Published in final edited form as:

Psychopharmacology (Berl). 2015 April ; 232(7): 1207–1217. doi:10.1007/s00213-014-3754-4.

Contributions of $\beta 2$ subunit-containing nAChRs to chronic nicotine-induced alterations in cognitive flexibility in mice

Robert D. Cole, Rachel L. Poole, Dawn M. Guzman, Thomas J. Gould, and Vinay Parikh^{*}
Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122

Abstract

Rationale—Deficits in executive functions underlie compulsive drug use and understanding how nicotine influences these cognitive processes may provide important information on neurobiological substrates of nicotine addiction. Accumulating evidence suggests that $\beta 2$ subunit-containing nicotinic receptors (nAChRs) are involved in the reinforcing process of nicotine addiction. Whether these nAChRs also contributes to the detrimental effects of chronic nicotine on flexible decision-making is not known.

Objectives—In the present study, the effects of chronic nicotine were assessed in mice with partial or complete deletion of the $\beta 2$ -subunit containing nAChR gene ($\beta 2+/-$ or $\beta 2-/-$) performing an operant cognitive flexibility task.

Results—Visual discrimination learning was not affected in saline-treated $\beta 2$ nAChR mutants as compared to the wild-type ($\beta 2+/+$) mice; yet, chronic nicotine facilitated acquisition of visual discrimination in all genotypes. The acquisition of new egocentric response strategy set-shifting remained similar in all genotypes and there was no effect of treatment. Chronic nicotine treatment impaired reversal learning in $\beta 2+/+$ mice by increasing response perseveration to the previously rewarded stimulus. Moreover, the acquisition of inverted stimulus-reward contingencies did not differ between $\beta 2+/+$ and $\beta 2-/-$ mice exposed to chronic nicotine. Interestingly, nicotine-induced reversal learning deficits were not observed in $\beta 2+/-$ mice.

Conclusions—Collectively, these findings suggest that $\beta 2$ subunit-containing nAChRs are not critical for visual discrimination learning and extradimensional rule shift. However, sustained activation of these nAChRs with nicotine may interfere with inhibitory control processes influencing affective shifts in stimulus-reward contingencies.

Keywords

nicotine; nAChRs; cognitive flexibility; addiction; mice

^{*}Corresponding author: Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122. Phone: 215-204-1572, vinay.parikh@temple.edu.

Conflicts of Interest: None

Introduction

Tobacco use continues to be one of the leading causes of preventable death worldwide and is considered a global health issue. Nicotine is one of the primary psychoactive ingredients associated with tobacco products and is also considered to largely contribute to developing addictive behaviors related to cigarette smoking. Although there are a large number of treatments targeted at smoking cessation, smokers attempting to quit continue to experience high rates of relapse (Hughes et al. 2008). It is therefore pivotal that research continues to focus on the specific neural mechanisms of nicotine addiction.

Nicotine exerts its physiological and behavioral effects by binding to nicotinic receptors (nAChRs), which are a family of ligand-gated ionotropic receptors mediating fast synaptic transmission throughout the central nervous system. Neuronal nAChRs consists of heteropentameric assemblies of $\alpha 2$ - $\alpha 6$ and $\beta 2$ - $\beta 4$ subunits, and homopentameric assemblies of $\alpha 7$ -10 subunits (Gotti et al. 2006; Dani and Bertrand 2007). Specifically, $\beta 2$ subunit-containing nAChRs are known to modulate reward-motivated behaviors and may play a critical role in nicotine addiction (Changeux 2010). For example, $\beta 2$ nAChR null mutant mice did not self-administer nicotine (Picciotto et al. 1998); however, re-expression of this receptor subunit restored the nicotine self-administration response (Pons et al. 2008). Rewarding effects of nicotine tested using the conditioned place preference paradigm were also not observed in mice lacking the $\beta 2$ subunits of nAChRs (Walters et al. 2006). Extensive evidence suggests that the reinforcing effects of nicotine primarily involve the activation of mesolimbic dopamine (DA) neurons in the ventral tegmental area (VTA) and consequent release of DA in striatal synapses via the high-affinity $\alpha 4\beta 2$ nAChRs (Dani and De Biasi 2001; Laviolette and van der Kooy 2004; Leslie et al. 2013). The presence of other $\beta 2$ subunit-containing nAChRs such as $\alpha 6\alpha 4\beta 2$ and $\alpha 6\beta 2$ in the VTA has also been reported; these receptors persistently activate mesolimbic DA neurons by nicotine at smoking relevant concentrations (Grady et al. 2012; Liu et al. 2012) and produce nicotine-induced reinforcement (Pons et al. 2008). Previous animal and human research indicates that chronic nicotine-induced neuroadaptations underlying dependence involves desensitization and long-lasting upregulation of $\alpha 4\beta 2$ nAChRs contributing to heightened nicotine reward sensitivity and increase relapse vulnerability (Kenny and Markou 2006; Cosgrove et al. 2009; Hilario et al. 2012). These findings suggest that $\beta 2$ -containing nAChRs contribute to the rewarding and reinforcing effects of nicotine.

In addition to modulating reward circuitry, stimulation of $\alpha 4\beta 2$ nAChRs in other cortical-subcortical regions, such as the prefrontal cortex (PFC) and hippocampus, is linked to alterations in cognitive processes including attention (McGaughy et al. 1999; Howe et al. 2010; Guillem et al. 2011), working memory (Levin et al. 2002; Chan et al. 2007) and contextual fear learning (Kenny and Markou 2006; Portugal et al. 2008). Due to the nature of overlap and interactions between the brain circuits underlying addiction and cognition, it is believed that these competing overlapping circuits produce an internal conflict between the rewarding effects of drugs and cognitive processes that suppress the urge to take drugs (Everitt and Robbins 2005; Robinson and Berridge 2008; Gould 2010). Disruptions in these converging circuits may lead to the loss of cognitive control and produce maladaptive patterns of drug use in addicts (Kalivas and Volkow 2005; Volkow et al. 2011). Because $\beta 2$

subunit-containing nAChRs are vastly distributed in these circuits (Gotti et al. 2007; Feduccia et al. 2012), it is important to determine the contribution of these nAChR subtypes in chronic nicotine-induced alterations in cognitive control.

Cognitive flexibility is defined as the ability to switch strategic responses adaptively between changing environmental stimuli, and is a fundamental component of fronto-executive function used to probe cognitive dysfunction in addiction (Stalnaker et al. 2009; Izquierdo and Jentsch 2012). We previously demonstrated that chronic nicotine treatment impaired reversal learning in C57BL/6J mice (Ortega et al. 2013). In the present study, the effects of chronic nicotine were examined in mice lacking the $\beta 2$ -subunit containing nAChRs performing an operant cognitive flexibility task. This task required the animals to progress sequentially through multiple phases including visual discrimination, set-shifting and reversal learning. Because the upregulation of nAChRs is linked to nicotine addiction (Govind et al., 2009), and it is not possible to determine whether nicotine-induced overactivation of $\beta 2$ subunit-containing nAChRs produce cognitive control deficits in null mutants, we utilized $\beta 2$ nAChR heterozygous mice to test this hypothesis. Our results indicate that $\beta 2$ nAChRs are neither involved in nicotine-induced facilitation of discrimination learning nor contribute to strategy set-shifting. However, $\beta 2$ subunit-containing nAChRs do partially contribute to nicotine-induced impairments in reversal learning.

Materials and Methods

Animals and Genotyping

A mutant mouse line with the targeted deletion of the $\beta 2$ -subunit containing nAChR gene was originally generated at the Baylor College of Medicine (Xu et al., 1999). Chimeric mice were backcrossed for seven generations on the C57BL/6J background. A breeding pair of $\beta 2$ nAChR heterozygous mice was generously provided by Dr. Arthur Beaudet (Baylor College of Medicine) and maintained on C57BL/6J mice for three additional generations prior to generating the wild-type ($\beta 2^{+/+}$), heterozygous ($\beta 2^{+/-}$) and knock-out ($\beta 2^{-/-}$) mice. Mice were genotyped by subjecting the DNA extracted from tail biopsies to the PCR using the following primers: $\beta 2$ wild-type forward, 5'-CTCTGACTGTAAAGGCAGTGGTTGCTATAG -3'; $\beta 2$ wild-type reverse, 5'-TAGCTATTGACGACGTCTTTAAGATCC -3'; and $\beta 2$ mutant reverse, 5'-GAGACTAGTGAGACGTGCTACTTCCATTTG -3'. The amplification products were 250 bp for the $\beta 2^{+/+}$, 400 bp for the null mutants, and both 250 and 400 bp for the heterozygous mice (Figure 1A).

Mice were group housed by sex post-weaning in a humidity/temperature controlled colony room with a 12h light/dark cycle (lights on at 07:00). One week prior to the beginning of experiments, wild-type and $\beta 2$ nAChR mutant mice of either sex were individually housed and progressively water-restricted to 5-min of water/day. Animals were 8-9 weeks (weight: 20-24 g) old at the beginning of the task onset. All behavioral training and testing took place 7 days/week between 9:00 and 16:00 h. At the completion of each behavioral session, mice received 5 min of water in addition to sweetened water received as a reward for each correct response (see below). Food (PMI LabDiet) was available *ad libitum* throughout the

experiment. All experimental procedures were approved by the Institutional Care and Use Committee (IACUC) of Temple University and were in accordance with the National Institute of Health guidelines.

Mouse Operant Procedure

Mouse operant conditioning chambers (MED Associates; St. Albans, VT, USA) equipped with a standard grid floor and house light (28V, 100mA), and a panel consisting of two large cue lights (2.5cm; 28V, 100mV), a central reward port attached to a fluid dipper, and two ultra-sensitive retractable levers were used. Control of all events, including light presentation, lever operations, and reward delivery, utilized a SmrtCtrl interface running MED-PC IV software on Dell PC (Optiplex 960).

Mice were trained in an automated operant cognitive flexibility task as described previously (D'Amore et al. 2013; Ortega et al. 2013). This task places heavier emphasis on response conflicts and shares features similar to the operant tasks previously established to assess cognitive flexibility in rodents (Floresco et al. 2008; Haluk and Floresco 2009; Brigman et al. 2010; Scheggia et al. 2014). Briefly, animals were autoshaped on a FR-1 schedule of reinforcement to acquire the lever press response and subsequent reward (10 μ l of .066% saccharin solution). After attaining at least 30 lever press responses with a 30-min session for three consecutive days, animals advanced to a pretraining phase that involved responding to an activated lever randomly associated with an unpredictably occurring illumination of the panel light within 10s to get the reward. Mice were implanted with mini-osmotic pumps for chronic drug delivery (see below) after reaching pretraining criterion (30 rewards and 10% omissions).

Mice that retained criterion following recovery were progressed sequentially to three different phases of training; visual discrimination, strategy set-shifting, and reversal learning (see Figure 1B for schematic diagram of experiment). During the visual discrimination phase, mice were required to discern the lever with an activated cue light. The session began with the illumination of the house light and presentation of 30 trials with an ITI of 9 ± 3 s. All trials were started with the illumination of 7s visual cue (either from the left or right panel), followed by the presentation of both levers 2s later. Levers were present for 5s and both the stimulus light and levers were co-terminated. A lever press response on the cued lever was scored as a "correct response" and was followed by reward delivery. Responses on the incorrect lever were not rewarded and resulted in a "timeout" (negative punishment) phase characterized by a 10s extinguishing of the house light. Punishment on incorrect responses was introduced to discourage indiscriminate responding to levers. It is important to note that the ITI and punishment durations used for this study were selected to allow the animals to clearly distinguish between correct trials (i.e., reward + ITI) and incorrect trials (i.e., time out + ITI). The house light remained illuminated throughout the entire session except for the punishment phase. Animals trained to criterion (80% correct responses for 3 consecutive days) were advanced to the next phases of training.

During the set-shifting phase, experimental parameters remained identical to the previous phase except that the contingencies were altered in such a way that the animals were required to adopt an egocentric response strategy to achieve rewards. Animals were required

to press the correct lever (either left or right; lever assignment was counterbalanced within a group) to earn a reward irrespective of cue presentation which remained pseudorandom. Mice that successfully attained criterion at this stage of training were moved to the reversal learning phase. During this phase of training, the proprioceptive rule was reversed; animals were required to press the lever opposite of the one assigned to them during preceding phases irrespective of the position of the activated cue light. Daily training on 30-trial sessions continued until the animals attained criterion by exhibiting 80% correct responses for 3 consecutive days on both the strategy set-shifting and reversal learning phases of the task.

Behavioral Measures

The number of correct responses, errors, omissions, response latencies and reward retrieval latencies was obtained for each behavioral session. Response accuracies were calculated for each session using the formula: correct responses/(correct+incorrect responses)*100. The total number of performed trials to criterion, errors to criterion and omissions were obtained for each training phase using the above described criteria. Strategy shifting performance was characterized by distinguishing whether an incorrect response occurred due to the perseverance of a previously learned strategy or failure to acquire/maintain a new strategy. Errors were classified as perseverative, regressive and never-reinforced based on criterion reported in earlier studies (Floresco et al. 2008; Howe et al. 2010; D'Amore et al. 2013). A perseverative error occurred if the animal responded to the incorrect lever when the visual cue was illuminated above it on 60% of trials within a session. Depending on the training performance in the preceding session, an error was scored as a regressive error if the animal made < 60% incorrect responses in subsequent sessions. At this point, the animals were making fewer errors and considered to be inhibiting the previously learned strategy and acquiring the new strategy. Never-reinforced errors occur if an animal respond on the incorrect lever while the visual cue is presented from the opposite side. Both regressive and never-reinforced errors were categorized as “learning errors” as they reflected an index of the acquisition/execution of a new strategy. Errors during the reversal learning phase occur when the animal responded on the lever opposite to the assigned one. Reversal errors were classified as perseverative if the animals made 60% incorrect lever presses on performed trials in a session. If the animals made < 60% incorrect responses, errors were scored as regressive (i.e. learning errors) in all subsequent sessions.

Chronic nicotine administration and experiments

Once the animals attained pretraining criterion, as described above, they were randomly assigned either to the saline or nicotine group. Twenty-four hours prior to surgeries, mice were given *ad libitum* water to prevent dehydration. Mini-osmotic pumps (model 1004, DURECT Corp., Cupertino, CA) designed to administer drug/vehicle solution at a rate of 0.11µL/h for 28 days were used. Pumps were filled with 100µL of either sterile saline or nicotine hydrogen tartrate (Sigma Co, St. Louis, MO) dissolved in saline, and implanted subcutaneously in isoflurane-anesthetized mice under aseptic surgical conditions. Nicotine was administered at a dose of 18mg/kg/day (reported as free base) previously reported to desensitize nAChRs (Turner et al. 2010; Turner et al. 2013; Wilkinson et al., 2013) and produce reversal learning deficits in mice (Ortega et al. 2013).

Pretraining was resumed 48 hrs after recovery. Once animals regained criterion performance at this phase (2-4 days), they were progressed through the subsequent stages of the task (i.e. visual discrimination, strategy set-shift, and reversal learning). Osmotic pumps remained implanted throughout the duration of the task that varied between 17-24 days across animals following the onset of visual discrimination training. One osmotic pump was sufficient to deliver nicotine throughout the acquisition of three task stages. Immediately following the last session of the task, blood samples were collected through intra-cardiac puncture and analyzed via a cotinine ELISA kit. A total of 52 mice ($n = 7-11$ /genotype/treatment group; see Table I for sample sizes per group) received osmotic minipumps and acquired all the stages of the task.

Cotinine ELISA

Cotinine, a major metabolite of nicotine, is used to quantify tobacco exposure due to its stability and longer half-life than nicotine (Jarvis et al. 1988; Benowitz 1996; Hatsukami et al. 2007). Immediately following the last session of the task, blood samples were collected through intra-cardiac puncture from mice randomly selected from each group ($n = 3-5$ /genotype/treatment group) to measure serum cotinine levels. Blood was allowed to coagulate for 30 min at room temperature following which samples were centrifuged at $3000 \times g$ for 10-min to collect the serum. Isolated serum was stored at -80°C until further analysis. To determine whether nicotine exposure differed between the earlier and later task stages, we also analyzed serum cotinine levels following 7-days of nicotine treatment. For this experiment, a separate cohort of naïve $\beta 2^{+/+}$ mice ($n = 4$) received nicotine-containing mini-osmotic pumps and blood samples were collected 7-days later. Serum cotinine levels were quantified using an ELISA kit (Orasure Technology) as per recommendations from the manufacturer.

Statistical Analysis

Statistical analysis was performed with SPSS/PC+V21.0 (IBM SPSS Software, Armonk, NY). Data for trials to criterion, errors to criterion, error types, omissions, correct and incorrect response latencies, and cotinine levels were analyzed using Two-way ANOVA using treatment (two levels; nicotine or saline) and genotype (three levels: $\beta 2^{+/+}$, $\beta 2^{+/-}$, or $\beta 2^{-/-}$) as between-subject variables. As prior statistical analysis did not indicate a main effect of sex (trials to criterion: $F(1,40) < 1.22$, $p > 0.27$ all stages; errors to criterion: $F(1,40) < 1.67$, $p > 0.20$ all stages) or sex $\times$ genotype interaction (trials to criterion: $F(2,40) < 0.71$, $p > 0.50$ all stages; errors to criterion: $F(2,40) < 0.88$, $p > 0.41$ all stages), data between sexes were aggregated across all groups to increase the statistical power. Learning analyses were conducted by comparing response accuracies using mixed-factor repeated measures ANOVA with training sessions as the within-subject factor, and treatment and genotype as the between-subject factors. For these analyses, response accuracy data for the first 5 training sessions was compared for each stage of the task. *Post hoc* comparisons were performed using the Tukey's honest significant difference (*HSD*) test. For all statistical tests, p values ≤ 0.05 were considered significant.

Results

Serum cotinine levels

Due to performance variability, the duration of chronic nicotine exposure on average varied between 3-4 weeks following the acquisition of all three stages of the task. Serum cotinine concentration was $131.35, \pm 1.47$ ng/mL in $\beta 2^{+/+}$ mice at the completion of behavioral testing. These values fall within the range of 100-200 ng/mL estimated in active smokers who consumes >3 cigarettes/day (Benowitz 1996). Moreover, serum cotinine levels estimated after the completion of the study in chronic nicotine-treated $\beta 2^{+/+}$ mice remained similar to cotinine levels assessed in wild-type mice that received nicotine for 7-days ($F(1,14)=1.36, p=0.26$). These data indicate that steady state nicotine concentration was presumably attained using subcutaneous mini-osmotic implants within a week. Absence of any difference in serum cotinine between the genotypes ($F(2,14)=0.85, p=0.45$) reflect that the rate of nicotine metabolism was not influenced by the level of $\beta 2$ -subunit containing nAChR expression. Serum cotinine levels in saline-treated animals remained below detectable levels (<14 ng/mL).

Nicotine-induced facilitation of visual discrimination learning does not involve $\beta 2$ -subunit containing nAChRs

Chronic nicotine-treated mice required reduced number of trials to attain visual discrimination criterion as compared to saline-treated mice (main effect: $F(1,46)=13.33, p<0.001$; Figure 2A). Moreover, nicotine-exposed animals committed less errors (main effect: $F(1,46)=8.87, p=0.005$ vs. saline; Figure 2B). Trials to criterion and incorrect responses did not differ by genotypes (main effect for trials: $F(2,46)=0.29, p=0.75$; main effect for errors: $F(2,46)=0.17, p=0.85$) nor there was a significant interaction with treatment (genotype $\times$ treatment interaction; trials: $F(2,46)=0.08, p=0.93$; errors: $F(2,46)=0.31, p=0.74$). The rate at which animals acquired visual discrimination during the first five discrimination training sessions was also examined by analyzing response accuracies. Repeated-measure ANOVAs show a main effect of training sessions illustrating effective discrimination learning ($F(4,184)=21.75, p<0.001$). As expected, response accuracy remained significantly higher in nicotine-treated animals as compared to the saline group (means for main effect; nicotine: $87.8\%, \pm 2\%$; saline: $78.6\%, \pm 2\%$; $F(1,46)=10.37, p=0.002$). However, learning rates remained similar by genotype ($F(2,46)=0.15, p=0.86$) and this effect did not interact with the training sessions (genotype $\times$ session interaction: $F(8,184)=1.13, p=0.35$). These data imply that $\beta 2$ subunit-containing nAChRs are not critical for visual discrimination performance. Moreover, these receptors do not appear to contribute to nicotine-induced facilitation of discrimination learning. Nicotine-treated mice omitted less trials than saline-treated mice (nicotine: $1.22, \pm 0.28$; saline: $4.76, \pm 1.39$; $F(1,46)=5.64, p=0.02$) plausibly reflecting slightly higher motivation to pay attention to the discriminative stimulus in the former. Average latencies for correct responses and errors did not differ between saline and nicotine-treated mice (correct response latency: ($F(1,46)=2.41, p=0.12$); incorrect response latencies: ($F(1,46)=0.36, p=0.55$). We did not find a main effect of genotype, nor a genotype $\times$ treatment interaction for omissions or response latencies (all main effects and interactions; $F<2.15; p>0.12$).

$\beta 2$ nAChR mutants and the acquisition of strategy set-shifting

All animals, regardless of the treatment or genotype, required similar number of trials to attain 80% correct responses for 3 consecutive days on the set-shifting phase (main effect of treatment: $F(1,46)=1.17$, $p=0.29$; main effect of genotype: $F(2,46)=1.75$, $p=0.19$; genotype $\times$ treatment: $F(2,46)=1.38$, $p=0.26$). Session-dependent learning during the set-shift procedure remained similar between the genotypes with increased response accuracies as the training progressed ($F(4,184)=213.13$, $p<0.001$). Learning rates remained unaffected by treatment ($F(1,46)=0.64$, $p=0.42$) and genotype ($F(2,46)=0.78$, $p=0.46$) and the two factors did not interact (treatment $\times$ genotype interaction: $F(8,184)=0.98$, $p=0.38$). These data are supported by error analysis, which showed that total errors committed during strategy set-shifting remained similar between saline and nicotine treated animals ($F(1,46)=1.01$, $p=0.32$). Moreover, set-shift errors neither differed by genotype ($F(2,46)=1.22$, $p=0.30$) nor did this effect interact with treatment ($F(2,46)=1.43$, $p=0.25$). Incorrect responses collapsed by error types also show similar results for perseverative errors (main effect of treatment: $F(1,46)=1.17$, $p=0.29$; main effect of genotype: $F(2,46)=0.26$, $p=0.77$; treatment $\times$ genotype interaction: $F(2,46)=1.56$, $p=0.22$) and learning errors (main effect of treatment: $F(1,46)=0.06$, $p=0.81$; main effect of genotype: $F(2,46)=2.49$, $p=0.09$; treatment $\times$ genotype interaction: $F(2,46)=2.18$, $p=0.13$). Latencies for correct and incorrect responses, and omissions during the set-shifting phase remained unaffected between genotypes and treatment ($p>0.05$; all main effects and interactions). Collectively, these data indicate that partial or complete deletion of the gene for $\beta 2$ subunit-containing nAChRs does not affect strategy shifting performance.

Nicotine-induced reversal learning deficits were attenuated in $\beta 2$ nAChR heterozygous mice

The main results on reversal learning performance are summarized in Figure 3. A 2×2 ANOVA on trials to criterion revealed no significant main effects of treatment ($F(1,46)=415$, $p=0.52$) or genotype ($F(2,46)=1.04$, $p=0.36$), but a significant interaction was found between the two factors (treatment $\times$ genotype interaction: $F(2,46)=3.26$, $p=0.04$). Although *post hoc* analysis using Tukey's HSD test could not locate the source of this interaction, we did observe a trend towards higher number of trials to criterion in nicotine-treated $\beta 2^{+/-}$ mice ($p=0.09$ vs saline-treated $\beta 2^{+/+}$ mice; Figure 3A).

Response accuracies for training sessions of all genotypes treated with saline or nicotine showing session-dependent learning are depicted in Figures 3B-D (main effect of training sessions: $F(4,184)=325.54$, $p<0.001$). Although, learning rates did not differ by genotype ($F(2,46)=2.05$, $p=0.14$) or treatment ($F(1,46)=0.72$, $p=0.40$), mixed factor ANOVAs revealed a significant interaction between the two factors ($F(2,46)=5.89$, $p=0.005$). A closer examination of response accuracy data shows a rightward shift of the learning curve in nicotine-treated wild-type mice reflecting reversal learning impairments. Average response accuracies were significantly lower in nicotine-treated $\beta 2^{+/+}$ animals as compared to the saline animals (saline: 64.9%, $\pm 2.9\%$; nicotine: 51.3%, $\pm 2.8\%$; $p=0.003$). This effect presumably may be interpreted to reflect difficulty in shifting strategies rather than deficits in the *rate* of incremental learning as session-dependent increases in response accuracy did not interact with the genotype (session $\times$ genotype interaction: $F(4,184)=0.33$, $p=0.95$).

Slower task acquisition was not observed in $\beta 2$ nAChR heterozygous mice that received chronic nicotine treatment ($p=0.99$ vs saline- and $p=0.003$ vs nicotine-treated wild-type mice); in fact, learning performance was marginally better in these mice as compared to the saline-treated $\beta 2+/-$ mice ($p=0.1$; Figure 3C). On the other hand, response accuracies in $\beta 2-/-$ mice treated with nicotine remained similar to $\beta 2+/+$ mice given a similar treatment ($p=0.28$). Moreover, learning curves of saline and nicotine-treated $\beta 2-/-$ mice seemed to overlap with each other (Figure 3D).

In terms of reversal errors, statistical analysis show no main effects of treatment ($F(1,46)=0.62$, $p=0.42$) or genotype ($F(2,46)=1.11$, $p=0.34$), but an interaction between the two variables ($F(2,46)=3.98$, $p=0.02$). Multiple comparisons revealed that nicotine-treated $\beta 2+/+$ animals committed more errors than their saline-treated $\beta 2+/+$ counterparts (nicotine: 69.18 , ± 4.84 ; saline: 50.2 , ± 5.07 ; $p=0.04$; Figure 3E). However, this effect was not observed in chronic nicotine-treated $\beta 2+/-$ mice ($p=0.97$ vs saline-treated $\beta 2+/+$ mice). Additionally, reversal errors remained substantially lower in nicotine-exposed heterozygous mice as compared to the wild-type animals that received similar treatment ($p=0.02$) indicating that the former actually benefited from nicotine. Reversal errors in $\beta 2$ null mutants remained similar to the wild-type mice exposed to chronic nicotine ($p=0.69$). A separate analysis on different types of reversal errors showed that chronic nicotine increased perseverative errors reflecting impairments in the ability to acquire previously learned strategy. This effect was primarily observed in the $\beta 2+/+$ and $\beta 2-/-$ mice (see Figure 3F for multiple comparisons). Again, perseverative errors remained substantially lower in nicotine-treated mice $\beta 2+/-$ mice exposed to nicotine ($p<0.001$) further explaining the lack of detrimental effects of nicotine on reversal learning. Regressive errors on the other hand show a different trend. Nicotine treatment significantly reduced regressive errors (main effect: $F(1,46)=5.15$, $p=0.02$). Moreover, the effect of genotype on this behavioral measure and its interaction with treatment approached significance (main effect: $F(2,46)=3.00$, $p=0.06$; genotype $\times$ treatment interaction: $F(2,46)=2.61$, $p=0.08$). It is evident from Figure 3G that saline-treated $\beta 2$ nAChR mutants committed more regressive errors during the reversal phase reflecting difficulty in acquiring new reward contingency. Chronic nicotine exposure seemed to attenuate the effect of $\beta 2$ nAChR genotype on regressive errors.

Analysis of omissions for reversal learning showed no main effect of treatment ($F(1,46)=0.01$, $p=0.99$) or genotype ($F(2,46)=0.08$, $p=0.92$), but yielded a significant interaction ($F(2,46)=3.87$, $p=0.02$). Despite the fact that multiple comparisons did not attain significance presumably due to higher variability in the data, it is interesting to note that nicotine-treated $\beta 2+/+$ mice exhibited a trend towards higher omissions ($p=0.09$ vs saline-exposed $\beta 2+/+$ mice; Figure 3H). On the other hand, an inverse trend was noted for the nicotine-treated $\beta 2-/-$ nAChR mice; omissions remained marginally lower in these animals as compared to wild-type mice that received a similar treatment ($p=0.1$). Absence of treatment and genotype effects on the speed of responding indicate that alterations in omissions were not influenced by sensorimotor processing (correct response latencies; main effects and interactions: $F<2.18$; $p>0.12$; incorrect response latencies; main effects and interactions $F<1.62$; $p>0.21$)

Discussion

We utilized transgenic mice to determine the contribution of $\beta 2$ subunit-containing nAChRs to chronic nicotine-induced alterations in cognitive flexibility. Mice were exposed to either nicotine or saline through the use of subcutaneous mini-osmotic pumps, and progressed through multiple stages of an operant task to receive reinforcement by a) visually discriminating the stimulus, b) switching to a response strategy while ignoring the visual stimulus, and c) reversing the previously acquired strategy. We show that nicotine facilitates visual discrimination and $\beta 2$ subunit-containing nAChRs do not seem to play a critical role in this effect. The ability to shift from a visual cue-based strategy to a new egocentric response strategy remained unaltered in $\beta 2^{+/-}$ and $\beta 2^{-/-}$ mice. Although, chronic nicotine did not influence strategy shifting, it impaired reversal learning by increasing the prepotent responding to the previously rewarded stimulus. Deficits in the acquisition of rule reversal were not observed in chronic nicotine-treated $\beta 2^{+/-}$ mice; but these cognitive impairments persisted in $\beta 2$ nAChR null mutants.

Faster acquisition of visual discrimination training criterion in chronic nicotine-treated $\beta 2^{+/+}$ mice replicated our previous data that chronic nicotine facilitate this form of learning (Ortega et al. 2013). There is a plethora of evidence indicating that acute or subchronic nicotine administration exerts beneficial effects on attentional processes by activating nAChRs in rodents (Stolerman et al. 2000; Newhouse et al. 2004; Young et al., 2004; Pattij et al., 2007; Semenova et al. 2007; Amitai and Markou 2009; Young et al., 2013). As attention serves as an important variable in discrimination learning by expediting the selection of goal-relevant stimuli from the environment (Lovejoy 1965; Muir 1996), our data support the view that chronic nicotine may enhance fundamental attentional operations. However, visual discrimination learning was not impacted in $\beta 2^{+/-}$ and $\beta 2^{-/-}$ mice. Moreover, nicotine-induced facilitation of this form of learning persisted in these mutants. These data reflect that perhaps activation of $\beta 2$ subunit-containing nAChRs is not mandatory for performance in discrimination learning tasks that engages bottom-up attentional processes to support perceptual learning. This interpretation is supported by the idea that activation of $\alpha 4\beta 2$ nAChRs, support top-down attentional processes and not performance under conditions requiring lower attentional demands (Sarter and Paolone 2011, Demeter and Sarter 2013, Howe et al., 2013). Previous studies have demonstrated that mice with complete elimination of either the $\alpha 7$ homomeric or $\alpha 5$ subunit-containing nAChRs show impaired visual attention (Young et al. 2004; Young et al. 2007; Bailey et al. 2010). Therefore, nicotine-induced activation of non- β subunit-containing nAChRs may still facilitate performance under conditions that do not tax attentional processes.

Attentional set-shifting functions are known to be affected with chronic psychostimulant exposure (Beveridge et al. 2008; Featherstone et al. 2008). In our study, these cognitive capacities were assessed using a strategy shifting paradigm that required mice to divert attention from one stimulus dimension (visual cue) that previously predicted reward to a new dimension (egocentric spatial location of the lever) to attain reinforcement. This type of behavioral shifting between discrimination strategies engages attentional set-shifting functions (Floresco et al. 2006; Ragozzino 2007). In line with our previous study, chronic nicotine exposure did not affect the ability of $\beta 2^{+/+}$ mice to adapt to the new response

strategy. These data are also in agreement with a human study that did not find effects of nicotine dependence on extradimensional set-shifting (Nesic et al. 2011). Additionally, the acquisition of strategy shifting was not influenced by the $\beta 2$ genotype; both saline- and nicotine-treated $\beta 2+/-$ and $\beta 2-/-$ mice suppressed the previously acquired strategy and executed the newer strategy to acquire the task similar to the wild-type mice. These data imply that $\beta 2$ subunit-containing nAChRs are not critical in the formation of attentional sets but may be important for selective processing of task-relevant information under conditions of distractors as noted previously.

Deficits in cognitive control and response inhibition are commonly observed in drug addicts and contribute to compulsive behavior (Dalley et al. 2011; Izquierdo and Jentsch 2012). The reversal learning task is a widely accepted paradigm that is used to measure the ability to abandon previously rewarding contingencies that interfere with newly presented contingencies (Kehagia et al. 2010; Laughlin et al. 2011; McCracken and Grace 2013). In our task, the strategy shifting procedure served as response discrimination and the reinforcement contingencies were inverted during the reversal phase. Acquisition of the reversal of response discrimination was slower in chronic nicotine-treated $\beta 2+/+$ mice. Moreover, these animals committed more perseverative errors than saline-treated $\beta 2+/+$ mice indicating that reversal learning deficits were primarily associated with exacerbation of prepotent responding to the previously reinforced stimulus. Together, these data suggest that chronic nicotine-induced reversal learning deficits reflect disruption of inhibitory control processes under a response-conflict condition. Previous studies that demonstrated behavioral disinhibition with chronic nicotine exposure further support this interpretation (Sacco 2001; Kolokotroni et al. 2012). Since nicotine has also been shown to increase proactive interference (Dunnett and Martel, 1990; Levin et al 1997), we cannot rule out the possibility that nicotine-induced reversal learning deficits might have occurred due to memory transfer from the previous stages of the task.

Chronic nicotine-treated $\beta 2+/-$ mutants did not exhibit reversal learning deficits; unlike $\beta 2+/+$ mice, perseverative responding remained restrained in these animals and they successfully acquired the reversal of response discrimination strategy. In contrast, $\beta 2-/-$ mutants exposed to chronic nicotine displayed higher perseverance to the penultimate strategy similar to $\beta 2+/+$ mice. As noted in the introduction, there have been numerous animal studies indicative of desensitization and compensatory upregulation of $\beta 2$ subunit-containing nAChRs following chronic nicotine exposure (Marks et al. 1985; McCallum et al. 2006). Moreover, human postmortem studies have also shown higher densities of $\alpha 4\beta 2$ nAChRs in the cortex and striatum of smokers (Perry et al. 1999; Staley et al. 2006). Multiple corticostriatal circuits including distinct prefrontal regions such as the orbitofrontal cortex, and discrete regions of the striatum are implicated in reversal learning (Ragozzino 2007; Schoenbaum et al. 2007; Clarke et al. 2008; Darvas and Palmiter 2011), and these circuits are also disrupted in drug addicts (Volkow et al., 2011). Therefore, we speculate that neuroadaptations in frontostriatal circuits produced by chronic nicotine-induced desensitization/upregulation of $\beta 2$ subunit-containing nAChRs may partly contribute to reversal learning deficits. Attenuation of chronic nicotine-induced reversal learning deficits in $\beta 2+/-$ mice might result from the restoration of $\alpha 4\beta 2$ and other $\beta 2$ subunit-containing nAChR densities similar to saline-treated wild-type mice. It is important to note that

increases in epibatidine-binding sites reflecting higher densities of $\alpha 4\beta 2$ nAChRs following chronic nicotine exposure has previously been demonstrated in $\beta 2$ nAChR heterozygous mice; however, receptor upregulation was lower in these animals as compared to nicotine-treated wild-type mice (McCallum et al. 2006). This scenario is consistent with the view that partial blockade of $\alpha 4\beta 2$ nAChRs with drugs like varenicline may offer therapeutic benefits for nicotine dependence (Coe et al. 2005; Crunelle et al. 2010). However, the persistence of reversal learning deficits in chronic nicotine-treated $\beta 2^{-/-}$ mice would suggest that besides $\beta 2$ subunit-containing nAChRs, sustained activation and desensitization of non- $\beta 2$ subunit containing nAChRs may also be involved in these effects. Such an interpretation would still not explain the differences in reversal learning performance between $\beta 2^{+/-}$ and $\beta 2^{-/-}$ mice exposed to chronic nicotine. Therefore, the involvement of potential compensatory changes in $\beta 2^{-/-}$ mice to possibly account for these behavioral effects may not be entirely dismissed.

Saline-treated $\beta 2$ nAChR mutants displayed subtle impairments in the learning/execution of the new strategy during reversal learning. Moreover, $\beta 2^{-/-}$ mice also omitted more trials during this stage of the task. Chronic nicotine administration eliminated genotype-dependent effects on regressive errors illustrating some beneficial learning effects of nicotine in these animals. Additionally, overall omissions that were markedly increased in nicotine-treated $\beta 2^{+/+}$ mice were significantly reduced in $\beta 2$ null mutants. Collectively, these data suggest that endogenous $\beta 2$ nAChR activation may play an essential role in the resolution of conflicting stimulus-response reinforcement strategies that require motivation to reliably execute competing new response patterns. Moreover, chronic nicotine may moderate the consequences of $\beta 2$ nAChR deficiency by supporting motivational and cognitive mechanisms via activation of non- $\beta 2$ nAChRs.

There are some limitations that must not be ignored while interpreting our data. First, the nicotine exposure was different during the acquisition of multiple phases of the task. Although the treatment regimen and the sequence of task stages remained uniform across the genotypes, the rates of nAChR activation and desensitization may be influenced by the duration of nicotine exposure and affect the behavior in a different manner. In an ideal situation, the effect of genotype on performance at each task stage should be assessed after a specific treatment duration period. Second, our interpretation that blood nicotine levels attained a steady state and remained similar throughout the task was based on the observation that cotinine concentration did not differ in serum samples collected from two separate cohorts of animals: a) treated with nicotine for one week, and b) that completed the entire behavioral study (~3-4 weeks). Therefore, we cannot rule out if there were any fluctuations in blood nicotine levels during each task stage. Finally, our sample sizes were too small to reveal any sex effects on performance in the cognitive flexibility task. These data should be treated with caution and studies with a larger number of animals for each sex per genotype should be designed to study sex differences.

To conclude, the presented evidence illustrates that sustained activation of $\beta 2$ subunit-containing nAChRs contributes to chronic nicotine-induced impairments in inhibitory control processes that are critical in reversing a stimulus-reward association. As the value of the reward and punishment stimulus switched during reversals in our paradigm (see Methods), these deficits could also reflect impairments in affective shifting. Attenuation of

nicotine-induced reversal learning deficits in $\beta 2$ +/- mice suggest that a balanced activation of $\beta 2$ subunit-containing nAChRs is required to adjust behavior in response to changing stimulus-reward contingencies. However, impairments in reversal learning persisted in nicotine-exposed mice with complete deletion of $\beta 2$ subunit nAChR gene exemplifying the involvement of other nAChR subtypes in this detrimental effect. These findings are consistent with the view that sustained activation and desensitization states of multiple nAChRs, most likely $\alpha 4\beta 2$ and $\alpha 7$ nAChRs, and functional coordination between nAChR subtypes may contribute to the behavioral effects of chronic nicotine (Besson et al. 2007; Picciotto et al. 2008; Changeux 2010; De Biasi and Dani 2011). Further investigation is required to explore the interplay of multiple nAChR subtypes in producing neuroadaptations in the frontostriatal circuits and how these processes alter executive control during nicotine dependence.

Acknowledgments

The studies were supported by Brain and Behavior Research Foundation (V.P.) and partly by grants from the National Institute of Health DA # 017949 and CA 143187 (T.J.G). D.G was supported by MARC Undergraduate Student Training in Academic Research (NIH 5T34 GM 087239). We thank David Braak for assistance with the genotyping.

References

- Amitai N, Markou A. Chronic nicotine improves cognitive performance in a test of attention but does not attenuate cognitive disruption induced by repeated phencyclidine administration. *Psychopharmacology*. 2009; 202:275–286. [PubMed: 18618099]
- Bailey CD, De Biasi M, Fletcher PJ, Lambe EK. The nicotinic acetylcholine receptor $\alpha 5$ subunit plays a key role in attention circuitry and accuracy. *Journal of neuroscience*. 2010; 30:9241–9252. [PubMed: 20610759]
- Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiologic reviews*. 1996; 18:188–204. [PubMed: 9021312]
- Besson M, Granon S, Mameli-Engvall M, Cloez-Tayarani I, Maubourguet N, Cormier A, Cazala P, David V, Changeux JP, Faure P. Long-term effects of chronic nicotine exposure on brain nicotinic receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:8155–8160. [PubMed: 17470777]
- Beveridge TJ, Gill KE, Hanlon CA, Porrino LJ. Review. Parallel studies of cocaine-related neural and cognitive impairment in humans and monkeys. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2008; 363:3257–3266.
- Brigman JL, Wright T, Talani G, Prasad-Mulcare S, Jinde S, Seabold GK, Mathur P, Davis MI, Bock R, Gustin RM, Colbran RJ, Alvarez VA, Nakazawa K, Delpire E, Lovinger DM, Holmes A. Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. *Journal of Neuroscience*. 2010; 30:4590–4600. [PubMed: 20357110]
- Chan WK, Wong PT, Sheu FS. Frontal cortical $\alpha 7$ and $\alpha 4\beta 2$ nicotinic acetylcholine receptors in working and reference memory. *Neuropharmacology*. 2007; 52:1641–1649. [PubMed: 17482650]
- Changeux JP. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nature Reviews Neuroscience*. 2010; 11:389–401.
- Clarke HF, Robbins TW, Roberts AC. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *Journal of Neuroscience*. 2008; 28:10972–10982. [PubMed: 18945905]
- Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollema H, Lu Y, Mansbach RS, Chambers LK,

- Rovetti CC, Schulz DW, Tingley FD 3rd, O'Neill BT. Varenicline: an $\alpha 4 \beta 2$ nicotinic receptor partial agonist for smoking cessation. *Journal of Medicinal Chemistry*. 2005; 48:3474–3477. [PubMed: 15887955]
- Cosgrove KP, Batis J, Bois F, Maciejewski PK, Esterlis I, Kloczynski T, Stiklus S, Krishnan-Sarin S, O'Malley S, Perry E, Tamagnan G, Seibyl JP, Staley JK. $\beta 2$ -Nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. *Archives of General Psychiatry*. 2009; 66:666–676. [PubMed: 19487632]
- Crunelle CL, Miller ML, Booij J, van den Brink W. The nicotinic acetylcholine receptor partial agonist varenicline and the treatment of drug dependence: a review. *European Neuropsychopharmacology*. 2010; 20:69–79. [PubMed: 19959340]
- D'Amore DE, Tracy BA, Parikh V. Exogenous BDNF facilitates strategy set-shifting by modulating glutamate dynamics in the dorsal striatum. *Neuropharmacology*. 2013; 75:312–323. [PubMed: 23958449]
- Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity, and top-down cognitive control. *Neuron*. 2011; 69:680–694. [PubMed: 21338879]
- Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annual Review of Pharmacology and Toxicology*. 2007; 47:699–729.
- Dani JA, De Biasi M. Cellular mechanisms of nicotine addiction. *Pharmacol Biochem Behav*. 2001; 70:439–446. [PubMed: 11796143]
- Darvas M, Palmiter RD. Contributions of striatal dopamine signaling to the modulation of cognitive flexibility. *Biological Psychiatry*. 2011; 69:704–707. [PubMed: 21074144]
- De Biasi M, Dani JA. Reward, addiction, withdrawal to nicotine. *Annual Review of Neuroscience*. 2011; 34:105–130.
- Demeter E, Sarter M. Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology*. 2013; 64:294–304. [PubMed: 22796110]
- Dunnett SB, Martel FL. Proactive interference effects on short-term memory in rats: I. Basic parameters and drug effects. *Behavioral Neuroscience*. 1990; 104:655–665. [PubMed: 2244974]
- Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*. 2005; 8:1481–1489.
- Featherstone RE, Rizos Z, Kapur S, Fletcher PJ. A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. *Behavioural Brain Research*. 2008; 189:170–179. [PubMed: 18299157]
- Feduccia AA, Chatterjee S, Bartlett SE. Neuronal nicotinic acetylcholine receptors: neuroplastic changes underlying alcohol and nicotine addictions. *Frontiers in Molecular Neuroscience*. 2012; 5:83. [PubMed: 22876217]
- Floresco SB, Block AE, Tse MT. Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural Brain Research*. 2008; 190:85–96. [PubMed: 18359099]
- Floresco SB, Ghods-Sharifi S, Vexelman C, Magyar O. Dissociable roles for the nucleus accumbens core and shell in regulating set shifting. *Journal of Neuroscience*. 2006; 26:2449–2457. [PubMed: 16510723]
- Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. Heterogeneity and complexity of native brain nicotinic receptors. *Biochemical Pharmacology*. 2007; 74:1102–1111. [PubMed: 17597586]
- Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends in Pharmacological Sciences*. 2006; 27:482–491. [PubMed: 16876883]
- Govind AP, Vezina P, Green WN. Nicotine-induced upregulation of nicotinic receptors: underlying mechanisms and relevance to nicotine addiction. *Biochemical Pharmacology*. 2009; 78:756–765. [PubMed: 19540212]
- Gould TJ. Addiction and cognition. *Addiction Science & Clinical Practice*. 2010; 5:4–14. [PubMed: 22002448]
- Grady SR, Wageman CR, Patzlaff NE, Marks MJ. Low concentrations of nicotine differentially desensitize nicotinic acetylcholine receptors that include $\alpha 5$ or $\alpha 6$ subunits and that

mediate synaptosomal neurotransmitter release. *Neuropharmacology*. 2012; 62:1935–1943. [PubMed: 22239849]

- Guillem K, Bloem B, Poorthuis RB, Loos M, Smit AB, Maskos U, Spijker S, Mansvelder HD. Nicotinic acetylcholine receptor beta2 subunits in the medial prefrontal cortex control attention. *Science*. 2011; 333:888–891. [PubMed: 21836018]
- Haluk DM, Floresco SB. Ventral striatal dopamine modulation of different forms of behavioral flexibility. *Neuropsychopharmacology*. 2009; 34:2041–2052. [PubMed: 19262467]
- Hatsukami DK, Ebbert JO, Anderson A, Lin H, Le C, Hecht SS. Smokeless tobacco brand switching: a means to reduce toxicant exposure? *Drug Alcohol Depend*. 2007; 87:217–224. [PubMed: 16996230]
- Hilario MR, Turner JR, Blendy JA. Reward sensitization: effects of repeated nicotine exposure and withdrawal in mice. *Neuropsychopharmacology*. 2012; 37:2661–2670. [PubMed: 22828747]
- Howe WM, Ji J, Parikh V, Williams S, Mocaer E, Trocme-Thibierge C, Sarter M. Enhancement of attentional performance by selective stimulation of alpha4beta2(*) nAChRs: underlying cholinergic mechanisms. *Neuropsychopharmacology*. 2010; 35:1391–1401. [PubMed: 20147893]
- Hughes JR, Peters EN, Naud S. Relapse to smoking after 1 year of abstinence: a meta-analysis. *Addict Behav*. 2008; 33:1516–1520. [PubMed: 18706769]
- Izquierdo A, Jentsch JD. Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology*. 2012; 219:607–620. [PubMed: 22134477]
- Jarvis MJ, Russell MA, Benowitz NL, Feyerabend C. Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. *Am J Public Health*. 1988; 78:696–698. [PubMed: 3369603]
- Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. *American Journal of Psychiatry*. 2005; 162:1403–1413. [PubMed: 16055761]
- Kehagia AA, Murray GK, Robbins TW. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. *Current Opinion in Neurobiology*. 2010; 20:199–204. [PubMed: 20167474]
- Kenny PJ, Markou A. Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology*. 2006; 31:1203–1211. [PubMed: 16192981]
- Kolokotroni KZ, Rodgers RJ, Harrison AA. Effects of chronic nicotine, nicotine withdrawal and subsequent nicotine challenges on behavioural inhibition in rats. *Psychopharmacology*. 2012; 219:453–468. [PubMed: 22124670]
- Laughlin RE, Grant TL, Williams RW, Jentsch JD. Genetic dissection of behavioral flexibility: reversal learning in mice. *Biological Psychiatry*. 2011; 69:1109–1116. [PubMed: 21392734]
- Laviolette SR, van der Kooy D. The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. *Nature Reviews Neuroscience*. 2004; 5:55–65.
- Leslie FM, Mojica CY, Reynaga DD. Nicotinic receptors in addiction pathways. *Molecular Pharmacology*. 2013; 83:753–758. [PubMed: 23247824]
- Levin ED, Bradley A, Addy N, Sigurani N. Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. *Neuroscience*. 2002; 109:757–765. [PubMed: 11927157]
- Levin ED, Christopher NC, Briggs SJ. Chronic nicotine agonist and antagonist effects on T-maze alternation. *Physiology and Behavior*. 1997; 61:863–866. [PubMed: 9177558]
- Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR. Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing alpha4 and alpha6 subunits. *Molecular Pharmacology*. 2012; 81:541–548. [PubMed: 22222765]
- Lovejoy, E. *Attention in discrimination learning; a point of view and a theory*. San Francisco: Holden-Day; 1968.
- Marks MJ, Stitzel JA, Collins AC. Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. *Journal of Pharmacology and Experimental Therapeutics*. 1985; 235:619–628. [PubMed: 4078726]
- McCallum SE, Parameswaran N, Bordia T, Fan H, Tyndale RF, Langston JW, McIntosh JM, Quik M. Increases in alpha4* but not alpha3*/alpha6* nicotinic receptor sites and function in the primate

- striatum following chronic oral nicotine treatment. *Journal of Neurochemistry*. 2006; 96:1028–1041. [PubMed: 16412091]
- McCracken CB, Grace AA. Persistent cocaine-induced reversal learning deficits are associated with altered limbic cortico-striatal local field potential synchronization. *Journal of Neuroscience*. 2013; 33:17469–17482. [PubMed: 24174680]
- McGaughy J, Decker MW, Sarter M. Enhancement of sustained attention performance by the nicotinic acetylcholine receptor agonist ABT-418 in intact but not basal forebrain-lesioned rats. *Psychopharmacology*. 1999; 144:175–182. [PubMed: 10394999]
- Muir JL. Attention and stimulus processing in the rat. *Brain Research Cognitive Brain Research*. 1996; 3:215–225. [PubMed: 8806024]
- Nesic J, Rusted J, Duka T, Jackson A. Degree of dependence influences the effect of smoking on cognitive flexibility. *Pharmacol Biochem Behav*. 2011; 98:376–384. [PubMed: 21281663]
- Newhouse PA, Potter A, Singh A. Effects of nicotinic stimulation on cognitive performance. *Current Opinion in Pharmacology*. 2004; 4:36–46. [PubMed: 15018837]
- Ortega LA, Tracy BA, Gould TJ, Parikh V. Effects of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. *Behavioural Brain Research*. 2013; 238:134–145. [PubMed: 23103711]
- Pattij T, Janssen MC, Loos M, Smit AB, Schoffeleer AN, van Gaalen MM. Strain specificity and cholinergic modulation of visuospatial attention in three inbred mouse strains. *Genes, Brain and Behavior*. 6:579–587.
- Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ. Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *Journal of Pharmacology and Experimental Therapeutics*. 1999; 289:1545–1552. [PubMed: 10336551]
- Piccio MR, Addy NA, Mineur YS, Brunzell DH. It is not “either/or”: activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Progress in Neurobiology*. 2008; 84:329–342. [PubMed: 18242816]
- Piccio MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998; 391:173–177. [PubMed: 9428762]
- Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, Fratta W. Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *Journal of Neuroscience*. 2008; 28:12318–12327. [PubMed: 19020025]
- Portugal GS, Kenney JW, Gould TJ. Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiology of Learning and Memory*. 2008; 89:106–113. [PubMed: 17584502]
- Ragozzino ME. The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. *Annals of the New York Academy of Sciences*. 2007; 1121:355–375. [PubMed: 17698989]
- Robinson TE, Berridge KC. Review. The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society of London Series B, Biological sciences*. 2008; 363:3137–3146.
- Sacco KA. Nicotine-induced behavioral disinhibition and ethanol preference correlate after repeated nicotine treatment. *European Journal of Pharmacology*. 2001; 417:117–123. [PubMed: 11301066]
- Sarter M, Paolone G. Deficits in attentional control: cholinergic mechanisms and circuitry-based treatment approaches. *Behavioral Neuroscience*. 2011; 125:825–835. [PubMed: 22122146]
- Scheggia D, Bebensee A, Weinberger DR, Papaleo F. The ultimate intra-/extra-dimensional attentional set-shifting task for mice. *Biological Psychiatry*. 2014; 75:660–670. [PubMed: 23810621]
- Schoenbaum G, Saddoris MP, Stalnaker TA. Reconciling the roles of orbitofrontal cortex in reversal learning and the encoding of outcome expectancies. *Annals of the New York Academy of Sciences*. 2007; 1121:320–335. [PubMed: 17698988]
- Semenova S, Stoleran IP, Markou A. Chronic nicotine administration improves attention while nicotine withdrawal induces performance deficits in the 5-choice serial reaction time task in rats. *Pharmacol Biochem Behav*. 2007; 87:360–368. [PubMed: 17582477]

- Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzler E, Frohlich E, Perry E, Dubin JA, Estok K, Brenner E, Baldwin RM, Tamagnan GD, Seibyl JP, Jatlow P, Picciotto MR, London ED, O'Malley S, van Dyck CH. Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. *Journal of neuroscience*. 2006; 26:8707–8714. [PubMed: 16928859]
- Stalnaker TA, Takahashi Y, Roesch MR, Schoenbaum G. Neural substrates of cognitive inflexibility after chronic cocaine exposure. *Neuropharmacology*. 2009; 56(Suppl 1):63–72. [PubMed: 18692512]
- Stolerman IP, Mirza NR, Hahn B, Shoaib M. Nicotine in an animal model of attention. *European Journal of Pharmacology*. 2000; 393:147–154. [PubMed: 10771008]
- Turner JR, Castellano LM, Blendy JA. Nicotinic partial agonists varenicline and sazetidine-A have differential effects on affective behavior. *Journal of Pharmacology and Experimental Therapeutics*. 2010; 334:665–672. [PubMed: 20435920]
- Turner JR, Wilkinson DS, Poole RL, Gould TJ, Carlson GC, Blendy JA. Divergent functional effects of sazetidine-a and varenicline during nicotine withdrawal. *Neuropsychopharmacology*. 2013; 38:2035–2047. [PubMed: 23624742]
- Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: beyond dopamine reward circuitry. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:15037–15042. [PubMed: 21402948]
- Walters CL, Brown S, Changeux JP, Martin B, Damaj MI. The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology*. 2006; 184:339–344. [PubMed: 16416156]
- Wilkinson DS, Turner JR, Blendy JA, Gould TJ. Genetic background influences the effects of withdrawal from chronic nicotine on learning and high-affinity nicotinic acetylcholine receptor binding in the dorsal and ventral hippocampus. *Psychopharmacology*. 2013; 225:201–208. [PubMed: 22836371]
- 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 beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptors. *Journal of Neuroscience*. 1999; 19:9298–9305. [PubMed: 10531434]
- Young JW, Crawford N, Kelly JS, Kerr LE, Marston HM, Spratt C, Finlayson K, Sharkey J. Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *European Neuropsychopharmacology*. 2007; 17:145–155. [PubMed: 16650968]
- 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 alpha7 nicotinic acetylcholine receptor. *Neuropsychopharmacology*. 2004; 29:891–900. [PubMed: 14970827]
- Young JW, Meves JM, Geyer MA. Nicotinic agonist-induced improvement of vigilance in mice in the 5-choice continuous performance test. *Behavior Brain Research*. 2013; 240:119–133.

Figure 1.

Genotyping and general experimental design. (A) PCR-based genotyping strategy confirming amplified DNA fragments from the target gene. $\beta 2^{+/+}$ mice revealed a DNA product of 250 bp as compared to 400 bp for the $\beta 2^{-/-}$ mice. $\beta 2^{+/-}$ mice displayed both gene products at 250 bp and 400 bp, respectively. (B) Schematic representation of the experimental design. Animals of each genotype were initially autoshaped and then pretrained to press an activated lever within allotted time to receive reinforcement (see Methods). Animals that reached pretraining criterion were implanted with subcutaneous osmotic minipumps (Alzet, Model #1004) to administer chronic nicotine (18mg/Kg/day) or saline for 28 days. Following recovery, mice were trained and tested for the retention of pretraining criterion. Animals that matched criterion performance were progressed through subsequent stages of the task (i.e. visual discrimination, strategy set-shifting, and reversal learning). The range of training days required to complete each stage of the task is indicated in the schematic. After completion of behavioral testing, serum samples were collected from few animals per genotype to determine cotinine levels.

Figure 2. Effects of $\beta 2$ nAChR genotype and chronic nicotine administration on visual discrimination learning. All data are presented as Mean, $\pm$ S.E.M. (A) Number of trials required to reach visual discrimination criterion. (B) Errors to criterion. (*, ** $p < 0.01, 0.001$; main effect of treatment)

Figure 3.

Effects of chronic nicotine on the reversal of response strategy and interactions with the $\beta 2$ nAChR genotype. (A) Trials to criterion during the reversal learning phase. Learning curves for reversal sessions depicting response accuracies for the first 5 training sessions of saline- and nicotine-treated $\beta 2^{+/+}$ (B), $\beta 2^{+/-}$ (C) and $\beta 2^{-/-}$ (D) mice. (E) Reversal errors to criterion. Bar charts representing types of errors: perseverative errors (F) and regressive errors (G). (H) Total number of omissions during the reversal sessions. (*, **, *** $p < 0.05$, 0.01, 0.001; *post hoc* tests; #, $p < 0.05$; main effect of treatment)

Table 1

The number of mice per experimental group. These animals were implanted with osmotic minipumps for chronic nicotine or saline delivery, and progressed through all the stages of the cognitive flexibility task. M: Male; F: Female

Genotype	Saline	Nicotine
$\beta 2^{+/+}$	N = 10 (8 M; 2F)	N = 11 (10 M; 1F)
$\beta 2^{+/-}$	N = 8 (5 M; 3F)	N = 8 (3 M; 5F)
$\beta 2^{-/-}$	N = 7 (4 M; 3F)	N = 8 (5 M; 3F)