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Nicotine exposure leads to deficits in differential cued fear conditioning in mice and humans: A potential role of the anterior cingulate cortex

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Abstract

Stress and anxiety disorders such as posttraumatic stress disorder (PTSD) are characterized by disrupted safety learning. Tobacco smoking has been strongly implicated in stress and anxiety disorder symptomatology, both as a contributing factor and as a vulnerability factor. Rodent studies from our lab have recently shown that acute and chronic nicotine exposure disrupts safety learning. However, it is unknown if these effects of nicotine translate to humans. The present studies addressed this gap by administering a translational differential cued fear conditioning paradigm to both mice and humans. In mice, we found that chronic nicotine exposure reduced discrimination between a conditioned stimulus (CS) that signals for danger (CS+) and another CS that signals for safety (CS-) during both acquisition and testing. We then employed a similar differential cued fear conditioning paradigm in human smokers and non-smokers undergoing functional magnetic resonance imaging (fMRI). Smokers showed reduced CS+/CS- discrimination during fear conditioning compared to non-smokers. Furthermore, using fMRI, we found that subgenual and dorsal anterior cingulate cortex activations were lower in smokers than in non-smokers during differential cued fear conditioning. These results suggest a potential biological mechanism underlying a dysregulated ability to discriminate between danger and safety cues. Our results indicate a clear parallel between the effects of nicotine exposure on safety learning in mice and humans and therefore suggest that smoking might represent a risk factor for inability to process information related to danger and safety related cues.

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Conflict of interest

We declare no potential conflict of interest.

Keywords

Nicotine; Fear conditioning; Skin conductance responses; PTSD; fMRI

Stress and fear-based disorders such as posttraumatic stress disorder (PTSD) are characterized by dysregulated emotional responses such as re-experiencing the traumatic memories, avoidance of trauma-related cues and contexts, as well as hyperarousal even in contexts that were previously perceived as safe [1,2]. Various laboratory paradigms have been used to probe some features associated with the pathophysiology of PTSD, notably contextual and cued fear conditioning paradigms. Results from multiple studies have demonstrated that patients with fear-based and anxiety disorders exhibit impaired fear extinction [3–5] as well as disrupted safety discrimination [6–9]. Specifically, these results indicated that war veterans and civilians with PTSD failed to reduce fear response to stimuli that signal safety [7–9]. Moreover, Craske et al., [10] showed that children with a lower ability to learn safety had a higher risk of developing anxiety disorders as adults. Overall, these studies suggest that impairments in safety learning may contribute to the development and maintenance of fear and anxiety-related symptoms. Importantly, neuroimaging reports have identified key brain structures involved in the fear circuitry, such as the amygdala, the hippocampus, the dorsal anterior cingulate cortex, the subgenual cingulate cortex, and the medial prefrontal cortex [11,68,12]. Multiple studies have shown that the activation of these brain regions is dysregulated in patients suffering from PTSD [13–16,5,17–21].

Tobacco smoking has been strongly implicated as a contributing factor in various psychopathologies, notably anxiety disorders and PTSD [22–24]. For example, smoking rates are significantly higher in PTSD patients compared to the healthy population (45.3% vs. 22.5%; [22,24]). Studies also showed that prior smoking behavior increased the likelihood of developing PTSD and smoking initiation rate increased following the development of PTSD [25–27]. Moreover, there is evidence showing that PTSD symptom severity is positively correlated with severity of nicotine dependence, suggesting that smoking may directly regulate PTSD symptomatology [28]; see [29] for a review). The extent to which nicotine is associated with particular features of PTSD, notably the ability to discriminate between safe and dangerous cues or contexts, still remains to be elucidated.

Recently, studies from our laboratories investigated the effects of acute and chronic nicotine exposure on animal safety learning paradigms such as contextual fear extinction and contextual safety discrimination [30–34]. Our results showed that acute and chronic nicotine impaired encoding and retrieval of contextual fear extinction memories [30,32–34]. Moreover, we showed that acute nicotine resulted in disruption of contextual safety discrimination in mice [31]. That is, mice that received acute nicotine injections prior to both training and testing failed to learn to reduce fear in safe contexts. Together with previous results from our group showing that acute nicotine augments fear conditioning in mice [35–39], these results suggest that nicotine exposure may significantly contribute to anxiety disorder symptomatology by modulating fear conditioning, fear extinction, and safety learning processes; though the neural substrates involved in the contribution of nicotine is largely unknown.

While results from rodent models clearly demonstrate that nicotine is an important factor modulating discrimination between dangerous and safe contexts, it is not clear if nicotine exposure also affects cued fear discrimination and whether there is any translational value to humans. Therefore, in the present study we used cued fear conditioning paradigms that have been shown to yield robust differential cued conditioning in each species [40,21]. Specifically, we investigated the effects of chronic nicotine administration in mice, using freezing levels, as well as the effects of chronic smoking in humans, using skin conductance responses (SCR) and blood-oxygenated-level-dependent (BOLD) signal. Based on our previous results showing that acute nicotine impairs safety learning, we predicted that nicotine exposure would disrupt discrimination between danger cues and safety cues. Given the extensive neuroimaging literature on the brain structures involved in the fear circuitry, we focused our attention on two subcortical (amygdala and hippocampus) and three cortical (dorsal anterior cingulate cortex [dACC], subgenual anterior cingulate cortex [sgACC], and ventromedial prefrontal cortex [vmPFC]) brain structures. We predicted that relative to the non-smoker group, smokers would exhibit dysregulated activity in these nodes of the fear circuitry.

1. Methods and materials

1.1. Mouse study

1.1.1. Subjects—For the rodent experiments, subjects were adult male C57BL6/J mice (8–10 weeks old, Jackson Laboratory, Bar Harbor, ME). All mice were group-housed and maintained in a 12 h light/dark cycle with access to food and water ad-libitum. Training and testing occurred during the light period, between 9:00 am and 7:00 pm. Behavioral procedures used in this study were approved by the Temple University Institutional Animal Care and Use Committee.

1.1.2. Osmotic mini-pump surgeries and chronic nicotine administration—In order to model long-term nicotine exposure in human smokers, nicotine hydrogen tartrate salt 12.6 mg/kg/d; (freebase concentration) dissolved in 0.9% saline was subcutaneously delivered via osmotic mini-pumps (Alzet, Model 1002, Durect, Cupertino, CA). For the chronic nicotine experiment, mice received 12.6 mg/kg per day for 14 days. In order to administer nicotine chronically, osmotic mini-pumps delivering continuous nicotine at a 0.26 μ l/hr rate were surgically inserted via an incision made on the lower back of the mouse. Sham control mice received the same surgery but no mini-pumps were placed subcutaneously. Mice were under 2–5% isoflurane anesthetic during surgeries. We chose the 12.6 mg/kg/day dose because previous reports showed that rodent plasma nicotine and cotinine levels resulting from the administration of this dose were comparable to the levels observed in human smokers [41–47].

1.1.3. Apparatus—Differential cued fear conditioning and testing took place in two different contexts: training context and testing context. Training context consisted of 4 identical conditioning chambers (18.8 $\times$ 20 $\times$ 18.3 cm) with Plexiglas front wall and aluminum side walls, placed in sound-attenuating boxes (MED Associates, St. Albans, VT). Testing context also consisted of 4 identical testing chambers (23.5 $\times$ 20.3 $\times$ 19.1 cm)

distinguished from the training context by the dimensions, round aluminum sides. Also, a black-and white-striped visual cue was attached to the back Plexiglas wall of the chambers. The floors were metal grids (0.20 cm and 1.0 cm apart) connected to a shock generator that produced a 2 s, 0.57 mA foot-shock unconditioned stimulus (US). Ventilation fans were located at the back of the boxes providing a background noise (65 dB). The two different conditioned stimuli (CS) were an 85 db white noise and an 85 ddB tone, both produced by a speaker located on the right wall of the conditioning chambers. All conditioned and unconditioned stimuli were controlled by an IBM-PC compatible computer running MED-PC software. All chambers were cleaned with 70% ethanol between each subject.

1.1.4. Procedure—The dependent variable for the rodent experiments was freezing behavior, defined as the absence of movement except respiration [48]. Freezing scores were collected using a time-sampling technique where each subject was observed for a duration of 1 s out of every 10 s and scored as either freezing or not freezing. Then freezing scores were converted to percent freezing (%freezing) for statistical analysis. Experimenters were blind to drug conditions throughout the study.

During the training phase, all mice were trained in differential cued fear conditioning [49] wherein one CS was paired with the foot-shock US (CS+) and another CS signaled the absence of the US (CS−). The white noise and tone stimuli were counterbalanced between CS+ and CS−. Consequently, half of the subjects received white noise as CS+ and tone as CS−, and the other half received tone as CS+ and white noise as CS−. The CS+ presentations were 30 s in duration and co-terminated with a 2 s 0.57 mA foot-shock US. Following a 120 s baseline period, 5 presentations of each CS+ and CS− were pseudorandomly presented with a 30 s inter-trial interval in average. Freezing responses were measured for the first 120 s baseline period and during each CS presentation. Testing occurred 24 h after the training session. Mice were removed from the training cages 30 s after the last CS presentation and returned to their homecages. During the testing phase, all mice were placed in the testing context and, following a 120 s baseline period, received 5 CS+ and 5 CS− presentations. For testing, the US presentations were omitted during CS+ trials. Mice received 12.6 mg/kg per day for 14 days. Differential cued fear conditioning training and testing occurred on the 13th and 14th days of nicotine administration.

1.1.5. Statistical analysis—Freezing levels for CS+ and CS− during training and testing were examined using separate 3-way repeated-measure ANOVAs (Drug X CS X Trial). Planned comparison *t*-tests at $\alpha = 0.05$ level were used for comparisons between CS+ and CS− for each drug group. Group sizes are indicated in figure captions. All statistical analyses were run using SPSS 21.0 (IBM, Armonk, NY).

1.2. Human study

1.2.1. Participants—Data from 23 men (mean age = 61.3, SEM = 1.2) are included in the current manuscript. These participants were recruited as part of a larger study of combat-exposed men and their combat-unexposed identical co-twins. For the current study, we included only the combat-unexposed co-twins. All participants provided written informed consent in accordance with the Partners Healthcare System. Of these 23 participants, 7

reported being regular smokers and 16 reported being non-smokers. At the time of the study, none of the participants met criteria for any psychopathologies (excluding nicotine dependence), based on Structured Clinical Interview for DSM-IV.

1.2.2. Procedure—In an fMRI setting, participants completed a well-validated differential cued fear conditioning paradigm as BOLD signal of brain and concurrent skin conductance levels (SCL) were collected. The paradigm has been described in previous publications (e.g., [50–53,54,21,55]). Briefly, participants viewed images projected onto a screen as they lay supine in an fMRI scanner (3T Siemens Magnetom Trio) using a 12-channel head coil (TR = 3 s) to acquire BOLD signal from brain. Before entering the scanner, shock electrodes were placed on the second and third finger of the participant's dominant hand and each participant was asked to select a level of electrical stimulation that was highly annoying but not painful. The experimenter then placed two SCL-recording Ac/Ag electrodes on the palm of the participant's non-dominant hand. In this paradigm, all trials had a similar structure- an image of a context (either an office with a lamp or a library with a lamp) appeared for 3 s with the lamp light off. The lamp light then turned on to one of three colors (red, yellow or blue) and stayed on for 6 s. These three colored lamps served as the CS and the finger shock served as the US. Two of the lamp colors were partially reinforced (62.5% of the time) with the delivery of a 0.5 s electrical stimulation (CS+) at the offset of the CS presented. One lamp color was never paired with a shock (CS–). Intertrial intervals consisted of a black screen lasting 12–18 s with an average of 15 s.

1.2.3. Data processing

Psychophysiology: SCR were computed for each trial of the fear-conditioning paradigm by using the maximum SCL reached during the CS presentation and subtracting from it the average SCL of the last 2 s of the context presentation. Raw values were then square root transformed in order for the data to follow a normal distribution and to allow the use of parametric statistical tests. Differential scores were computed for each trial (SCR to the CS+ minus SCR to the corresponding CS– trial). Averages were computed for the first four and the last four differential trials of each CS+, resulting in 8 early conditioning trials (first four trials of each CS+) and 8 late conditioning trials (last four trials of each CS+).

Imaging: Coordinates are based on the Montreal Neurological Institute (MNI) system. SPM8 (The Wellcome Trust; www.fil.ion.ucl.ac.uk) was used for processing the imaging data. Functional MR images were realigned, corrected for slice timing, co-registered with a T1 image, normalized in MNI space and smoothed with a 9 mm full width half-maximum Gaussian kernel to increase the signal-to-noise ratio. High-pass temporal filtering with a cutoff of 128 s was applied to remove low frequency signal drift. Serial correlations in the fMRI time series due to aliased biorhythms were accounted for using an autoregressive AR(1) model. Functional time series for each participant were modeled with regressors specifying stimulus onsets. Artifact detection toolbox (ART, <http://gablab.mit.edu>) was applied and the motion artifact data detected through the use of that tool were used in the first-level analysis.

Based on the literature, we identified five regions of interests (ROIs) that have been shown to be involved in fear conditioning paradigms: average of left and right amygdala, average of left and right hippocampus, dACC, sgACC, and vmPFC. For the amygdala and the hippocampus, the Anatomical Automatic Labeling Atlas [56] was used to create a mask of each of these ROIs. The masks for the three other ROIs (dACC, sgACC, and vmPFC) were created with the use of Neurosynth (neurosynth.org), which is a searchable online automated synthesis of fMRI data. By using the keywords ‘fear conditioning’; we identified clusters within 248 studies with estimated peaks at the following coordinates: dACC (MNI_{xyz} = 0; 24; 22); vmPFC (MNI_{xyz} = 6; 40; -20); sgACC (MNI_{xyz} = 0; 16; -18). In our functional data; a sphere of 5 mm centered around these coordinates was created for each region. Beta-weights; which are parameter estimates for brain activations; were then extracted from these ROIs for the following contrasts: early CS+ vs. early CS- contrast and late CS+ vs. late CS-. Positive beta-weights could be interpreted as an increased activation in a given ROI for the contrast of interest whereas negative beta-weights could be interpreted as a decrease activation in a given ROI for the contrast of interest.

2. Results

2.1. Mouse study

2.1.1. Effects of chronic nicotine on differential cued fear conditioning—

Separate $2 \times 2 \times 5$ repeated measures ANOVAs for training and testing phases showed that the Drug (Nicotine vs. Sham) X CS (CS+ vs. CS-) X Trial interaction was significant during training ($F(4128) = 2.523$, $p < 0.05$; Fig. 1A) and testing ($F(4128) = 2.816$, $p < 0.05$; Fig. 1B). Moreover, Bonferroni-corrected t -tests showed that the difference between CS+ and CS- percent freezing was significant for the 1st, 2nd, 3rd, and 5th training trials and for the first 3 testing trials in the sham controls. However, the chronic nicotine group showed no significance difference for any of the training trials and only for the 2nd trial during testing, which demonstrates that chronic nicotine disrupted both acquisition and recall of differential cued fear learning. Finally, we averaged CS+ and CS- freezing scores during training and computed differential cued fear conditioning scores by subtracting CS- freezing from CS+ freezing. A one-way ANOVAs showed that the main effect of Drug was significant for averaged differential cued fear conditioning scores during training ($F(1.32) = 8.795$, $p < 0.05$; Fig. 1C) and approached significance during testing ($F(1.32) = 3.262$, $p = 0.08$). Overall, these results demonstrate that chronic nicotine impaired differential cued fear conditioning (Fig. 1).

2.2. Human study

Independent samples t -tests were performed on age, years of education, and selected shock levels. The smoker and non-smoker groups did not differ significantly from each other on any of these variables, all $t(21) < 0.710$, all p s > 0.485 (data not shown). When looking at early differential SCR, there was a significant group difference, $t(21) = 2.24$, $p = 0.036$, with the smokers showing lower differential SCR during early conditioning relative to non-smokers (Fig. 2A). This trend-level difference was driven by an attenuated response to the early CS+ trials in the smoker group relative to the non-smoker group, $t(21) = 1.93$, $p = 0.067$ (data not shown). The two groups did not differ from each other for the CS- trials,

$t(21) = 0.58$, $p = 0.567$ (data not shown). The late conditioning differential SCR did not reveal any group differences, $t(21) = 1.55$, $p = 0.140$ (Fig. 2B).

For the early CS+ vs. early CS- neuroimaging contrast, the smoker group was found to exhibit lower activation in the following ROIs: sgACC ($t(21) = 3.63$, $p = 0.002$) and dACC ($t(21) = 2.94$, $p = 0.032$). No group differences were found in vmPFC, amygdala, or hippocampus (all $ps > 0.05$) (data not shown for these three ROIs). For the sake of completeness, beta-weights for the full contrast (early CS+ vs. early CS-), as well as for each condition of the contrast (early CS+ and early CS- separately), are plotted for sgACC (Fig. 3A) and dACC (Fig. 3B).

For the late CS+ vs. late CS- neuroimaging contrast, the smoker group showed significantly lower activation in the sgACC, $t(21) = 2.05$, $p = 0.053$, with that region activating less in the smoker group than in the non-smoker group (see Fig. 4A). No group differences were found for the other ROIs (amygdala, hippocampus, dACC, and vmPFC), all $ps > 0.05$ (data not shown for these four ROIs). For the sake of completeness, beta-weights of the sgACC during late CS+ vs. late CS- contrast as well as each condition separately (late CS+, late CS-) are plotted (Fig. 4A).

3. Discussion

The results of the present study show that chronic nicotine exposure resulted in impaired differential cued fear conditioning in mice. In humans, chronic smokers showed similar deficits as mice. That is, compared to non-smokers, chronic smokers showed disrupted early differential cued fear conditioning as their CS+ responses were blunted during training. Importantly, our fMRI results showed that the impaired differential cued fear conditioning found in chronic smokers was associated with decreased activation of the sgACC and dACC. Overall, these results demonstrate that chronic nicotine has similar effects on learning and memory for both rodents and humans, causing disrupted safety learning.

The finding that chronic nicotine results in impairment of differential cued fear conditioning by increasing freezing response to CS- extends our previous reports showing that acute nicotine impaired contextual safety discrimination [31]. This suggests that chronic nicotine could facilitate maladaptive fear learning and fear responses. In further support, several reports from our laboratory demonstrated that acute and chronic nicotine impaired acquisition of contextual fear extinction [30,32,33]. Inability to extinguish fear responses and to differentiate between safe and dangerous stimuli could induce and exacerbate fear and anxiety disorders in smokers.

Our results from the fMRI experiment suggest that sgACC and dACC may contribute to the effects of chronic nicotine on differential cued fear conditioning in humans. Although we do not have direct evidence for the involvement of ACC in nicotine's effects on differential cued fear conditioning in mice, some parallels may be drawn. For example, in rodent models, ACC is necessary for trace fear conditioning [57] and visual discrimination learning [58], both of which require a certain level of attentional capacity and awareness of contingency [59]. In support, ACC has been shown to be involved in conscious anticipation

of fearful stimuli in cued fear conditioning in humans [60–62]. Therefore, ACC may also be critically involved in differential cued fear conditioning in mice as the task requires awareness of the discrimination between dangerous and safe CSs. Moreover, Gulick and Gould [63] found that acute nicotine infusions into the ACC reversed the ethanol-induced deficits in a plus-maze discriminative avoidance task where mice were required to discriminate between safe and dangerous arms. This suggests that ACC may contribute to nicotine's effects on safety discrimination. The involvement of the ACC in the fear circuitry has been demonstrated extensively in the human literature. Activations in the ACC have been linked with fear learning and fear expression [11,68,12]. Thickness of the ACC has been shown to correlate with fear expression, measured by SCR, during cued fear conditioning [52]. The literature has suggested that the ACC plays a major role in error detection [64]. The partial reinforcement schedule that we used during fear conditioning in the human experiment increases the level of uncertainty regarding the delivery of a shock. This likely increases the activation of the ACC so it can track the contingencies and help in discriminating between the CS+ and the CS-. Nicotine might lower the ACC activation, which in turn decreases the ability to correctly discriminate between the threat and the safety cues. This is ultimately translated at the SCR level, where a decreased differential signal is observed. Importantly, during early conditioning, the activations of the sgACC and the dACC were significantly reduced in the smoker group relative to the non-smoker group and this was reflected by a lower differential signal in the SCR data. Towards the end of conditioning (late), only the sgACC showed reduced activation and no between-group differences were found at the SCR level. Previous studies have shown that the activation of the dACC was positively correlated with SCR during conditioning [52]. Therefore, given that the two groups differ from each other with regards to differential SCR and dACC activation only during early conditioning, this provides further support for the role of the dACC in sustaining autonomic responses. The sgACC involvement in differential fear learning might not be directly associated with SCR.

The current study has limitations. First, the human study was not designed to assess the impact of nicotine on fear conditioning. Based on the rodent results post-hoc analyses were performed to determine whether the same phenomenon occurs in humans and whether the pilot neuroimaging results could inform about potential neural mechanisms. Consequently, the groups were not balanced and the sample size for the smoker group was relatively small. Moreover, the degree of nicotine dependence was not assessed and no information was available with regards to the time elapsed between the scan and the last cigarette that the participants had. It is also important to note that while the mice in our animal study received only nicotine, human smokers are exposed to other substances in cigarette smoke (e.g., tar and carbon monoxide). Nevertheless, the converging behavioral effects of nicotine exposure in mice and smoking in humans indicate that nicotine may be a key substance in mediating changes in fear learning and memory in both species. Finally, our sample was exclusively composed of older men. Despite these limitations, it is important to highlight the uniqueness of our translational findings. In fact, to our knowledge, it is the first study to assess the impact of nicotine on threat and safety learning across species. Despite some minor differences in the fear conditioning procedures between both species, the converging data between the mouse and human studies suggest an important association between nicotine

consumption and how threat and safety signals are processed. Moreover, our pilot neuroimaging data suggest brain region that could underlie such phenomenon and that should be further investigated in more powered and better controlled studies.

Taken together, our results suggest that chronic nicotine may disrupt safety learning both in rodents and humans. These results are in line with studies showing that smokers exhibit higher vulnerability to developing anxiety and stress disorders [25,27] as deficits in safety learning are critically associated with the etiology of these psychopathologies [7]. Moreover, our results showing abnormal ACC activation during differential cued fear conditioning in smokers suggest that ACC activation patterns may contribute to these behavioral deficits. This conclusion is supported by studies linking ACC abnormalities with anxiety and stress disorders [65,66]. For example, similar to our results showing lower ACC activation in smokers during differential cued fear conditioning, several studies suggest that the ACC is dysregulated in PTSD patients during aversive learning, which was accompanied by inadequate aversive learning [65–67]. Overall, these results have important clinical implications as they suggest that smoking is potentially a risk factor for populations that experience traumatic episodes. Nevertheless, future studies are needed to identify the role of ACC in safety learning deficits in nicotine dependence.

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Fig. 1.

Chronic nicotine administration disrupts acquisition and recall of differential cued fear conditioning. Panel A: Trial by trial CS+ and CS- scores for the sham controls during training (Left Panel) and testing (Right Panel). Sham control mice (n = 12) show successful discrimination between CS+ and CS-. Panel B: Trial by trial CS+ and CS- scores for the chronic nicotine administered group during training (Left Panel) and testing (Right Panel). Chronic nicotine administered mice (n = 12) showed disrupted acquisition and recall of differential cued fear conditioning. Panel C: Differential cued fear conditioning scores (CS+ freezing – CS- freezing) for the chronic nicotine group and sham controls during training.

Error bars indicate Standard Error of the Mean (SEM). (*) denotes significant differences between nicotine group and sham controls at the $p < 0.05$ level.

Fig. 2.

Average differential skin conductance responses (SCR) during fear conditioning as a function of Time (Early Conditioning – Panel A; Late Conditioning – Panel B) and Group (Non-Smokers and Smokers). Differential SCR signal is calculated by using SCR to the CS+ minus SCR to the corresponding CS-. Early refers to the first four trials of each CS+ (total of 8 trials) whereas Late refers to the last four trials of each CS+ (total of 8 trials). The white bars represent the non-smoker group (n = 16) whereas the black bars represent the smoker group (n = 7). Error bars indicate standard error of the mean (SEM). (*) denotes significant group differences at $p < 0.05$ level. Units for SCR = microsiemens (μS).

Early Conditioning

Fig. 3.

Significant group differences for BOLD signal during early fear conditioning (early CS+ vs. early CS- contrast). Panel A: Subgenual anterior cingulate cortex (sgACC) seed ROI (5 mm sphere at 0,16,-18) from which BOLD signal was extracted from both groups. Averages beta-weights for the early CS+ vs. early CS- contrast, for non-smokers and smokers. Panel B: Dorsal anterior cingulate cortex (dACC) seed ROI (5 mm sphere at 0,24,22) from which BOLD signal was extracted for both groups. Averages beta-weights for the early CS+ vs. early CS- contrast, for non-smokers and smokers. For both panels, the average beta-weights for the CS+ signal and the CS- signal alone as a function of group are also graphed in order to give a more complete picture of the group difference found. The white bars represent the

non-smoker group whereas the black bars represent the smoker group. Error bars indicate standard error of the mean (SEM). (*) denotes significant group differences at $p < 0.05$ level.

Late Conditioning

Fig. 4.

Significant group differences for BOLD signal in the subgenual anterior cingulate cortex (sgACC) during late fear conditioning (late CS+ vs. late CS- contrast). Averages beta-weights for the late CS+ vs. late CS- contrast, for non-smokers and smokers. The average beta-weights for the CS+ signal and the CS- signal alone as a function of group are also graphed in order to give a more complete picture of the group difference found. The white bars represent the non-smoker group whereas the black bars represent the smoker group. Error bars indicate standard error of the mean (SEM). (*) denotes significant group differences at $p < 0.05$ level.