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Acute administration of nicotine into the higher order auditory Te2 cortex specifically decreases the fear-related charge of remote emotional memories

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ABSTRACT

Nicotine elicits several behavioural effects on mood as well as on stress and anxiety processes. Recently, it was found that the higher order components of the sensory cortex, such as the secondary auditory cortex Te2, are essential for the long-term storage of remote fear memories. Therefore, in the present study, we examined the effects of acute nicotine injection into the higher order auditory cortex Te2, on the remote emotional memories of either threat or incentive experiences in rats. We found that intra-Te2 nicotine injection decreased the fear-evoked responses to a tone previously paired with footshock. This effect was cue- and dose-specific and was not due to any interference with auditory stimuli processing, innate anxiety and fear processes, or with motor responses. Nicotine acts acutely in the presence of threat stimuli but it did not determine the permanent degradation of the fear-memory trace, since memories tested one week after nicotine injection were unaffected. Remarkably, nicotine did not affect the memory of a similar tone that was paired to incentive stimuli. We conclude from our results that nicotine, when acting acutely in the auditory cortex, relieves the fear charge embedded by learned stimuli.

1. Introduction

The pharmacological effects of nicotine – the major psychoactive ingredient in tobacco smoke – are mediated by the activation of nicotinic acetylcholine receptors (nAChRs), which are present in several brain regions and tied to different cellular processes (McGehee et al., 1995; Pontieri et al., 1996; Chiamulera, 2005; Laviolette and van der Kooy, 2004; Metherate, 2004). As a consequence, nicotine produces a wide variety of motivational and behavioural effects, including acting on mood, emotions, cognition and motor functions (Chiamulera, 2005; Laviolette and van der Kooy, 2004; McGehee et al., 1995; Pontieri et al., 1996; Stolerman and Jarvis, 1995; Tipps et al., 2014). Among these effects, the close interaction between nicotine and anxiety represents one of the main concerns, yet it is poorly understood.

Nicotine modulates the physiological responses to stress and anxiety processes in both animal models and human smokers (Anderson and Brunzell, 2012; Breslau, 1995; Brioni et al., 1993; Fidler and West, 2009; File et al., 1998; George et al., 2000a,b; George et al., 1998; Irvine et al., 2001; McGranahan et al., 2011; Perkins and Grobe, 1992; Varani et al., 2012). Stress is also a major precipitating factor for smoking relapse (Shiffman et al., 1997) and for the increase in cigarette use (Skara et al., 2001). This depiction is, however, complicated by the fact that nicotine, like many drugs of abuse, possesses both rewarding and aversive properties (Grieder et al., 2012; Laviolette and van der Kooy, 2003, 2004; Sun and Laviolette, 2014). Nicotine can also induce effects in anxiety disorders such as post-traumatic stress disorder (PTSD), as

Abbreviations: CS, Conditioned stimulus; GABA, γ-aminobutyric acid; nAChR, Nicotinic acetylcholine receptor; PTSD, Post-traumatic stress disorder; US, Unconditioned stimulus; VTA, Ventral tegmental area.

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evidenced by the high comorbidity between these diseases and nicotine dependence through habitual tobacco use (Breslau et al., 2004; Dalack et al., 1998; Hughes et al., 1986; Lasser et al., 2000; Tipps et al., 2014; Ziedonis et al., 2008; Ziedonis and George, 1997).

In animal models, a useful protocol to study psychological stress, as well as the mechanisms involved in PTSD-like manifestations, is provided by the fear-conditioning paradigm; whereby, the subject is exposed to a conditioned stimulus (CS), such as a tone, in association with an unconditioned stimulus (US), typically a footshock. Nicotine pretreatment does not modify the acquisition or the expression of conditioned fear-responses (George et al., 2001). Accordingly, it was reported that the acute systemic administration of nicotine or the direct infusion of nicotine into the dorsal hippocampus did not alter fear behaviours related to a CS, but did enhance the memory of the environment paired with the aversive event (Davis et al., 2007; Elias et al., 2010; Kenney et al., 2012; Raybuck and Gould, 2010; Tipps et al., 2014). Conversely, acute nicotine injection into the ventral hippocampus produced a deficit in contextual fear memories and in trace fear conditioning (Kenney et al., 2012; Kutlu and Gould, 2015; Gould and Leach, 2014; Raybuck and Gould, 2010). It was therefore suggested that the effects of nicotine vary according to the brain region (Kutlu and Gould, 2015; Gould and Leach, 2014; Raybuck and Gould, 2010).

To our knowledge, no data are available on the effects that nicotine induces when administered directly in brain structures involved in the encoding of fear memories related to explicit sensory cues. Recently, we found that the higher order components of the sensory cortex, such as the secondary auditory cortex Te2, are essential for the long-term storage of remote (i.e. 30 days) auditory fear memories (Sacco and Sacchetti, 2010). The involvement of this cortex is strictly related to emotional memory processes, and not is due to any interference with sensory or innate emotional processes (Grosso et al., 2015; Sacco and Sacchetti, 2010). Thus, this cortex is particularly suitable for investigating the effects, if any, that nicotine has on explicit emotional memories, without any confounding factors from the interference with emotional or motor processes. Therefore, in this study, we address the question of whether, and if so how, acute nicotine administration into Te2 can modulate the memory of cues that are predictive of threat events.

2. Materials and methods

2.1. Subjects

Male Wistar rats (age, 65–80 days; weight, 250–350 g) were used. The animals were housed in plastic cages with food and water available *ad libitum*, with a 12 h light/dark cycle at a constant temperature of 22 ± 1 °C. All the experiments were conducted in accordance with the European Communities Council Directive 2010/63/EU and approved by the Italian Ministry of Health (Authorisation No 265/2011) and by the local Bioethical Committee of the University of Turin.

2.2. Drugs and infusion procedure

Nicotine hydrogen tartrate salt (Glentham Life Sciences, Corsham, UK) was dissolved in physiological saline (0.9% NaCl) and the pH was adjusted to 7.4. Different doses of nicotine (0.54, 27 and 54 nmol/μl; 0.6 μl per site, as calculated on the tartrate salt weight) were applied, based on previous studies (Laviolette et al., 2008; Sun and Laviolette, 2014) and on our preliminary experiments. In particular, we applied a dose of 54 nmol/μl, as this is similar to that of Laviolette et al. (2008) who applied a dose of 24 nmol/0.5 μl, and which has been reported to elicit rewarding effects when injected into the ventral tegmental area (VTA) (Laviolette and van der Kooy, 2003; Paxinos and Watson, 1986). Muscimol (Tocris Bioscience, Bristol, UK) was prepared at 1 mg/ml in physiological saline (Letzkus et al., 2011; Martin and Ghez, 1999). The rats were infused bilaterally with either saline, nicotine or muscimol at a rate of 0.25 μl/min. To allow the diffusion of the drug, the injection needle was removed after waiting for 1 min. The behavioural experiments were started at 15–20 min after completion of the nicotine injection procedures or 60 min after muscimol administration.

2.3. Cannulae placement

The rats were surgically implanted with bilateral, chronic, intracranial stainless steel guide-cannulae (4 mm long, 26 gauge, Plastic One, Roanoke, USA). First, the animals were anaesthetised with an intraperitoneal administration of ketamine (100 mg/kg; Ketavet; Bayer, Leverkusen, Germany) supplemented by xylazine (5 mg/kg; Rompun; Bayer) and mounted in the stereotaxic apparatus. Bilateral cannulae aimed at 2.1 mm above the Te2 cortex were implanted at the following stereotaxic coordinates: 6.8 mm posterior to the bregma and ±6.5 mm lateral to the midline (Paxinos and Watson, 1986). The cannulae were lowered below the skull surface at an angle of 19° to the vertical axis in the coronal plane (medial to lateral). To inactivate the ventral hippocampus adjacent to the Te2 cortex, bilateral cannulae were implanted at 2.1 mm above this structure at the following stereotaxic coordinates: 6.8 mm posterior to the bregma ±4.5 mm lateral to midline (Paxinos and Watson, 1986). The cannulae were anchored to the skull by two anchor screws and dental cement. Once secured, cannula dummies (Plastic One) were used to obdurate the guide cannulae. After post-surgical recovery (8–10 days), injection cannulae (31 gauge) were inserted through the guide cannulae. The injector was connected through polyethylene tubing to a Hamilton syringe (10 μl), which was mounted on an infusion pump (Harvard Apparatus, Holliston, USA). After completing the experiments, the cannulae placements were confirmed using standard histological methods.

2.4. The fear-conditioning paradigm

2.4.1. Fear-memory acquisition

A Skinner box module was employed as a conditioning chamber, as in our previous work (Sacco and Sacchetti, 2010). The box floor was made of stainless steel rods (1 cm in diameter, spaced 5 cm apart) connected to a shock delivery apparatus. The apparatus was enclosed within a sound attenuating chamber. Once inside, the animals were left undisturbed for 2 min. After this time, a series of sensory stimuli acting as CSs were administered. The final 1 s of each CS was accompanied with an US consisting of a scrambled electric footshock (intensity, 0.7 mA). The rats were left in the chamber for an additional 1 min, then returned to the home cage. In the fear conditioning to acoustic stimuli, seven pure tones (8 s, 78 dB, 3000 Hz, 22-s inter-trial interval) were delivered as CSs by a loudspeaker located 20 cm above the grid floor. In olfactory fear conditioning, seven almond odours (8 s, 22-s inter-trial interval) were presented using a flow-dilution olfactometer. Clean air (1.5 L/min) was directed to a solenoid valve, which when operated, passed the air to a 15 ml bottle containing 10 ml of almond odour. Odourised air was then directed to the conditioning chamber via ¼-in Tygon tubing. Weaker olfactory fear memories were obtained by employing a footshock intensity of 0.4 mA.

2.4.2. Fear-memory retention

Remote fearful memories were tested at 4 weeks after memory acquisition. The animals were handled for three days (5 min per

day) before the memory retention experiments. Memory was tested using a different apparatus located in a separate experimental room in order to avoid conditioned fear behaviour to contextual cues (Kim and Fanselow, 1992; Sacchetti et al., 1999, 2002, 2004; Sacco and Sacchetti, 2010). The apparatus consisted of a plastic cage with the floor and sides made of transparent plastic and enclosed within a sound attenuating chamber equipped with an exhaust fan, which eliminated odourised air from the enclosure and provided background noise of 60 dB. Once inside, the subject was left undisturbed for 2 min. After this time, CSs were administered that were identical to those used during conditioning. Rat behaviour was recorded via digital video camera. A freezing response was taken as a fear index (Sacco and Sacchetti, 2010), where freezing was defined as the complete absence of somatic mobility, except for respiratory movements. For each animal, the amount of time (in seconds) spent freezing during the CSs was measured offline. The freezing behaviour was analysed by two independent observers who were blind to the animal groups (inter- and intra-rater reliabilities ≥90%). Freezing during the 120 s period preceding the first tone was also recorded to measure any generalisation of fear (preCS period).

2.5. Appetitive conditioning

2.5.1. Incentive-memory acquisition

The rats were placed on a restricted diet to maintain their body weight at approximately 90% of their free-feeding weight. A day before the behavioural protocol began, the rats were given ~1 g of chocolate-flavoured food pellets (Bio-Serv, F07256, Flemington, USA) in the home cage to familiarise them with the pellets. Animals were conditioned in the standard Skinner box module described in the fear-conditioning protocol (Section 2.4). The rats underwent Pavlovian conditioning sessions in which the presentation of a CS was coupled with an US, consisting of the delivery of one sucrose pellet into a food cup within the chamber. The CS–US pairing was presented 28 times per session at variable intervals. Each conditioning session lasted a total of 60 min. All the animals were conditioned for 3 consecutive days, consisting of one conditioning session per day.

Memory retention was tested 4 weeks post conditioning by presenting the CSs, which were not paired with any US. To minimise contextual influences, the animals were tested in an environment completely different from that employed during the learning trial (see Section 2.4, the fear-conditioning paradigm). Moreover, to reduce the contribution of within-group variations in baseline responding, we analysed the differences between the CS responses and preCS responses (Saddoris et al., 2009). The conditioned discriminative approach was calculated as the time the animals spent with their head in the food cup during the first 25 s of CS (8 s) and post-CS presentation (17 s) minus the 25 s preceding CS onset. The CSs were identical to those employed in the fear-conditioning paradigm conditioning (pure tones, 8 s, 78 dB, 3000 Hz).

2.6. Open-field paradigm

In this paradigm, the rats faced a conflict between an innate aversion to an open space and the motivation to explore it. A greater amount of time spent in the brightly lit space was linked to an index of decreased anxiety-like behaviour. The open-field apparatus consisted of a plastic opaque box (50 × 80 × 40 cm). The rats were placed in the centre of the apparatus and their behaviour was recorded for 10 min. The analyses were conducted using the Smart 3.0 software (Panlab, Cornellà, Spain).

2.7. Startle analysis

The acoustic startle response was measured as an input/output function (Valsamis and Schmid, 2011) using a startle chamber (SPSG, La Jolla, California, USA). Startle responses were induced by the presentation of white noise stimuli of increasing amplitude. After an acclimation period of 5 min with a constant background white noise of 50 dB, startle stimuli (1 s white noise) were displayed every 20 s, starting at 57 dB. The startle stimulus intensity was increased between each stimulus until it reached 88 dB, thus resulting in 10–30 trials with startle stimuli (Sacco and Sacchetti, 2010).

2.8. Histology

Following the experiments, the injected sites were histologically verified. Rats were deeply anesthetized with intraperitoneal administration of ketamine (100 mg/kg; Ketavet; Bayer, Leverkusen, Germany) supplemented by xylazine (5 mg/kg; Rompun; Bayer) and intracardially perfused with saline, followed by 4% formaldehyde. Brains were cut with a freezing microtome, and injection needle tracks were identified in Nissl-stained serial sections. Subjects whose histological evidence was not adequate were excluded from the data processing.

2.9. Statistical analysis

Student's t-tests, one-way (with the total time spent freezing or the conditioned incentive responses as a dependent variable) and repeated-measure (with groups as a between-subjects variable and retention trials as a within-subjects variable) ANOVA tests and Newman–Keuls multiple comparisons test were employed for comparing the different behavioural groups. Significant differences were reported at a $P = 0.05$ level. The degrees of freedom were n−1 throughout. The statistical analyses were performed using the SPSS statistics 22.0 software.

3. Results

3.1. Experiment 1: nicotine injection into the Te2 cortex impairs fearful memory retrieval

Initially, we addressed the question of whether and how nicotine administered locally in the Te2 cortex interferes with the retrieval of remote fearful memories. Remote memory retention was assessed 1 month after fear conditioning by measuring the freezing behaviour elicited by the auditory CSs that had been previously paired with the US (Lesburguères et al., 2011; Sacco and Sacchetti, 2010) (Fig. 1A). To minimise contextual influences, the rats were tested in a new environment (Kim and Fanselow, 1992; Sacchetti et al., 1999, 2002, 2004; Sacco and Sacchetti, 2010) (Fig. 1A).

Fig. 1B shows the total time the animals spent freezing before the 2 min preceding and during the presentation of the seven CSs. Freezing responses during the 2 min preceding the CS presentation were low and similar between groups, thus indicating the absence of generalisation phenomena (Kim and Fanselow, 1992; Sacchetti et al., 1999). During the CS presentation, we found that nicotine decreases the fear-evoked responses at doses of 54 (n = 8) and 27 (n = 6) nmol/μl, while the concentration of 0.54 nmol/μl (n = 6) did not modify the freezing response with reference to the saline-injected (n = 8) rats. One-way ANOVA showed a significant difference between groups ($F_{3,24} = 24.70$; $P < 0.001$). The subsequent Newman–Keuls test revealed differences between the freezing responses of the animals injected with 54 and 27 nmol/μl doses of

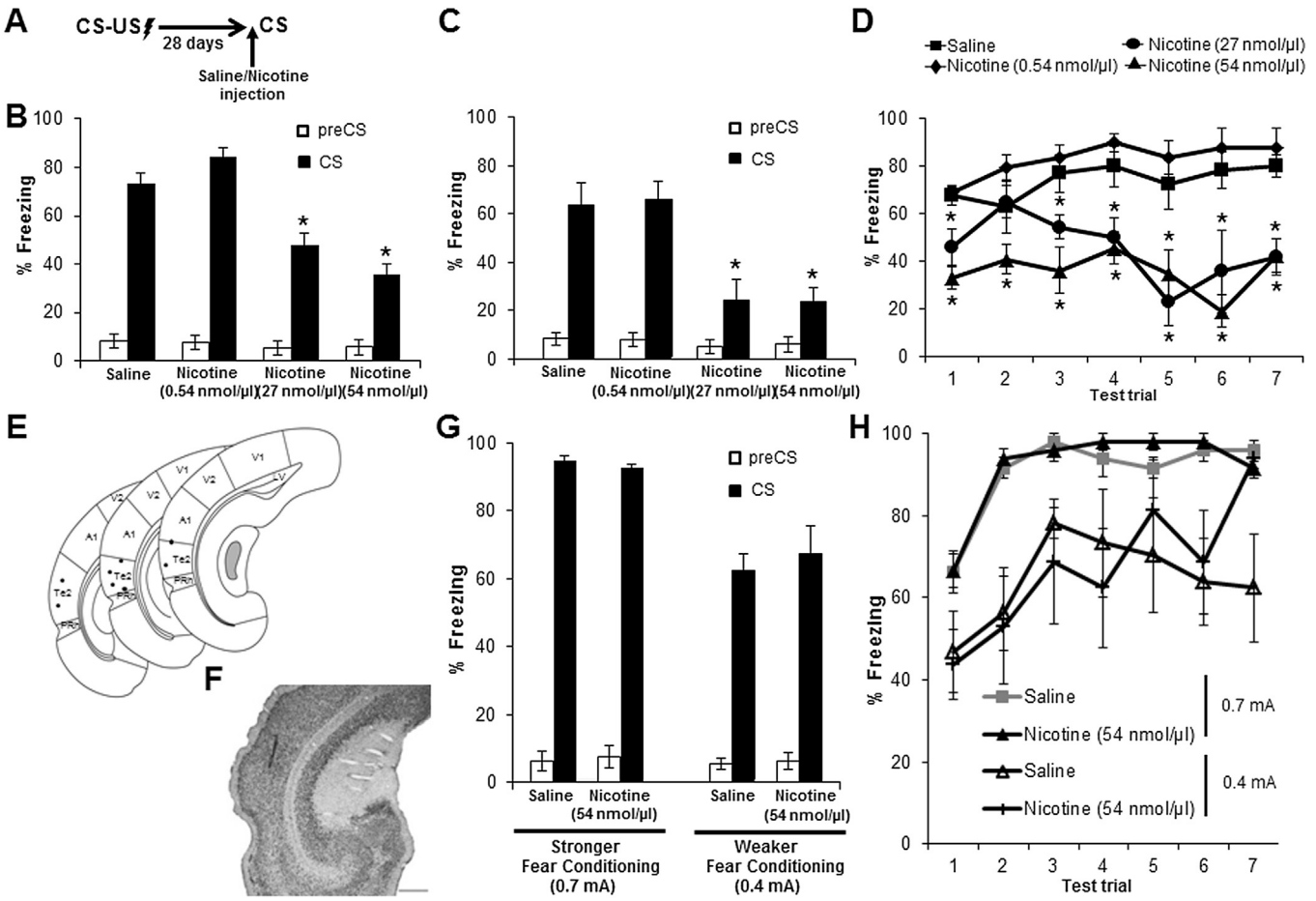

Fig. 1. Nicotine-induced dose-dependent effects on remote fearful memories. (A) Schematic diagram illustrating the behavioural experiment. Saline or Nicotine at different dosages were injected into the Te2 cortex at 15–20 min before the retention of remote auditory fear memories. (B) Fear response measured as a percentage of total immobility (freezing) both at 2 min before (preCS) and during the overall CS presentation (CS) in saline and nicotine-injected rats at the different doses. (C) Similar results were observed by measuring overall freezing response during CSs presentation and inter trial time intervals. (D) Total percentage of the freezing responses across the seven CS presentation in all groups. (E) Location of the needle tips of nicotine (54 nmol/μl) in the Te2. A1, primary auditory cortex; Ent, entorhinal cortex; PRh, perirhinal cortex; Te2, secondary auditory cortex; V1 and V2, primary and secondary visual cortices; VL, lateral ventricle. Plates are adapted from the map of Paxinos and Watson (Paxinos and Watson, 1986). The Te2 was defined according to the map of Zilles (Zilles, 1985). (F) Representative histology of the needle track in the Te2, Scale bars, 500 μm. (G) Nicotine (54 nmol/μl) did not affect conditioned freezing toward an odour CS obtained either after strong or weaker conditioning. (H) Similar results were obtained by analysing total percentage of the freezing responses across the seven CS presentation in all groups. *P < 0.05. All values are reported as mean ± SEM.

nicotine and the freezing of the saline-injected rats ($P < 0.05$), but not between the control group and the animals injected with 0.54 nmol/μl of nicotine ($P > 0.05$). Similar effects were observed by analysing the total freezing time of the animals during CSs presentation and the inter-trial time intervals ($F_{3,24} = 9.10$; $P < 0.001$) (Fig. 1C).

Given that we presented seven CSs, the observed decreases in conditioned fear behaviour may be due to an effect on the threat memory retrieval or alternatively to an enhancement of the extinction processes that may occur during repeated presentation of CSs (Elias et al., 2010; Kutlu and Gould, 2015). We therefore analysed the freezing responses during each CS presentation to test whether the freezing response changed across trials. The ANOVA test for the repeated measures showed differences between the groups ($F_{3,24} = 24.72$; $P < 0.001$), but not within each group across the CS presentation ($F_{3,18} = 1.49$; $P > 0.05$) (Fig. 1D). This revealed that nicotine acts early during memory retrieval, i.e. during the first CS presentation, and similarly decreases the freezing responses to all other CSs. A subsequent Nissl-stained inspection revealed that intra-cortical nicotine administration did not elicit permanent neuronal damage (Fig. 1E–F).

To investigate whether the effects of nicotine were limited to the neural network specifically engaged by the auditory memory processes, we repeated the previous experiment in animals conditioned to an odour CS, a process in which the Te2 is not involved (Sacco and Sacchetti, 2010). There was no significant difference between saline- (n = 6) and nicotine (n = 6)-injected rats during the overall CS presentation (Student's t-test, $t_{10} = -1.41$, $P > 0.05$) (Fig. 1G), or during each CS presentation ($F_{1,10} = 0.28$; $P > 0.05$) (Fig. 1H). However, the odour-conditioned animals displayed very strong conditioned responses, potentially masking the interference of nicotine. To rule out this possibility, nicotine administration was repeated in rats conditioned by pairing odour CSs with lower intensity USs. This procedure resulted in a weaker conditioning (n = 8) that was not affected by nicotine administration (n = 8), with regard to the overall CSs presentation ($t_{14} = -0.53$, $P > 0.05$) (Fig. 1G) and across each CS presentation ($F_{1,14} = 0.07$; $P > 0.05$) (Fig. 1H). This indicates that the nicotine effects are strictly related to memory processes occurring in Te2 and are not secondary to interferences from other structures, such as the amygdala or the VTA, which are known to play a general role in emotional memory. Furthermore, these findings show that nicotine injection into Te2

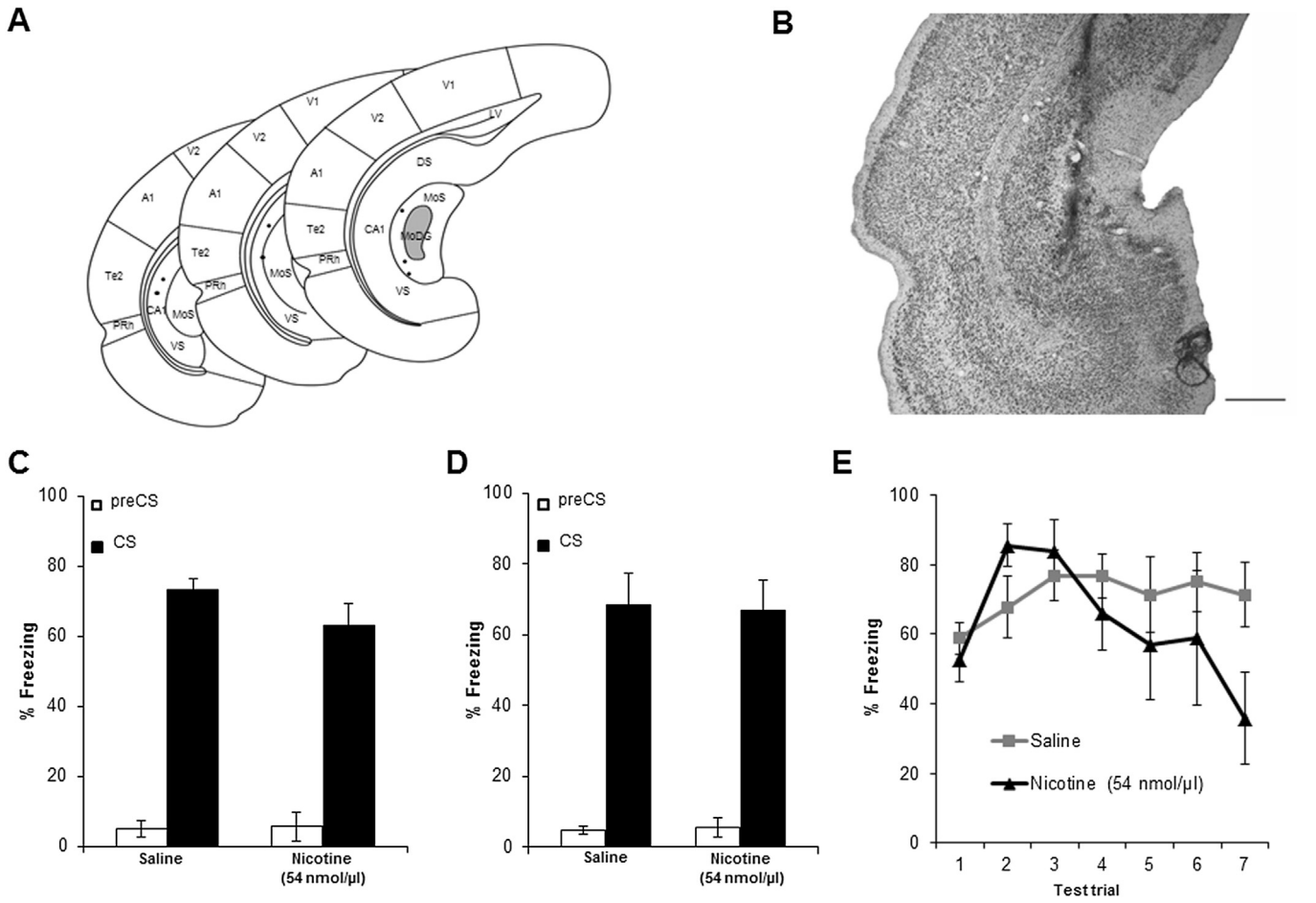

Fig. 2. Nicotine administered locally into the ventral hippocampal region adjacent to the Te2 cortex did not affect remote fear memories. (A) Location of the needle tips of nicotine (54 nmol/µl) in the ventral hippocampus adjacent to the Te2 cortex. Plates are adapted from the map of Paxinos and Watson (Paxinos and Watson, 1986). (B) Representative histology of the needle track in the ventral hippocampal region, Scale bars, 500 µm (C–E) Nicotine did not affected the retrieval of remote fear memories to the overall CSs presentation (C), to the total time spent freezing during CSs presentation and intertribal intervals (D) and to each CS (E). All values are reported as mean ± SEM.

did not affect the freezing response or emotional process; rather it interferes selectively with local emotional memory processes in Te2.

3.2. Experiment 2: nicotine injection into the ventral hippocampus cortex did not hamper remote fearful memory retrieval

Several previous studies have shown that nicotine administration into the ventral hippocampus impairs contextual and trace cued fear memories (Kenney et al., 2012; Kutlu and Gould, 2015; Gould and Leach, 2014; Raybuck and Gould, 2010). Given that the Te2 cortex is adjacent to the caudal portion of the ventral hippocampus, it may be that the nicotine effects observed in our experiment are due to nicotine spreading to this region. Despite the fact that in the aforementioned studies nicotine was administered in a region of the ventral hippocampus far from that adjacent to the Te2 cortex, to clarify this point, we repeated the previous experiment by injecting a similar dose of nicotine into the region of the ventral hippocampus that is immediately adjacent to the Te2 (Figs. 2A–B). When compared to saline-injected rats (n = 7), nicotine (n = 7) did not alter remote fearful memories to CS administration ($t_{12} = 1.37$, $P > 0.05$) (Fig. 2C), the total freezing time during the CSs and the inter-trial time intervals ($t_{12} = 0.12$, $P > 0.05$) (Fig. 2D), or freezing across each CS ($F_{1,12} = 1.05$; $P > 0.05$) (Fig. 2E).

3.3. Experiment 3: nicotine administration into the Te2 cortex did not alter sensory, motor or emotional unconditioned responses

Nicotine has also been reported to modulate sensory stimuli perception (Liang et al., 2008; Metherate et al., 2012). Nevertheless, the Te2 auditory cortex is not necessary for processing simple auditory stimuli, such as those employed in the present study with the CSs (Sacco and Sacchetti, 2010). To better define whether intra-Te2 nicotine administration affects auditory stimuli perception, we analysed the auditory input/output curve by assessing the startle responses as a function of the auditory stimulus intensity (dB) (Valsamis and Schmid, 2011). On day 1, we measured the startle response in the absence of any treatment (Fig. 3A). The following day, we administered the highest effective dose of nicotine (54 nmol//µl; n = 8) or saline (n = 8) (Fig. 3B). ANOVA for the repeated measures revealed no differences between groups before ($F_{1,14} = 0.12$; $P > 0.05$) or after ($F_{1,14} = 0.15$; $P > 0.05$) nicotine injection (Fig. 3B). Therefore there were no significant effects of cortical nicotine administration on the perception and processing of auditory stimuli.

Nicotine can enhance spontaneous motor activity and modify innate emotional behaviour when administered systemically or in specific brain regions, such as the VTA and the nucleus accumbens (Ferrari et al., 2002; Panagis et al., 1996; Trigo et al., 2009). However, the Te2 cortex is neither involved in motor responses nor in innate anxiety behaviour (Sacco and Sacchetti, 2010). The data

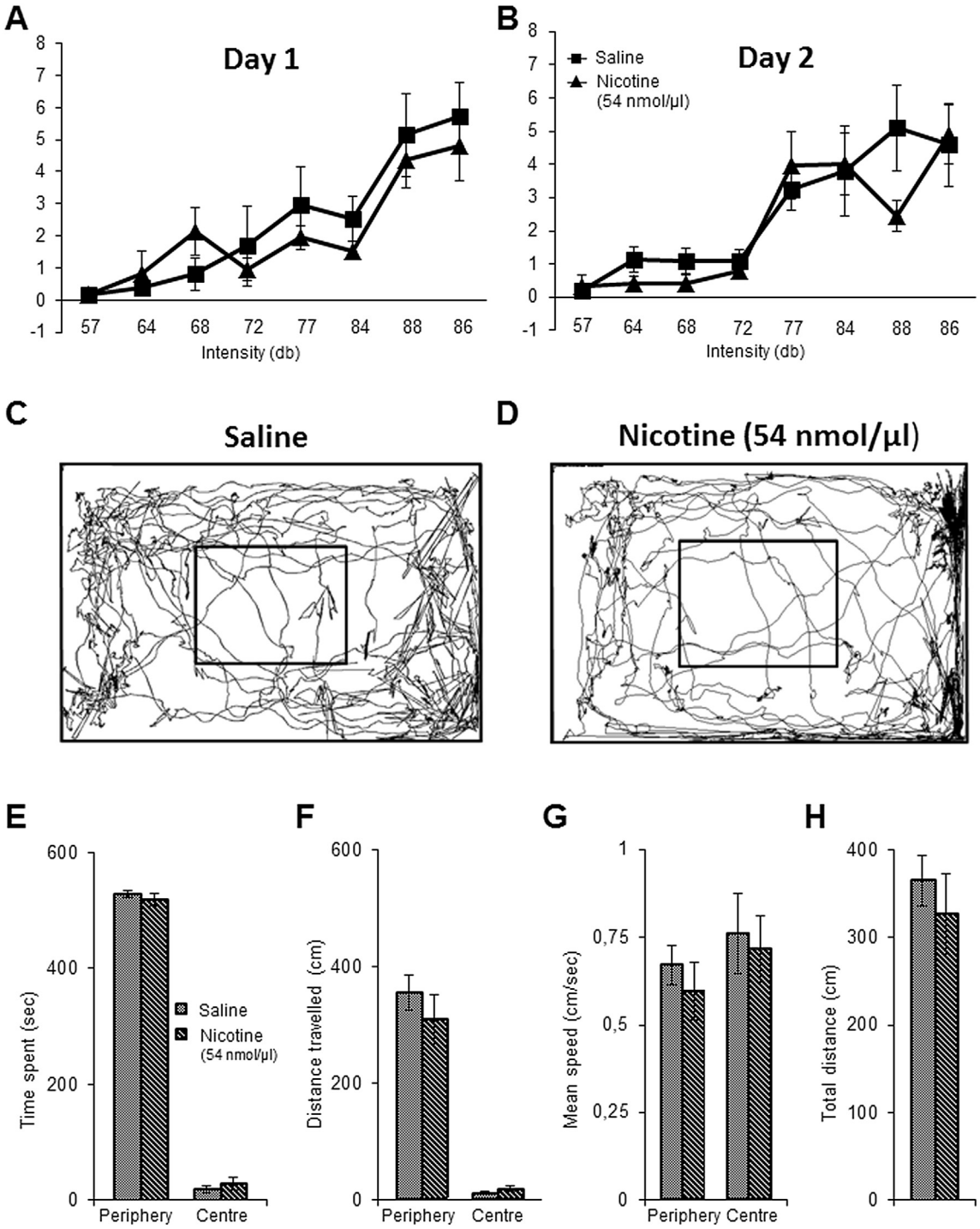

Fig. 3. Intra-Te2 nicotine did not affect auditory stimuli perception or motor and anxiety processes. (A) Input–output auditory curve showing the average startle amplitudes in arbitrary units (a.u.) at increased auditory stimulus intensity (dB) measured the day before (DAY 1) and after (B) nicotine (54 nmol/µl) or saline (DAY 2) injection. ANOVA for repeated measures revealed no differences between groups before or after nicotine injection. (C–D) Representative example of saline (C) and nicotine (D)-injected rats activity during the open-field test. Innate fear and anxiety behaviours were similar between saline and nicotine-injected animals, as shown by the time spent (E) and the similar distance travelled (F) in both the periphery and the centre of the arena. (G–H). Also the spontaneous motor activity was similar between saline and nicotine-treated animals, as shown by the mean speed (G) and the total distance (H) travelled. All values are reported as mean ± SEM.

obtained from the animals conditioned to odour CS support this view. However, to further rule out any possible interference of nicotine administration in Te2 on these processes, we tested the effects of such a manipulation on the open-field paradigm, a well-established model of anxiety. Nicotine (n = 6) or saline (n = 6) were administered in the Te2 before the open-field test (Fig. 3C–D). The two groups did not differ in the time spent in the centre (Student's t-test, $t_{10} = -0.79$, $P > 0.05$) or in the periphery ($t_{10} = 0.80$, $P > 0.05$) of the open field, two measures of the innate level of anxiety (Fig. 3E). Similarly, there were no differences in the distance travelled in the centre ($t_{10} = -1.31$, $P > 0.05$) or in the periphery ($t_{10} = 0.2$, $P > 0.05$) (Fig. 3F). These data support the view that the reduction in the conditioned fear-evoked response is not due to a change in the spontaneous fear state.

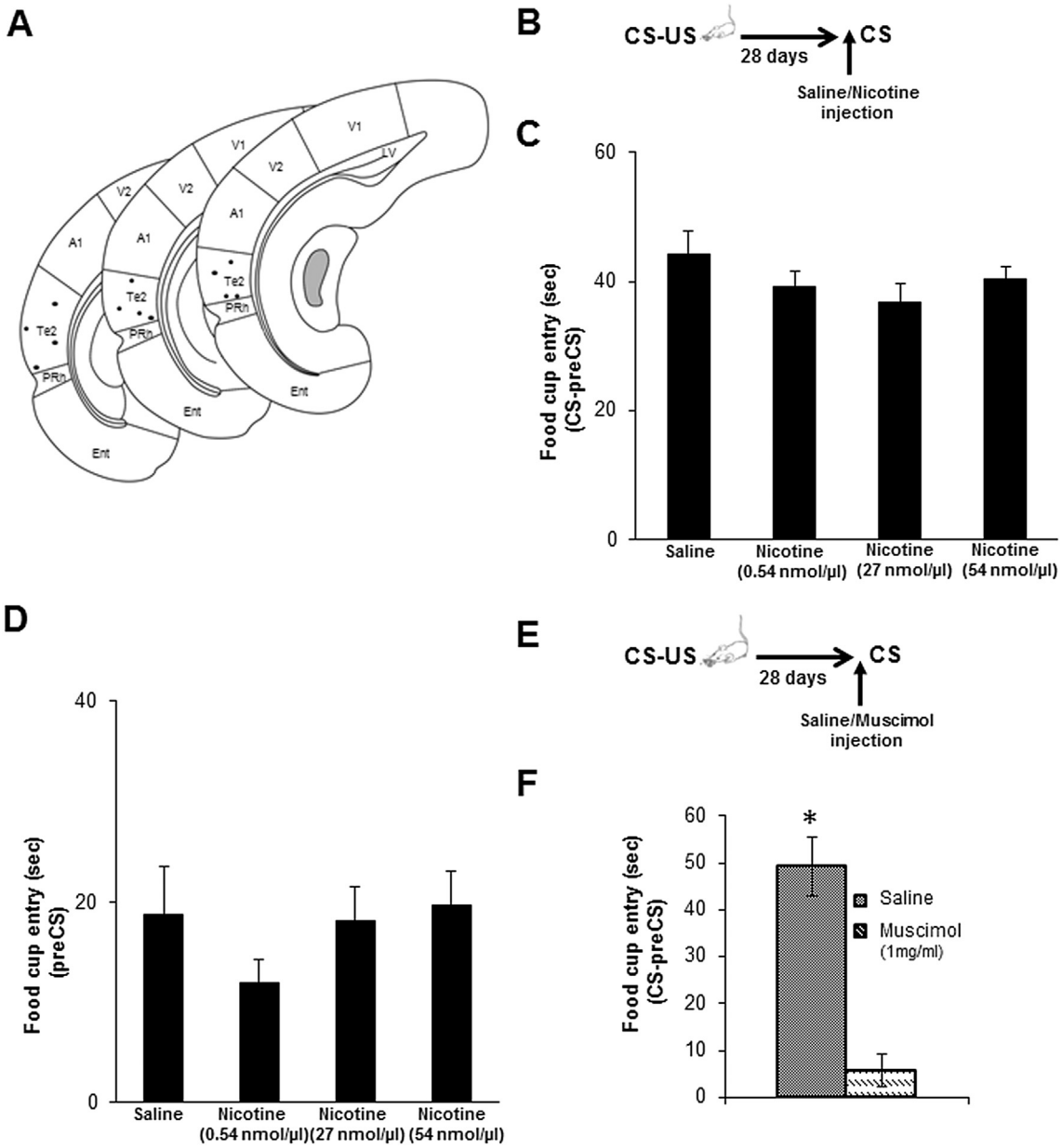

Fig. 4. Nicotine did not affect remote auditory memories to incentive stimuli. (A) Location of the needle tips of nicotine (54 nmol/µl) in Te2. A1, primary auditory cortex; Ent, entorhinal cortex; PRh, perirhinal cortex; Te2, secondary auditory cortex; V1 and V2, primary and secondary visual cortices; VL, lateral ventricle. Plates are adapted from the map of Paxinos and Watson (36). The Te2 was defined according to the map of Zilles (Zilles, 1985). (B) Schematic diagram illustrating the behavioural experiment. Saline or Nicotine at different doses were injected into the Te2 cortex at 15–20 min before the retention of remote auditory incentive memories. (C) The total time that the animals spent in the food cup during the overall CSs administration minus the preCS period was the same in all behavioural groups (D) The overall food cup approach before CS presentation was similar between all groups. (E) Schematic representation showing the Te2 inactivation through the local injection of the GABAA (γ-aminobutyric acid)-receptor agonist muscimol (1.0 µg/µl) at 1 h before remote appetitive memory recall. (F) Muscimol-injected rats showed reduced conditioned incentive responses to the CS. *P < 0.05. All values are reported as mean ± SEM.

In addition, we also measured two parameters in the two groups related to spontaneous motor activity, namely the total distance travelled ($t_{10} = -0.71$) and the averaged velocity ($t_{10} = -0.29$). No differences were detected for all instances ($P > 0.05$) (Fig. 3G–H). These data confirm that our manipulation did not modify the spontaneous motor activity.

3.4. Experiment 4: investigating the intra-Te2 nicotine effects on remote incentive memories

Our findings raised the question as to which cortical processes are modulated by nicotine. The effects we observed could be due to a disturbance of the memory retrieval processes occurring locally in the Te2. Alternatively, it may be that nicotine induces a selective decrement in the fear-evoked responses elicited by CS presentation. In addition, are these effects strictly related to threat memories or, alternatively, does nicotine also interact with emotional memories characterised by a positive, incentive emotional content? To investigate these issues, we tested the impact of Te2-nicotine administration in remote emotional memories obtained by pairing auditory CSs, identical to those previous employed (frequency, amplitude and duration), with delivery of a palatable food. Remote memories were tested 1 month after conditioning (Fig. 4A–B). The total time that Te2-nicotine injected subjects spent with their heads in the food cup during the presentation of CSs minus the preCS periods did not differ at all the tested dosages (54 nmol/µl,

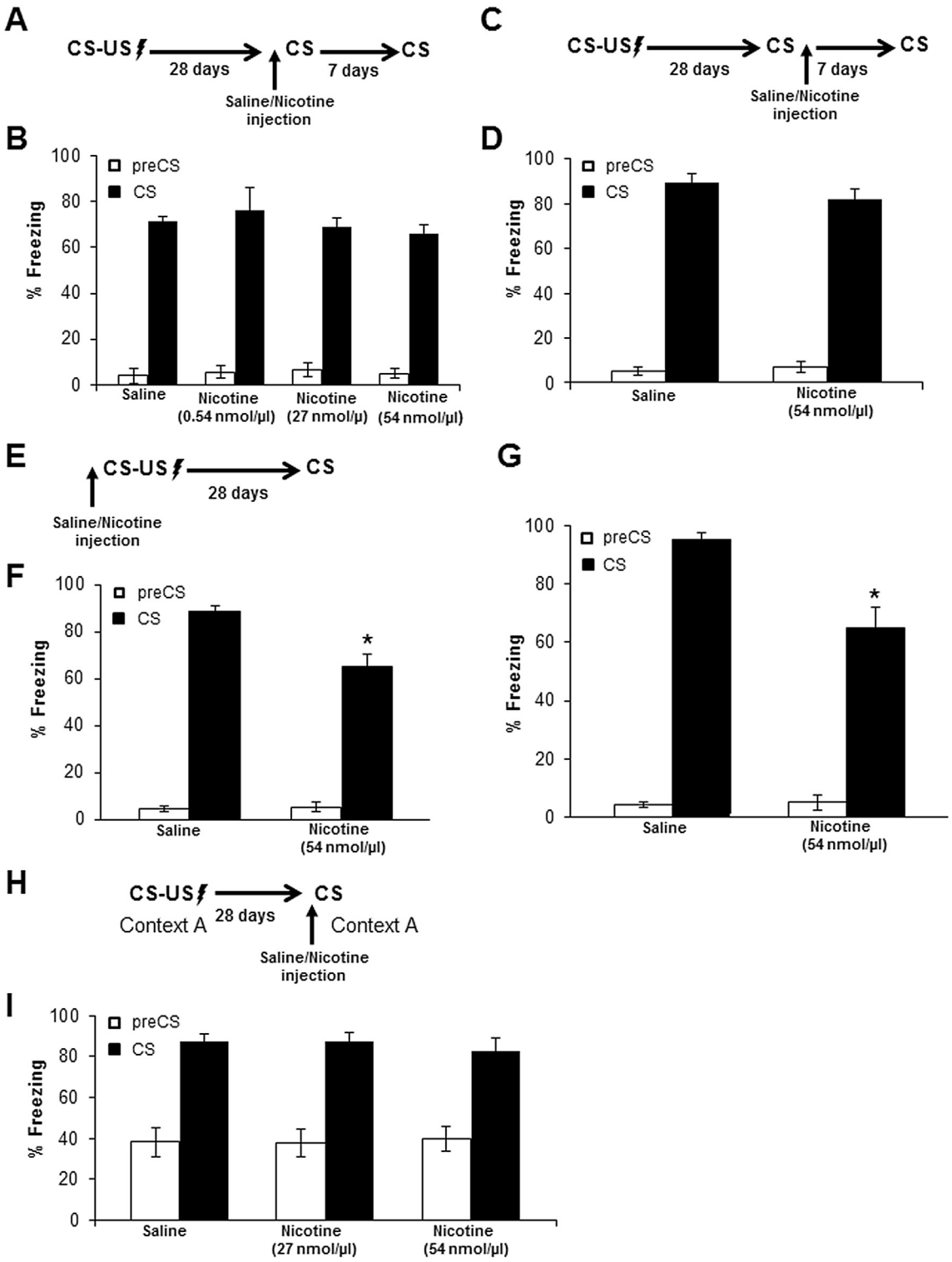

Fig. 5. Nicotine did not permanently affect remote fear memories. (A) Schematic diagram illustrating the behavioural procedure aimed at testing the effects of nicotine at 1 week after its injection. (B) Rats which received saline or different doses of nicotine in Experiment 1. were subsequently tested 1 week later. No differences were detected between groups in the conditioned freezing responses to the overall CSs. (C) Schematic diagram showing the reconsolidation procedure. (D) Percentage of freezing responses before (preCS) and during (CS) the CS presentation is similar between control and nicotine (54 nmol/µl)-injected rats. (E) Schematic diagram showing the pre-acquisition experiment. (F) Nicotine (54 nmol/µl) administered before acquisition trial decreased the freezing response to CSs tested one month later. (G) Similar results were observed by analysing the total time spent freezing during both CSs presentation and the intertribal time intervals. (H–I) Total percentage of freezing responses displayed by the animals before (preCS) and during (CS) the CS administration in the context where they were originally conditioned. All values are reported as mean ± SEM.

n = 12; 27 nmol/µl, n = 12, 0.54 nmol/µl, n = 12) from the saline-injected (n = 12) rats ($F_{3,44} = 1.27$, $P > 0.05$) (Fig. 4C). No differences were detected between groups in the baseline preCS responding ($F_{3,44} = 0.44$, $P > 0.05$), thus suggesting that nicotine did not alter motor or motivational processes (Fig. 4D). In the case of the lowest dosage (0.54 nmol/µl), the low level of preCS responding

may be related to a potential floor effect. However, several observations argued against this possibility. Firstly, in the latter group the preCS responding, even if low, is still statistically higher than zero (Student's t-test, $t_{11} = 5.14$, $P < 0.001$). More important, in this group the time that animals spent in the food cup during CS presentation (51.10 ± 4.63) was similar either to that of saline-injected rats (59.75 ± 4.10) and of the other groups that received nicotine (54 nmol/μl, 57.79 ± 3.81; 27 nmol/μl, 50.82 ± 5.12) ($F_{3,44} = 1.24$, $P > 0.05$, data not shown), thus showing the lack of nicotine's effects on appetitive memories.

An alternative explanation of the present data may be that Te2 is necessary for threat but not for incentive remote memories. To address this issue, we tested whether blocking Te2 general activity would impair remote appetitive memories. We thus inactivated Te2 through the local injection of the $GABA_A$ (γ-aminobutyric acid)-receptor agonist muscimol (Letzkus et al., 2011; Martin and Ghez, 1999) (Fig. 4E). When compared with the saline-injected (n = 5) animals, the muscimol-injected (n = 6) rats showed a strongly reduced conditioned incentive response to the CSs (Student's t-test, $t_9 = 5.67$, $P < 0.001$) (Fig. 4F). These data indicate that the Te2 cortex is necessary for the retrieval of remote appetitive memories and, therefore, that any eventual disturbance in its activity would lead to detrimental effects on such emotional memories.

Overall, these results show that nicotine, at all the tested doses, did not affect incentive-memory retention, thus suggesting that intra-Te2 nicotine injection did not elicit a specific memory disturbance. Thus, nicotine selectively results in a decrease in learned fear, while incentive memories remain unaffected. We therefore conclude that acutely administered nicotine in the higher order auditory cortex does not interfere with memory retrieval processes at a general level, but instead, it selectively relieves the fear aversive emotional content associated with auditory CSs.

3.5. *Experiment 5: investigating the long-term effects of nicotine on fear-memory traces*

We then addressed the question of whether nicotine is only able to reduce fearful memories acutely, or if its effects endure for a period of time after nicotine administration. For this aim, the rats that displayed a significant reduction in conditioned freezing were re-tested 1 week after nicotine injection, in a drug-free state (Fig. 5A). The freezing responses of the nicotine-treated rats were similar to those of the control animals ($F_{3,24} = 0.70$, $P > 0.05$) (Fig. 5B). The data revealed that the effects of nicotine are not long-lasting, but are instead limited to the actual presence of nicotine in the brain.

To further characterise the impact of nicotine on memory processes, we investigated whether nicotine can interfere with the memory reconsolidation processes that occur shortly after memory retrieval. It has been shown that well-established fear memories are altered when active compounds are applied immediately after memory retrieval (Misanin et al., 1968; Nader et al., 2000; Sacchetti et al., 2007; Tedesco et al., 2014), a process referred to as memory reconsolidation (Nader et al., 2000; Schwabe and Nader, 2014; Tedesco et al., 2014). Therefore, nicotine was administered shortly after remote fear-memory recall. After 1 week, memory was tested by re-presenting the CSs (Fig. 5C). No differences were detected between the nicotine- (n = 6) and saline (n = 6)-injected animals (Student's t-test, $t_{10} = -1.09$, $P > 0.05$) (Fig. 5D), thus indicating that nicotine did not interfere with the reconsolidation mechanisms occurring after memory recall.

In addition, we investigated whether nicotine interferes with the acquisition of remote memories. To this purpose, nicotine was administered before the acquisition trial, and then memory was tested 1 month later in a drug-free state (Fig. 5E).When compared to saline −injected animals (n = 6), rats that received nicotine before the acquisition trial displayed a statistically significant decrement in the fear-evoked responses to the CSs (Student's t-test, $t_{10} = 4.23$, $P < 0.05$) and in the total time spent freezing during CSs and the inter trial intervals ($t_{10} = 3.96$, $P < 0.05$) (Fig. 5F). Taken together with the results obtained in the Experiment 1, the data indicate that nicotine affects either the formation and the retrieval of remote fear memories.

So far, the data were obtained by testing memory in a contextual environment that was completely different from that in which the conditioning trials occurred. Nonetheless, as previously mentioned, the animals that systemically received nicotine did not display any significant fear-relief effects when the threat memory was tested in the same environment to which the conditioning occurred (Elias et al., 2010; George et al., 2001). We therefore tested the impact of nicotine administration in the Te2 on the auditory fear memories in the context of which the animals were originally conditioned (Fig. 5G). In this case, the presence of contextual cues determined an elevated fear state, as indicated by the freezing of the saline- and nicotine-injected animals during the 2 min before CS presentation (Fig. 5H). During CS presentation, the freezing response was higher than during the 2 min preceding it, but similar between the saline- (n = 7) and nicotine (27 nmol/μl, n = 6; 54 nmol/μl, n = 7)-injected animals ($F_{2,17} = 0.31$, $P > 0.05$) (Fig. 5I).

Collectively, these data indicate that nicotine acutely affected the auditory fear memories when tested in a neutral environment. This effect was strictly attributed to the presence of nicotine and was not followed by any consequence of the original memory trace. The data suggest that nicotine relieves the fear charge embedded by auditory CSs but does not affect the original memory trace. This view is further supported by the evidence that, when the fear state of the animals was enhanced by the presence of contextual cues, the fear-relief effect of nicotine was abolished.

4. Discussion

In this study, we have shown that acute nicotine administration into the Te2 auditory cortex before the retrieval of remote fearful memories decreases fear-evoked responses. This effect was cue- and dose-specific. A similar treatment did not affect remote incentive memories to auditory CSs previously paired with reward stimuli. We conclude that nicotine administration in the Te2 selectively decreases the aversive charge of fear-predictive cues.

Several observations have allowed us to rule out many confounding factors that may have interfered with the emotional memory processes. First, the effects of nicotine are not secondary to any interference with innate fear and anxiety behaviour, as indicated by the open-field test and by the high level of conditioned freezing to odour CSs. In addition, nicotine administration does not affect auditory sensory perception and processing, as shown by the startle reflex paradigm as well as by the high level of memory retention to similar auditory CSs in appetitive-conditioned rats. We can also rule out the possibility that intra-Te2 nicotine administration interferes with spontaneous motor activity, thus counteracting the freezing response. In fact, nicotine injection did not affect conditioned freezing to an odour CS, and it did not change the spontaneous motor activity in the open-field paradigm – results which are in line with a previous study (Sacco and Sacchetti, 2010). Nicotine's effects may be due to state-dependent effects. Although we cannot rule out this possibility, it should be noted that nicotine administration did not reduce auditory incentive memories or fear conditioning to odour CSs. These data suggest that nicotine's effects are specifically related to fear memories. Collectively, therefore, our findings indicate that nicotine injection into the Te2 selectively interacts with memory information encoded at the level of the

auditory cortex. Furthermore, the different effects elicited by nicotine on threat and incentive memories indicate that this drug is not involved in simply disturbing the memory retrieval process, nor does it affect the memory of the physical features of the tone acting as a CS. Therefore, we suggest that nicotine, when injected into the Te2, selectively interferes with the negative aversive valence acquired by the CS.

Many studies have tested the effects of nicotine on innate spontaneous behaviour. In rodent behavioural assays of anxiety-like behaviour, nicotine can either decrease (Anderson and Brunzell, 2012; File et al., 1998; McGranahan et al., 2011; Varani et al., 2012) or promote (Cheeta et al., 2001; File et al., 1998; Irvine et al., 2001; Ouagazzal et al., 1999; Zarrindast et al., 2008) anxiety. Accordingly, nicotine elicits anxiolytic and stress-dampening, in a dose- and context-dependent manner, as well as having mood-enhancing properties in humans (Evatt and Kassel, 2010; Laje et al., 2001; Morissette et al., 2007; Picciotto, 1998). Regarding the current findings, we found that nicotine acts directly on the aversive emotional charge endowed in the perceived stimuli, independently of its action on spontaneous behaviours.

Several studies have also investigated the effects of nicotine on learned threat stimuli. The systemic administration of nicotine does not induce any effects on learned fear obtained by pairing an auditory stimulus to footshock, however it affects the extinction of contextual cues and cued fear memories (Elias et al., 2010; Kutlu and Gould, 2015; George et al., 2001; Gould and Wehner, 1999). However, when administered systemically nicotine can cause peripheral drug-associated effects, such as a modulation of the motor and/or sensory perception processes, as well as interacting with a large variety of brain regions and neuronal mechanisms, producing a complex combination of effects.

Nicotine, when administered to selected brain regions, elicits a contextual, but not cued, fear-memory strengthening, mostly due to an interaction with the memory and/or attentive processes that occur in the dorsal hippocampus (Davis et al., 2007; Kenney et al., 2012; Raybuck and Gould, 2010). Conversely, these authors also reported that nicotine injection into the ventral hippocampus impaired rather than potentiated contextual and trace fear memories (Kenney et al., 2012; Kutlu and Gould, 2015; Gould and Leach, 2014; Raybuck and Gould, 2010). Therefore, it is concluded that the different behavioural effects seen with nicotine are related to regional specificity, receptor subtype specificity and/or interactions with diverse neurotransmitter systems (Raybuck and Gould, 2010). In this scenario, we showed that nicotine administration in the more posterior region of the ventral hippocampus did not affect auditory remote fear memories, whereas nicotine injection into the Te2 auditory cortex affected learned threat tones. The odour threat stimuli and the configuration of auditory CSs in the conditioning environment were however unaffected, i.e. the effects of nicotine are highly cue- and site specific.

It is worth mentioning that the doses employed in the present study are much higher than those employed in the aforementioned studies on the dorsal and ventral hippocampus (Davis et al., 2007; Kenney et al., 2012; Raybuck and Gould, 2010). It may be therefore, that the administration of lower doses in the Te2 could produce an enhancement of the CS responses, rather than a decrease. However, in most cases, nAChRs are formed by a combination of different alpha and beta subunits and are highly heterogeneous across different brain structures (Chiamulera, 2005; Laviolette and van der Kooy, 2004; McGehee et al., 1995; Metherate et al., 2012; Pontieri et al., 1996). Thus, memory-enhancing nicotine doses (if any) can markedly differ between the hippocampus and the auditory cortex. In the latter site, in the case of auditory memory enhancement induced by intra-cortical nicotine administration, it should be also clarified as to whether these effects are due to a potentiation of the memory and/or attentive processes or rather to an improvement of the auditory stimuli perception and processing. In fact, the systemic administration of nicotine modulates tone-evoked responses in the auditory cortex (Liang et al., 2008; Metherate, 2004; Metherate et al., 2012). Future studies should therefore address these issues.

On the other hand, the doses we employed in the present study resemble those previously employed to elicit rewarding effects on spontaneous behaviour when nicotine was administered into the VTA (Laviolette and van der Kooy, 2003; Sacchetti et al., 2002). Conversely, we found that nicotine administered into the Te2 did not increase the appetitive-conditioned responses to CSs. Given that we used CS of strong motivational value, the effects of nicotine on stimulus processing may be lost to ceiling effects. However, in our study the conditioned incentive responses of the saline-treated rats were not close to the maximum value. We therefore suggest that the observed fear-relief effects are most likely to be due to direct anxiolytic-like effects on threat memories. Nevertheless, despite unlikely, we cannot ruled out the possibility that nicotine's effects may be in part due to alteration of the motivational properties of the CSs.

We also observed an effect of nicotine on the formation of new remote fear memories. The data therefore suggest that this substance interferes with the attribution of aversive charge to sensory stimuli, may be though a decrease in the fear-evoked content of the aversive experience, as we suggested during memory retrieval.

Although a large number of studies have investigated the nAChRs in several subcortical structures, such as the VTA and the nucleus accumbens, much less is known about how nAChRs are formed within the auditory cortex. Several genes have been identified for a large number of receptor subunits in the cortex (Metherate, 2004). However, most nAChRs in the sensory cortex are thought to exist as heteromers formed by α4 and β2 subunits or as α7 homomers (Metherate, 2004; Bieszczad et al., 2012). High-affinity nicotine binding has been detected mostly in cortical layers 3 and 4, but also in layers 1 and 6 (Metherate, 2004). In layer 4, α4β2-containing nAChRs may be present at the presynaptic level where they can regulate thalamo-cortical transmission (Metherate, 2004). In line with these observations, the Te2 layer 4 is reported to be markedly activated following remote fear-memory retrieval (Kwon et al., 2012; Sacco and Sacchetti, 2010). nAChRs have also been detected in interneurons present in the superficial layers (Letzkus et al., 2011; Metherate, 2004). Notably, interneurons in superficial layers of the auditory cortex are involved in fear memory (Letzkus et al., 2011; Pi et al., 2013), the activity of which is shaped by nicotine administration (Letzkus et al., 2011).

Our findings have important implications for understanding the interactions between nicotine and anxiety- and fear-related disturbances, such as PTSD. Frequently, cigarette smokers have reported that they smoke to relieve anxiety (Fidler and West, 2009; Perkins and Grobe, 1992). Stress is also a major precipitating factor in smoking relapse (Shiffman et al., 1997) and in the escalation of cigarette use (Skara et al., 2001). The present data support this view, by showing that nicotine decreases fear responses associated with perceived stimuli. Indeed, our findings have raised the intriguing idea that nicotine can relieve fear-evoked processes without impairing memory retrieval processes or decreasing the subsequent fear-memory recall in the absence of nicotine.

Cigarette smoking is associated with many mental health disorders. PTSD in particular has high comorbidity, with over 45% of PTSD sufferers reported as being smokers (Breslau et al., 2004; Dalack et al., 1998; Hughes et al., 1986; Lasser et al., 2000; Ziedonis et al., 2008; Ziedonis and George, 1997). Moreover, the rates of smoking are higher in individuals with PTSD than the rates of abuse of other compounds (Breslau et al., 2004; Dalack et al., 1998; Hughes et al., 1986; Lasser et al., 2000; Ziedonis et al.,

2008; Ziedonis and George, 1997). Although it does not precisely reproduce the entire PTSD pathology, fear conditioning is widely used to study the neural mechanisms underlying threat memories and, therefore, to also gain novel insights into the pathophysiology of PTSD (Taubenfeld et al., 2009; Tipps et al., 2014). In the present study, we focused on the effects of nicotine in the retrieval phase of memory processes, which could be similar to the mental recall/avoidance of trauma-associated stimuli observed in PTSD-suffering individuals. In this framework, our data suggest that individuals with PTSD tend to use nicotine (by smoking cigarettes) as a self-medication to relieve fear and anxiety induced by sensory cues or intrusive memories. On the other hand, our data also showed that threat memories are unaffected by nicotine after its use, i.e. PTSD-like symptoms remain intact after nicotine use. It is worth mentioning that in our study nicotine was administered locally in the cortex and acutely in drugs-naïve animals. Indeed, nicotine has very different effects depending on administration time course, route and drug history. Thus, the relationship between nicotine use and stress-related disorders requires further evaluation.

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References

Anderson, S.M., Brunzell, D.H., 2012. Low dose nicotine and antagonism of β2 subunit containing nicotinic acetylcholine receptors have similar effects on affective behavior in mice. PLoS One 7, e48665. http://dx.doi.org/10.1371/journal.pone.0048665.

Bieszczad, K.M., Kant, R., Constantinescu, C.C., Pandey, S.K., Kawai, H.D., Metherate, R., et al., 2012. Nicotinic acetylcholine receptors in rat forebrain that bind ^{18}F-nifene: relating PET imaging, autoradiography, and behavior. Synapse 66, 418–434. http://dx.doi.org/10.1002/syn.21530.

Breslau, N., 1995. Psychiatric comorbidity of smoking and nicotine dependence. Behav. Genet. 25, 95–101.

Breslau, N., Novak, S.P., Kessler, R.C., 2004. Psychiatric disorders and stages of smoking. Biol. Psychiatry 55, 69–76.

Brioni, J.D., O'Neill, A.B., Kim, D., Decker, M.W., 1993. Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus-maze test. Eur. J. Pharmacol. 238, 1–8.

Cheeta, S., Tucci, S., File, S.E., 2001. Antagonism of the anxiolytic effect of nicotine in the dorsal raphe nucleus by dihydro-beta-erythroidine. Pharmacol. Biochem. Behav. 70, 491–496.

Chiamulera, C., 2005. Cue reactivity in nicotine and tobacco dependence: a "multiple-action" model of nicotine as a primary reinforcement and as an enhancer of the effects of smoking-associated stimuli. Brain Res. Brain Res. Rev. 48, 74–97.

Dalack, G.W., Healy, D.J., Meador-Woodruff, J.H., 1998. Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings. Am. J. Psychiatry 155, 1490–1501.

Davis, J.A., Kenney, J.W., Gould, T.J., 2007. Hippocampal alpha4beta2 nicotinic acetylcholine receptor involvement in the enhancing effect of acute nicotine on contextual fear conditioning. J. Neurosci. 27, 10870–10877. Drug Alcohol Depend 51:165–172.

Elias, G.A., Gulick, D., Wilkinson, D.S., Gould, T.J., 2010. Nicotine and extinction of fear conditioning. Neuroscience 165, 1063–1073.

Evatt, D.P., Kassel, J.D., 2010. Smoking, arousal, and affect: the role of anxiety sensitivity. J. Anxiety Disord. 24, 114–123.

Ferrari, R., Le Novère, N., Picciotto, M.R., Changeux, J.P., Zoli, M., 2002. Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. Eur. J. Neurosci. 15, 1810–1818.

Fidler, J.A., West, R., 2009. Self-perceived smoking motives and their correlates in a general population sample. Nicotine Tob. Res. 11, 1182–1188.

File, S.E., Kenny, P.J., Ouagazzal, A.M., 1998. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav. Neurosci. 112, 1423–1429.

George, T.P., Picciotto, M.R., Verrico, C.D., Roth, R.H., 2001. Effects of nicotine pretreatment on dopaminergic and behavioral responses to conditioned fear stress in rats: dissociation of biochemical and behavioral effects. Biol. Psychiatry 49, 300–306.

George, T.P., Verrico, C.D., Xu, L., Roth, R.H., 2000a. Effects of repeated nicotine administration and footshock stress on rat mesoprefrontal dopamine systems: evidence for opioid mechanisms. Neuropsychopharmacology 23, 79–88.

George, T.P., Verrico, C.D., Picciotto, M.R., Roth, R.H., 2000b. Nicotinic modulation of mesoprefrontal dopamine neurons: pharmacologic and neuroanatomic characterization. J. Pharmacol. Exp. Ther. 295, 58–66.

George, T.P., Verrico, C.D., Roth, R.H., 1998. Effects of repeated nicotine pretreatment on mesoprefrontal dopaminergic and behavioral responses to acute footshock stress. Brain Res. 801, 36–49.

Gould, T.J., Leach, P.T., 2014. Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. Neurobiol. Learn. Mem. 107, 108–132. http://dx.doi.org/10.1016/j.nlm.2013.08.004.

Gould, T.J., Wehner, J.M., 1999. Nicotine enhancement of contextual fear conditioning. Behav. Brain Res. 102, 31–39.

Grieder, T.E., George, O., Tan, H., George, S.R., Le Foll, B., Laviolette, S.R., van der Kooy, D., 2012. Phasic D1 and tonic D2 dopamine receptor signaling double dissociate the motivational effects of acute nicotine and chronic nicotine withdrawal. Proc. Natl. Acad. Sci. U. S. A. 109, 3101–3106.

Grosso, A., Cambiaghi, M., Concina, G., Sacco, T., Sacchetti, B., 2015. Auditory cortex involvement in emotional learning and memory. Neuroscience 299, 45–55. http://dx.doi.org/10.1016/j.neuroscience.2015.04.068.

Hughes, J.R., Hatsukami, D.K., Skoog, K.P., 1986. Physical dependence on nicotine in gum. A placebo substitution trial. JAMA 255, 3277–3279. http://dx.doi.org/10.3791/3446. J Vis Exp 1:e3446.

Irvine, E.E., Bagnalasta, M., Marcon, C., Motta, C., Tessari, M., File, S.E., Chiamulera, C., 2001. Nicotine self-administration and withdrawal: modulation of anxiety in the social interaction test in rats. Psychopharmacology (Berl) 153, 315–320.

Kenney, J.W., Raybuck, J.D., Gould, T.J., 2012. Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. Hippocampus 22, 1681–1690.

Kim, J.J., Fanselow, M.S., 1992. Modality-specific retrograde amnesia of fear. Science 256, 675–677.

Kutlu, M.G., Gould, T.J., 2015. Nicotinic receptors, memory, and hippocampus. Curr. Top. Behav. Neurosci. 23, 137–163. http://dx.doi.org/10.1007/978-3-319-13665-3_6.

Kwon, J.T., Jhang, J., Kim, H.S., Lee, S., Han, J.H., 2012. Brain region-specific activity patterns after recent or remote memory retrieval of auditory conditioned fear. Learn. Mem. 19, 487–494.

Laje, R.P., Berman, J.A., Glassman, A.H., 2001. Depression and nicotine: preclinical and clinical evidence for common mechanisms. Curr. Psychiatry Rep. 3, 470–474.

Lasser, K., Boyd, J.W., Woolhandler, S., Himmelstein, D.U., McCormick, D., Bor, D.H., 2000. Smoking and mental illness: a population-based prevalence study. JAMA 284, 2606–2610.

Laviolette, S.R., Lauzon, N.M., Bishop, S.F., Sun, N., Tan, H., 2008. Dopamine signaling through D1-like versus D2-like receptors in the nucleus accumbens core versus shell differentially modulates nicotine reward sensitivity. J. Neurosci. 28, 8025–8033.

Laviolette, S.R., van der Kooy, D., 2003. Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. Mol. Psychiatry 8, 50–59.

Laviolette, S.R., van der Kooy, D., 2004. The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. Nat. Rev. Neurosci. 5, 55–65.

Lesburguères, E., Gobbo, O.L., Alaux-Cantin, S., Hambucken, A., Trifilieff, P., Bontempi, B., 2011. Early tagging of cortical networks is required for the formation of enduring associative memory. Science 331, 924–928.

Letzkus, J.J., Wolff, S.B., Meyer, E.M., Tovote, P., Courtin, J., Herry, C., Lüthi, A., 2011. A disinhibitory microcircuit for associative fear learning in the auditory cortex. Nature 480, 331–335.

Liang, K., Poytress, B.S., Weinberger, N.M., Metherate, R., 2008. Nicotinic modulation of tone-evoked responses in auditory cortex reflects the strength of prior auditory learning. Neurobiol. Learn. Mem. 90, 138–146.

Martin, J.H., Ghez, C., 1999. Pharmacological inactivation in the analysis of the central control of movement. J. Neurosci. Methods 86, 145–159.

McGehee, D.S., Heath, M.J., Gelber, S., Devay, P., Role, L.W., 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269, 1692–1696.

McGranahan, T.M., Patzlaff, N.E., Grady, S.R., Heinemann, S.F., Booker, T.K., 2011. α4β2 nicotinic acetylcholine receptors on dopaminergic neurons mediate nicotine reward and anxiety relief. J. Neurosci. 31, 10891–10902.

Metherate, R., 2004. Nicotinic acetylcholine receptors in sensory cortex. Learn. Mem. 11, 50–59.

Metherate, R., Intskirveli, I., Kawai, H.D., 2012. Nicotinic filtering of sensory processing in auditory cortex. Front. Behav. Neurosci. 19 (6), 44.

Misanin, J.R., Miller, R.R., Lewis, D.J., 1968. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160, 554–555.

Morissette, S.B., Tull, M.T., Gulliver, S.B., Kamholz, B.W., Zimering, R.T., 2007. Anxiety, anxiety disorders, tobacco use, and nicotine: a critical review of interrelationships. Psychol. Bull. 133, 245–272.

Nader, K., Schafe, G.E., Le Doux, J.E., 2000. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406, 722–726.
Ouagazzal, A.M., Kenny, P.J., File, S.E., 1999. Modulation of behaviour on trials 1 and 2 in the elevated plus-maze test of anxiety after systemic and hippocampal administration of nicotine. Psychopharmacology (Berl) 144, 54–60.
Panagis, G., Nisell, M., Nomikos, G.G., Chergui, K., Svensson, T.H., 1996. Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. Brain Res. 730, 133–142.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.
Perkins, K.A., Grobe, J.E., 1992. Increased desire to smoke during acute stress. Br. J. Addict. 87, 1037–1040.
Pi, H.J., Hangya, B., Kvitsiani, D., Sanders, J.I., Huang, Z.J., Kepecs, A., 2013. Cortical interneurons that specialize in disinhibitory control. Nature 503, 521–524.
Picciotto, M.R., 1998. Common Aspects of the Action of Nicotine and Other Drugs of Abuse.
Pontieri, F.E., Tanda, G., Orzi, F., Di Chiara, G., 1996. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382, 255–257.
Raybuck, J.D., Gould, T.J., 2010. The role of nicotinic acetylcholine receptors in the medial prefrontal cortex and hippocampus in trace fear conditioning. Neurobiol. Learn. Mem. 94, 353–363.
Sacchetti, B., Baldi, E., Lorenzini, C.A., Bucherelli, C., 2002. Cerebellar role in fear-conditioning consolidation. Proc. Natl. Acad. Sci. U. S. A. 11, 8406–8411.
Sacchetti, B., Lorenzini, C.A., Baldi, E., Tassoni, G., Bucherelli, C., 1999. Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. J. Neurosci. 19, 9570–9578.
Sacchetti, B., Sacco, T., Strata, P., 2007. Reversible inactivation of amygdala and cerebellum but not perirhinal cortex impairs reactivated fear memories. Eur. J. Neurosci. 25, 2875–2884.
Sacchetti, B., Scelfo, B., Tempia, F., Strata, P., 2004. Long-term synaptic changes induced in the cerebellar cortex by fear conditioning. Neuron 42, 973–982.
Sacco, T., Sacchetti, B., 2010. Role of secondary sensory cortices in emotional memory storage and retrieval in rats. Science 329, 649–656.
Saddoris, M.P., Holland, P.C., Gallagher, M., 2009. Associatively learned representations of taste outcomes activate taste-encoding neural ensembles in gustatory cortex. J. Neurosci. 29, 15386–15396.
Schwabe, L., Nader, K., Pruessner, 2014. Reconsolidation of human memory: brain mechanisms and clinical relevance. Biol. Psychiatry 76, 274–280.
Shiffman, S., Hickcox, M., Paty, J.A., Gnys, M., Richards, T., Kassel, J.D., 1997. Individual differences in the context of smoking lapse episodes. Addict. Behav. 22, 797–811.
Skara, S., Sussman, S., Dent, C.W., 2001. Predicting regular cigarette use among continuation high school students. Am. J. Health Behav. 25, 147–156.
Stolerman, I.P., Jarvis, M.J., 1995. The scientific case that nicotine is addictive. Psychopharmacology (Berl) 117, 2–10 discussion 14–20.
Sun, N., Laviolette, S.R., Addiction Research Group, 2014. Dopamine receptor blockade modulates the rewarding and aversive properties of nicotine via dissociable neuronal activity patterns in the nucleus accumbens. Neuropsychopharmacology 39, 2799–2815.
Taubenfeld, S.M., Riceberg, J.S., New, A.S., Alberini, C.M., 2009. Preclinical assessment for selectively disrupting a traumatic memory via postretrieval inhibition of glucocorticoid receptors. Biol. Psychiatry 65, 249–257.
Tedesco, V., Mutti, A., Auber, A., Chiamulera, C., 2014. Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation. Behav. Pharmacol. 25, 725–731. http://dx.doi.org/10.1097/FBP.0000000000000088.
Tipps, M.E., Raybuck, J.D., Lattal, K.M., 2014. Substance abuse, memory, and post-traumatic stress disorder. Neurobiol. Learn. Mem. 112, 87–100. http://dx.doi.org/10.1016/j.nlm.2013.12.002.
Trigo, J.M., Martin-García, E., Berrendero, F., Robledo, P., Maldonado, R., 2009. The endogenous opioid system: a common substrate in drug addiction. Drug Alcohol Depend. 108, 183–194.
Valsamis, B., Schmid, S., 2011. Habituation and Prepulse Inhibition of Acoustic Startle in Rodents.
Varani, A.P., Moutinho, L.M., Bettler, B., Balerio, G.N., 2012. Acute behavioural responses to nicotine and nicotine withdrawal syndrome are modified in GABA(B1) knockout mice. Neuropharmacology 63, 863–872.
Zarrindast, M.R., Solati, J., Oryan, S., Parivar, K., 2008. Effect of intra-amygdala injection of nicotine and GABA receptor agents on anxiety-like behaviour in rats. Pharmacology 82, 276–284.
Ziedonis, D., Hitsman, B., Beckham, J.C., Zvolensky, M., Adler, L.E., Audrain-McGovern, J., et al., 2008. Tobacco use and cessation in psychiatric disorders: National Institute of Mental Health report. Nicotine Tob. Res. 10, 1691–1715.
Ziedonis, D.M., George, T.P., 1997. Schizophrenia and nicotine use: report of a pilot smoking cessation program and review of neurobiological and clinical issues. Schizophr. Bull. 23, 247–254.
Zilles, K., 1985. In: Zilles, K. (Ed.), The Cortex of the Rat. Springer-Verlag, , Berlin.