareham NJ, Zhao JH, Loos RJ, Barroso I, Khaw KT, Grundy S, Barter P, Mahley R, Kesaniemi A, McPherson R, Vincent JB, Strauss J, Kennedy JL, Farmer A, McGuffin P, Day R, Matthews K, Bakke P, Gulsvik A, Lucae S, Ising M, Brueckl T, Horstmann S, Wichmann HE, Rawal R, Dahmen N, Lamina C, Polasek O, Zgaga L, Huffman J, Campbell S, Kooner J, Chambers JC, Burnett MS, Devaney JM, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein S, Wilson JF, Wild SH, Campbell H, Vitart V, Reilly MP, Li M, Qu L, Wilensky R, Matthai W, Hakonarson HH, Rader DJ, Franke A, Wittig M, Schafer A, Uda M, Terracciano A, Xiao X, Busonero F, Scheet P, Schlessinger D, St Clair D, Rujescu D, Abecasis GR, Grabe HJ, Teumer A, Volzke H, Petersmann A, John U, Rudan I, Hayward C, Wright AF, Kolcic I, Wright BJ, Thompson JR, Balmforth AJ, Hall AS, Samani NJ, Anderson CA, Ahmad T, Mathew CG, Parkes M, Satsangi J, Caulfield M, Munroe PB, Farrall M, Dominiczak A, Worthington J, Thomson W, Eyre S, Barton A, Mooser V, Francks C, Marchini J. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet.* 2010; 42:436–440. [PubMed: 20418889]
- Malin DH, Lake JR, Schopen CK, Kirk JW, Sailer EE, Lawless BA, Upchurch TP, SHenoi M, Rajan N. Nicotine abstinence syndrome precipitated by central but not peripheral hexamethonium. *Pharmacol Biochem Behav.* 1997; 58(3):6595–6599.
- Markou A, Koob GF. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol Behav.* 1992; 51:111–119. [PubMed: 1741436]
- Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J Pharmacol Exp Ther.* 1983; 226:817–825. [PubMed: 6887012]
- McGehee DS, Heath MJ, Gelber S, Devay P, Role LW. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science.* 1995; 269:1692–1696. [PubMed: 7569895]
- Meyer EL, Xiao Y, Kellar KJ. Agonist regulation of rat $\alpha 3\beta 4$ nicotinic acetylcholine receptors stably expressed in human embryonic kidney 293 cells. *Mol Pharmacol.* 2001; 60:568–576. [PubMed: 11502889]
- Molinari EJ, Delbono O, Messi ML, Renganathan M, Arneric SP, Sullivan JP, Gopalakrishnan M. Up-regulation of human $\alpha 7$ nicotinic receptors by chronic treatment with activator and antagonist ligands. *Eur J Pharmacol.* 1998; 347:131–139. [PubMed: 9650859]
- Naylor C, Quarta D, Fernandes C, Stolerman IP. Tolerance to nicotine in mice lacking $\alpha 7$ nicotinic receptors. *Psychopharmacology (Berl).* 2005; 180:558–563. [PubMed: 15723228]
- Nguyen HN, Rasmussen BA, Perry DC. Subtype-selective up-regulation by chronic nicotine of high-affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. *J Pharmacol Exp Ther.* 2003; 307:1090–1097. [PubMed: 14560040]

- Olds ME, Fobes JL. The central basis of motivation: intracranial self-stimulation studies. *Annu Rev Psychol.* 1981; 32:523–574. [PubMed: 7015997]
- Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW, Beaudet AL. Mice deficient in the $\alpha 7$ neuronal nicotinic acetylcholine receptor lack α -bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci.* 1997; 17:9165–9171. [PubMed: 9364063]
- Pakkanen JS, Jokitalo E, Tuominen RK. Up-regulation of $\beta 2$ and $\alpha 7$ subunit containing nicotinic acetylcholine receptors in mouse striatum at cellular level. *Eur J Neurosci.* 2005; 21:2681–2691. [PubMed: 15926916]
- Papke RL, Boulter J, Patrick J, Heinemann S. Single-channel currents of rat neuronal nicotinic acetylcholine receptors expressed in *Xenopus oocytes*. *Neuron.* 1989; 3:589–596. [PubMed: 2484342]
- Papke RL, Sanberg PR, Shytle RD. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. *J Pharmacol Exp Ther.* 2001; 297:646–656. [PubMed: 11303054]
- Parker MJ, Beck A, Luetje CW. Neuronal nicotinic receptor $\beta 2$ and $\beta 4$ subunits confer large differences in agonist binding affinity. *Mol Pharmacol.* 1998; 54:1132–1139. [PubMed: 9855644]
- Paxinos, G.; Franklin, KBJ. The mouse brain in stereotaxic coordinates. 2nd edn.. Academic Press; San Diego: 2001.
- Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. $\alpha 7$ nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of *Acra7*-deficient mice. *Learn Mem.* 1998; 5:302–316. [PubMed: 10454356]
- Piasecki TM, Fiore MC, McCarthy DE, Baker TB. Have we lost our way? The need for dynamic formulations of smoking relapse proneness. *Addiction.* 2002; 97:1093–1108. [PubMed: 12199822]
- Piccio MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the $\beta 2$ subunit are involved in the reinforcing properties of nicotine. *Nature.* 1998; 391:173–177. [PubMed: 9428762]
- Piccio MR, Caldarone BJ, Brunzell DH, Zachariou V, Stevens TR, King SL. Neuronal nicotinic acetylcholine receptor subunit knockout mice: physiological and behavioral phenotypes and possible clinical implications. *Pharmacol Ther.* 2001; 92:89–108. [PubMed: 11916531]
- Quik M, Polonskaya Y, Gillespie A, Jakowec M, Lloyd GK, Langston JW. Localization of nicotinic receptor subunit mRNAs in monkey brain by in situ hybridization. *J Comp Neurol.* 2000; 425:58–69. [PubMed: 10940942]
- Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet.* 2007; 16:36–49. [PubMed: 17135278]
- Sack R, Gochberg-Sarver A, Rozovsky U, Kedmi M, Rosner S, Orr-Urtreger A. Lower core body temperature and attenuated nicotine-induced hypothermic response in mice lacking the $\beta 4$ neuronal nicotinic acetylcholine receptor subunit. *Brain Res Bull.* 2005; 66:30–36. [PubMed: 15925141]
- Salas R, Orr-Urtreger A, Broide RS, Beaudet A, Paylor R, De Biasi M. The nicotinic acetylcholine receptor subunit $\alpha 5$ mediates short-term effects of nicotine in vivo. *Mol Pharmacol.* 2003a; 63:1059–1066. [PubMed: 12695534]
- Salas R, Pieri F, Fung B, Dani JA, De Biasi M. Altered anxiety-related responses in mutant mice lacking the $\beta 4$ subunit of the nicotinic receptor. *J Neurosci.* 2003b; 23:6255–6263. [PubMed: 12867510]
- Salas R, Cook KD, Bassetto L, De Biasi M. The $\alpha 3$ and $\beta 4$ nicotinic acetylcholine receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. *Neuropharmacology.* 2004a; 47:401–407. [PubMed: 15275829]
- Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the $\beta 4$ nicotinic acetylcholine receptor subunit. *J Neurosci.* 2004b; 24:10035–10039. [PubMed: 15537871]

- Salas R, Main A, Gangitano D, De Biasi M. Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the $\alpha 7$ nicotinic acetylcholine receptor subunit. *Neuropharmacology*. 2007; 53:863–869. [PubMed: 17920082]
- Salas R, Sturm R, Boulter J, De Biasi M. Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *J Neurosci*. 2009; 29:3014–3018. [PubMed: 19279237]
- Schilstrom B, Svensson HM, Svensson TH, Nomikos GG. Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of $\alpha 7$ nicotinic receptors in the ventral tegmental area. *Neuroscience*. 1998; 85:1005–1009. [PubMed: 9681941]
- Semenova S, Bespalov A, Markou A. Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration. *Eur J Pharmacol*. 2003; 472:99–110. [PubMed: 12860478]
- Shiffman SM. The tobacco withdrawal syndrome. *NIDA Res Monogr*. 1979; 23:158–184. [PubMed: 111135]
- Stoker, AK.; Markou, A. The intracranial self-stimulation procedure provides quantitative measures of brain reward function. In: Gould, TJ., editor. *Mood and anxiety related phenotypes in mice: characterization using behavioral tests*, vol II (series title: *Neuromethods*). Humana Press; Totowa, NJ: 2011. in press
- Stoker AK, Semenova S, Markou A. Affective and somatic aspects of spontaneous and precipitated nicotine withdrawal in C57BL/6J and BALB/cByJ mice. *Neuropharmacology*. 2008; 54:1223–1232. [PubMed: 18452957]
- Stolerman IP, Jarvis MJ. The scientific case that nicotine is addictive. *Psychopharmacology (Berl)*. 1995; 117:2–10. discussion 14–20. [PubMed: 7724697]
- Stolerman IP, Chamberlain S, Bizarro L, Fernandes C, Schalkwyk L. The role of nicotinic receptor $\alpha 7$ subunits in nicotine discrimination. *Neuropharmacology*. 2004; 46:363–371. [PubMed: 14975691]
- Tritto T, McCallum SE, Waddle SA, Hutton SR, Paylor R, Collins AC, Marks MJ. Null mutant analysis of responses to nicotine: deletion of $\beta 2$ nicotinic acetylcholine receptor subunit but not $\alpha 7$ subunit reduces sensitivity to nicotine-induced locomotor depression and hypothermia. *Nicotine Tob Res*. 2004; 6:145–158. [PubMed: 14982698]
- Walters CL, Brown S, Changeux JP, Martin B, Damaj MI. The $\beta 2$ but not $\alpha 7$ subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology (Berl)*. 2006; 184:339–344. [PubMed: 16416156]
- Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, Lindstrom J. Chronic nicotine treatment up-regulates human $\alpha 3\beta 2$ but not $\alpha 3\beta 4$ acetylcholine receptors stably transfected in human embryonic kidney cells. *J Biol Chem*. 1998; 273:28721–28732. [PubMed: 9786868]
- Watkins SS, Stinus L, Koob GF, Markou A. Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. *J Pharmacol Exp Ther*. 2000; 292:1053–1064. [PubMed: 10688623]
- Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, Beaudet A, Heinemann SF, Balogh SA. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. *Neuroscience*. 2004; 129:11–24. [PubMed: 15489024]
- Wei ZL, Xiao Y, Yuan H, Baydyuk M, Petukhov PA, Musachio JL, Kellar KJ, Kozikowski AP. Novel pyridyl ring C5 substituted analogues of epibatidine and 3-(1-methyl-2(S)-pyrrolidinylmethoxy)pyridine (A-84543) as highly selective agents for neuronal nicotinic acetylcholine receptors containing $\beta 2$ subunits. *J Med Chem*. 2005; 48:1721–1724. [PubMed: 15771418]
- Winzer-Serhan UH, Leslie FM. Codistribution of nicotinic acetylcholine receptor subunit $\alpha 3$ and $\beta 4$ mRNAs during rat brain development. *J Comp Neurol*. 1997; 386:540–554. [PubMed: 9378850]
- World Health Organization. [Accessed 20 April 2011] WHO report on the global tobacco epidemic. 2009. http://whqlibdoc.who.int/publications/2009/9789241563918_eng_full.pdf
- Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, Patrick JW, Role LW, Beaudet AL, De Biasi M. Multiorgan autonomic dysfunction in mice lacking the $\beta 2$ and the $\beta 4$ subunits of neuronal nicotinic acetylcholine receptors. *J Neurosci*. 1999; 19:9298–9305. [PubMed: 10531434]

Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, Sharkey J. Nicotine improves sustained attention in mice: evidence for involvement of the $\alpha 7$ nicotinic acetylcholine receptor. *Neuropsychopharmacology*. 2004; 29:891–900. [PubMed: 14970827]

Fig. 1.

Effects of chronic administration of nicotine (40 mg/kg/day base, 28 days) or saline on ICSS thresholds in $\alpha 7$ (a) and $\beta 4$ (b) knockout and wildtype mice. The data (mean $\pm$ SEM) are expressed as a percentage of baseline thresholds (see text for details). No statistically significant effects were observed. The effects of nicotine administration on ICSS thresholds are not shown for days 9–15 in this figure because during these days the effects of acute administration of various mecamylamine doses on ICSS thresholds were assessed using a Latin square design (see text for details)

Fig. 2.

Effects of mecamylamine-precipitated nicotine (40 mg/kg/day base, 28 days) or saline withdrawal on ICSS thresholds in $\alpha 7$ (a) and $\beta 4$ (b) knockout and wildtype mice. The data (mean $\pm$ SEM) are expressed as a percentage of baseline thresholds (see text for details). Asterisks indicate significant differences between nicotine- and saline-treated mice within the same genotype (* $P < 0.05$, *** $P < 0.001$). Pound signs indicate significant differences between nicotine-treated $\alpha 7^{-/-}$ and $\alpha 7^{+/+}$ mice (## $P < 0.001$). Delta signs ($\Delta\Delta\Delta P < 0.001$) indicate a significant main effect of *Mecamylamine* in an ANOVA

Fig. 3.

Effects of spontaneous nicotine (40 mg/kg/day base, 28 days) or saline withdrawal on ICSS thresholds in $\alpha 7$ (a) and $\beta 4$ (b) knockout and wildtype mice. The data (mean $\pm$ SEM) are expressed as a percentage of baseline thresholds (see text for details). The *pound sign* in (a) indicates a significant main effect of *Nicotine/Saline Exposure* ($\#P < 0.05$) in a three-way repeated-measures ANOVA. The *asterisk* indicates a significant difference between nicotine- and saline-treated wildtype mice in a *t* test ($*P < 0.05$). The *pound sign* in (b) indicates a significant difference between nicotine-withdrawing $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice ($\#P < 0.05$) in a *t* test

Fig. 4.

Effects of spontaneous withdrawal from nicotine (40 mg/kg/day base, 28 days) or saline on somatic signs in $\alpha 7$ (a) and $\beta 4$ (b) knockout and wildtype mice. The data are expressed as the mean number of somatic signs $\pm$ SEM. Asterisks indicate significant differences between nicotine- and saline-treated mice within the same genotype (* $P < 0.05$, ** $P < 0.01$). The pound sign indicates a significant main effect of *Nicotine/Saline Exposure* ($^{\#}P < 0.05$)

Fig. 5.

Effects of spontaneous nicotine withdrawal (80 mg/kg/day base, 28 days) or saline on somatic signs in $\beta 4^{-/-}$ and $\beta 4^{+/+}$ mice. The data are expressed as the mean number of somatic signs $\pm$ SEM. Asterisks indicate significant differences between groups linked by the horizontal lines (* $P < 0.05$, ** $P < 0.01$)

Table 1

Mean baseline thresholds (μ A) of the experimental groups used to express the data as percent of baseline

Experimental group	Nicotine/saline administration	Nicotine/saline withdrawal
$\alpha 7^{+/+}$ saline	76.36 $\pm$ 5.44	63.44 $\pm$ 3.65
$\alpha 7^{+/+}$ nicotine	74.34 $\pm$ 4.46	62.86 $\pm$ 6.94
$\alpha 7^{-/-}$ saline	77.21 $\pm$ 7.04	61.33 $\pm$ 5.03
$\alpha 7^{-/-}$ nicotine	71.91 $\pm$ 7.91	56.22 $\pm$ 4.28
$\beta 4^{+/+}$ saline	85.40 $\pm$ 7.44	68.36 $\pm$ 7.91
$\beta 4^{+/+}$ nicotine	84.96 $\pm$ 7.07	78.10 $\pm$ 7.18
$\beta 4^{-/-}$ saline	93.71 $\pm$ 8.29	87.18 $\pm$ 11.71
$\beta 4^{-/-}$ nicotine	97.57 $\pm$ 10.33	99.98 $\pm$ 13.08

Data are expressed as mean $\pm$ SEM