Supplementary Information

1. Supplementary Methods

1.1. Mini-osmotic pump surgery

Following the acquisition of pretraining criterion, mice were prepared for subcutaneous implantation of osmotic minipumps. All surgeries were conducted under isoflurane (1.5-3% mixture with oxygen) anesthesia. Osmotic minipumps (model 1004; DURECT Corporation, Cupertino, CA) were filled either with nicotine hydrogen tartrate (Sigma Co., St Louis, MO) dissolved in sterile saline to deliver 18 mg/kg/d of nicotine (as free base) solution at a rate of 0.11 µL/h for 28 days. This dose yielded plasma cotinine levels in nicotine-treated mice similar to highly dependent smokers (100-150 ng/mL; Cole et al. 2015). Pumps were filled with sterile saline for the control groups. All pumps were inserted subcutaneously into the back by incision just below the neck region. After insertion, the wounds were closed with sutures. Mice were given *ad libitum* food and water and 48 hours of recovery time following surgery.

1.2. Assessment of withdrawal symptoms

Mice were observed for the somatic signs of withdrawal immediately after the mecamylamine challenge for 20-min in the home cage. Withdrawal symptoms included head shakes, paw tremors, retropulsion, writhing, scratching, backing, piloerection, and Straub tail. Somatic signs were calculated as the number of signs displayed by mice during the 20-min observation period after the first injection. Other control groups (see main text) including chronic nicotine challenged with saline (nic-sal), chronic saline challenged with mecamylamine (sal-mec), and chronic saline challenged with saline (sal-sal) were also examined for these symptoms for comparison with the nic-mec group. In few animals, withdrawal signs were also monitored on the seventh day following the last injection of mecamylamine.

2. Supplementary Results

2.1. Visual discrimination learning

We first analyzed the performance of chronic saline- and nicotine-treated animals during the visual discrimination stage. This stage preceded testing in the strategy switching phase during withdrawal. Although the number of trials to attain criterion remained similar between the two groups ($F_{1,37}$=1.57, p=0.22); nicotine-exposed mice committed significantly less errors as compared to the saline group (nicotine: 17.21±1.61; saline: 28.15±4.0; $F_{1,36}$=4.95, p=0.03, η^2=.12). Moreover, the response accuracy during the learning sessions was higher in nicotine-treated animals (vs saline). These data are in line with previous studies (Besheer and Bevins, 2000, Ortega et al., 2013) and imply that nicotine may facilitate certain aspects of visual attention by facilitating the perceptual selectivity of the discriminative stimulus. Comparison of discrimination learning performance between animals assigned to either the saline- or mecamylamine-challenge groups yielded no significant differences in trials to criterion ($F_{1,18}$=1.05, p=0.32 sal-sal vs. sal-mec; $F_{1,17}$=.1.36, p=0.26 nic-sal vs. nic-mec) or errors to criterion ($F_{1,18}$=0.05, p=0.83 sal-sal vs. sal-mec; $F_{1,17}$=0.87, p=0.77 nic-sal vs. nic-mec).

2.2. Withdrawal signs

Somatic signs of withdrawal observed on the first day of saline or mecamylamine treatment are summarized in Suppl Fig. 1. We observed significant group differences in the episodes of paw tremors ($F_{3,35}$=5.21, p=0.004, η^2=.31), head shakes ($F_{3,35}$=7.67, p<0.001, η^2=.40), and backing ($F_{3,35}$=10.83, p<0.001, η^2=.48). *Post hoc* analysis confirmed that these differences emerged due to robust increase in the number of episodes of these symptoms in chronic nicotine-treated mice injected with mecamylamine (all p<0.03 vs sal-sal)). Incidence of other symptoms was rare in all groups and therefore, total withdrawal symptom scores were primarily composed of tremors, head shakes and backing episodes. As expected, total scores were significantly higher in mecamylamine-precipitated withdrawal group as compared to the sal-sal mice (p<0.001). Withdrawal symptoms were not observed in the nic-sal and sal-mec groups and total scores in these animals remained similar to the control mice (both *p's*>0.90 vs. sal-sal). Withdrawal symptoms were also monitored on the last (seventh) day of mecamylamine injection and compared with the sal-sal group. Total mecamylamine precipitated withdrawal effects remained elevated in chronic nicotine treated animals compared to sal-mec and sal-sal treated animals *($F_{2,9}$*=17.06, p=0.001, η^2=.79; *post hoc*: p=0.003 and p=0,001 respectively). This is in concordance with previous reported findings following repeated mecamylamine injections in nicotine treated animals (Damaj et al., 2003).

Supplementary References

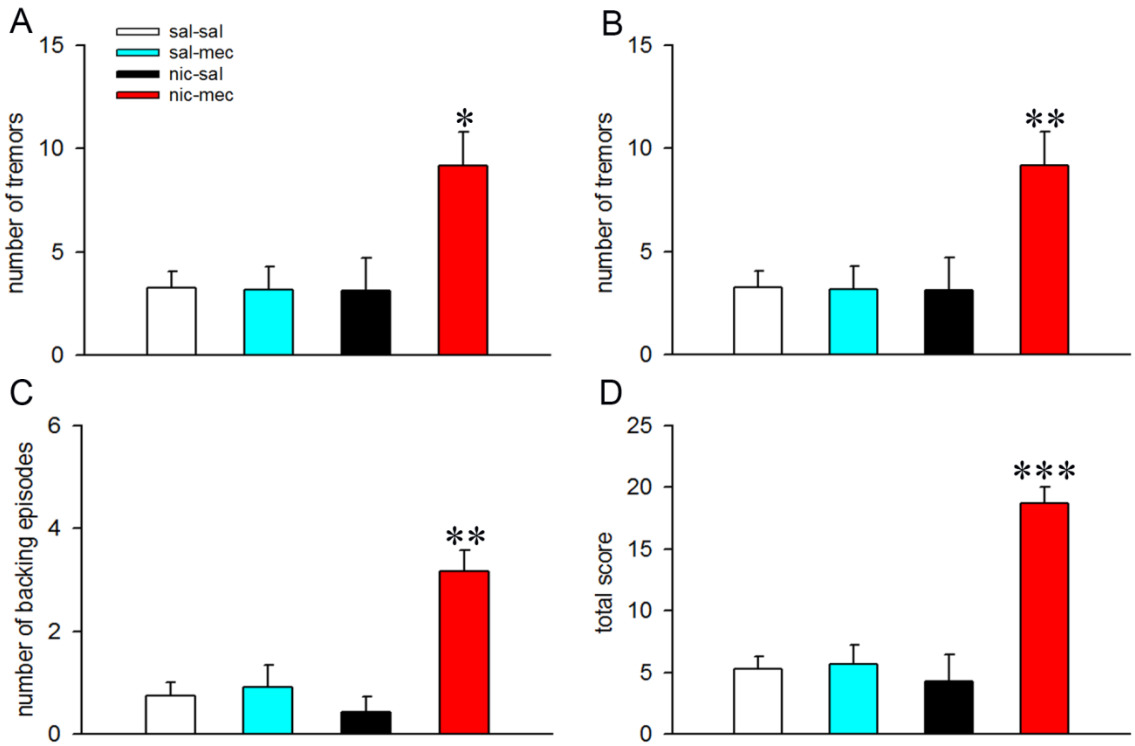
Besheer J, Bevins RA (2000) Nicotine enhances acquisition of a T-maze visual discrimination: assessment of individual differences. Behavioural Pharmacology 11:613-620.

Cole RD, Poole RL, Guzman DM, Gould TJ, Parikh V (2015) Contributions of beta2 subunit-containing nAChRs to chronic nicotine-induced alterations in cognitive flexibility in mice. Psychopharmacology 232:1207-1217.

Damaj MI, Kao W, Martin BR (2003) Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. Journal of Pharmacology and Experimental Therapeutics 307:526-534.

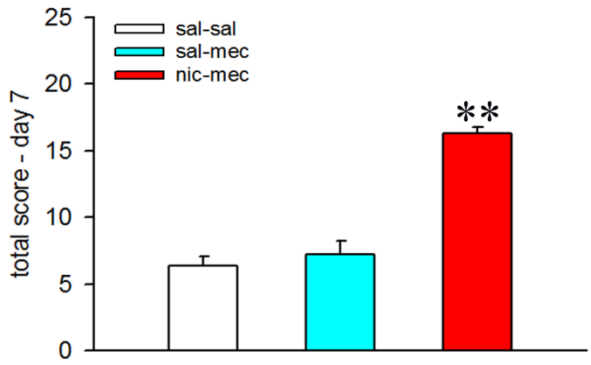
Ortega LA, Tracy BA, Gould TJ, Parikh V (2013) Effects of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. Behavioural Brain Research 238:134-145.

Supplementary Figure 1



Somatic signs of withdrawal observed during a 20-min observation period after mecamylamine or saline injection in chronic nicotine- or saline-treated mice. Withdrawal symptoms, characterized as the number of paw tremors, head shakes and backing episodes, were significantly increased in nic-mec mice. Chronic nicotine or mecamylamine challenge per se did not produce any visible signs of withdrawal. *, **, *** p<0.05, 0.01, 0.001 vs. sal-sal.

Supplementary Figure 2



Withdrawal symptoms observed on the seventh day following repeated mecamylamine injections. Total scores for the somatic signs remained significantly elevated in the nic-mec group. **, $p<0.01$ vs. sal-sal and sal-mec.