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Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses

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Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses

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Abstract:	Despite an increased understanding of nicotine addiction, there is a scarcity of research comparing the neural correlates of non-drug reward between smokers and ex-smokers. Long-term changes in reward-related brain functioning for non-drug incentives may elucidate patterns of functioning that potentially contribute to ongoing smoking behaviour in current smokers. Similarly, examining the effects of previous chronic nicotine exposure during a period of extended abstinence may reveal whether there are neural correlates responsible for non-drug reward processing that are different from current smokers. The current study, therefore, set out to examine the neural correlates of reward and loss anticipation, and their respective outcomes, in smokers, ex-smokers and matched controls using a monetary incentive delay task during functional MRI. Here we report that in the absence of any significant behavioural group differences, both smokers and ex-smokers showed a significantly greater activation change in the lateral orbitofrontal/anterior insular cortex compared to smokers when anticipating both potential monetary gains and losses. We further report that ex-smokers showed a significantly greater activation change in the ventral putamen compared to both controls and smokers, and in the caudate compared to controls during the anticipation of potential monetary losses only. The results suggest that smoking may sensitize striato-orbitofrontal circuitry subserving motivational processes for loss avoidance and reward gain in nicotine addiction.

Reviewer: 2

Comments to the Author
This is a revision of an article detailing brain response differences between smokers, ex-smokers and non-smokers using the monetary incentive delay task. The authors did a great job addressing previous concerns and clarifying the analysis methods.

A major limitation of the study is the sample size which could only be addressed by recruiting more subjects, despite this limitation the study is the first step towards dissociating theoretical models of reward processing in smokers.

The inclusion of the mask in the supplemental materials is helpful, however, the inclusion of the whole-brain analysis in the supplemental materials would be helpful (although not necessary). The exploratory whole brain analysis demonstrates that the ROI approach did not miss any key reward processing regions.

New Author Response: We have now included the ROI mask as a supplementary figure.

Reviewer: 1

Comments to the Author
Summary

This report titled 'Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses' tests the neural correlates of reward and loss anticipation and outcomes via the Monetary Incentive Delay task during fMRI in current cigarette smokers, ex-smokers, and non-smoking young adults. This reviewer continues to have no major concerns, but there were some responses that may require further thought and elaboration (outlined below) before this paper is accepted for publication.

Materials and Methods

fMRI Data Analysis

1. Coregistration and normalization: it is possible that, due to imperfection/imprecision of standard coregistration and normalization algorithms in standard fMRI statistical packages such as FSL, activations could appear in lateral OFC, but may actually originate from anterior insula or other areas in close approximation to lateral OFC. This could have consequences on the interpretation of the data (see Stoeckel et al., Addiction Biology, 2015) for brief discussion of this point and a suggested alternative coregistration and normalization approach, which should result in improved coregistration and normalization). This reviewer is not recommending that it is necessary to use this approach, but this issue should be addressed in some way – either with additional analyses or in text.

Author Response: We have reported the peak voxel coordinates that come from a cluster in the OFC following cluster-based unpaired t-test analyses in FSL when searching for group differences across the OFC and striatal (caudate, putamen and nucleus accumbens) ROI mask taken from the Harvard-Oxford atlas. We acknowledge that these OFC clusters also appear to cover the anterior edges of the insula, which likely comes from the Harvard-Oxford OFC masks partially abutting the anterior insular cortex. We also now refer to the activations as the OFC and the insula throughout the results and discussion sections. We used FLIRT (FMRIB's Linear Image Registration Tool), which is a fully

automated tool for linear (affine) intra- and inter-modal brain image registration in FSL. We also acknowledge that this method of registration might not be the most optimal for anatomical localisation. We are happy to acknowledge this as a potential limitation, and mention the anterior insular cortex as a region where the group differences may also be emerging. We have now inserted the following text under limitations in the discussion as follows:

"Imperfections of standard coregistration and normalization algorithms in FSL may also mean that the group differences reported in the lateral OFC may also have included a contribution from the anterior insular cortex, which is implicated in addiction, particular relapse."

Reviewer Response: It would be helpful if the authors provided more detail about the implications for activations originating from lateral OFC vs. anterior insula, as there are quite different functions in these different networks. For example, anterior insular networks may have special relevance for nicotine addiction (vs. addiction more broadly). The information in the Discussion should be added to the Introduction and the paper should be revisited to entertain potential hypotheses for what would be expected if activation changes were driven by lateral OFC vs. anterior insula networks (or both). The localization issue is not just a limitation, but may have implications for how these data are interpreted. More attention and thought should be given to this, especially given this was a concern raised by both reviewers.

New Author Response: We have now inserted the following information into the introduction:

"Previous and sustained substance use may also have a sensitizing effect in regions connected to, but outside, the striatum, such as the insular and orbitofrontal cortices, that represent motivational drive (Goldstein et al., 2007) and emotional and interoceptive states (Critchley et al., 2004; Terasawa et al., 2013)."

We have also entered the updated the discussion to include the following:

"Activations originating in the anterior insular cortex (versus the lateral OFC) may further suggest that there is a disproportionate weighting of interoception in response to cues that signal non-drug rewards. This weighting may represent a sensitization of the lateral insular cortex by previous nicotine exposure, which through its connections with the OFC, represents a heightened motivational drive (Goldstein et al., 2007) and emotional and interoceptive state (Critchley et al., 2004; Terasawa et al., 2013) during non-drug reward expectancy."

2. Motion parameters: there is no mention of whether groups were compared on motion parameters or other outlier volumes in the fMRI data that could differ by groups. Please discuss.

New Author Response: The groups did not differ with respect to motion. We have inserted the following text in the results section as follows:

"Finally, we did not observe any differences in motion (mean absolute displacement in millimetres) between the groups (F=1.4; df=37, 2; p=0.3; control 0.21 ± 0.03; smoker 0.28 ± 0.05; ex-smoker 0.29 ± 0.03) when acquiring the MID images."

Results

1. FTND scores are roughly equivalent between current and ex-smokers. This must be a mistake as ex-smokers should not have an FTND score mean ~3. Please discuss.

Author Response: The FTND score in ex-smokers is a retrospective score acquired during screening that reflects the dependence level of the ex-smokers when they were active smokers. We thought that it was important to match smokers and ex-smokers on this measure in order to allay concerns regarding the influence of former dependence levels on any potential neural differences that emerged between the two groups. We have now reported this rationale in the methods section (under questionnaires) as follows:

"We also administered the FTND to ex-smokers, retrospectively, in order to match their previous levels of nicotine dependence (when they were active smokers) to current smokers. The rationale for this was to eliminate previous dependency levels in ex-smokers as a potential contributing factor to neural differences with smokers."

Reviewer Response: The authors should also acknowledge the limitations of a retrospective reporting of FTND in ex-smokers, and should list (if the information is available) the mean and range in years since the last cigarette (to provide the reader with some sense for how much "remembering" was needed to report retrospective FTND). It is possible that these reports are quite inaccurate.

New Author Response: Information about average abstinence (time since last cigarette) is in table 1. We have also calculated the range of nicotine abstinence and entered this information into the results section (under demographics) as follows:

"The ex-smoker group had been abstinent from nicotine, on average, nearly 85 weeks (range: 52-180 weeks) at the time of testing."

We acknowledge that there may be some limitations to retrospectively recording a FTND score (i.e. inaccuracies in remembering in the ex-smoker); although as we state, the rationale was to match ex-smokers on their previous levels of nicotine dependence (when they were active smokers) to current smokers, and this was the only viable way in which to do so. We have, however, now inserted the following text under limitations in the discussion as follows:

"There may also have been a limitation of retrospectively recording previous dependency in our ex-smoker sample, given the large range of abstinence, potentially contributing to some inaccuracies in remembering."

2. FTND scores are in the low to moderate dependence range for current smokers, possibly due to the mean age of this group (young adults). This should be addressed in the Discussion section, especially how this may limit generalizability to more dependent and/or older smokers.

Author Response: We have discussed this as a limitation in the discussion as follows:

"The levels of dependency, for example, may reflect the young age of the smoker and ex-smoker groups, where a modest exposure to nicotine may have a sensitizing effect on brain circuitry subserving motivational and reward processes. These relatively modest levels of dependency,

therefore, may curtail the generalizability of the current findings to greater levels of nicotine dependence that are observed in older adults."

Reviewer Response: The authors should also add that this may curtail generalizability of the current findings to more dependent smokers in addition to "older" smokers (i.e., there is a confound between age and nicotine dependence).

New Author Response: We have now modified the previous insertion as follows:

"The levels of dependency, for example, may reflect the young age of the smoker and ex-smoker groups, where a modest exposure to nicotine may have a sensitizing effect on brain circuitry subserving motivational and reward processes. These relatively modest levels of dependency, therefore, may curtail the generalizability of the current findings to greater levels of nicotine dependence that are observed in older adults (i.e. the confound between age and nicotine dependence)."

Discussion

1. There is no direct comparison of drug and non-drug reward and loss anticipation and outcome. This may limit how these data can inform the relative importance of drug vs. non-drug reward alterations related to nicotine addiction. This is also a relatively low-to-moderately dependent sample, which also limits interpretation of these data. This should be discussed.

Author Response: We have now discussed the low-moderate nicotine dependence levels of the smoker and ex-smoker samples under limitations in the manuscript (also see above). A previous study comparing 13 current cigarette smokers, 10 ex-smokers and 13 controls (Nestor et al., 2011) did examine neural responses to drug cues (i.e. cigarette images). That study showed that exsmokers had significantly less BOLD activation change compared to smokers (but not controls) in the ventral striatum while viewing smoking images. These are the same subjects, apart from two additional volunteers recruited into the smoker and control groups. While we cannot make a direct comparison of drug and non-drug rewards in this study, we can speculate the same ten ex-smokers appeared to have a neural shift in the attribution of incentive salience between "drug" and non-drug reward. The inclusion of a drug condition, whereby cigarettes could also have been received as rewards (i.e. drug rewards), we believe, would not have been appropriate or ethical in the abstinent (ex-smoker) group. Therefore, we have inserted the following text in the discussion to address the reviewer's comment:

"Interestingly, this same sample of ex-smokers demonstrated reduced activation changes in the ventral striatum compared to smokers while viewing smoking stimuli (Nestor et al., 2011), suggesting a neural shift in the attribution of salience between drug and non-drug predictive cues in the striatum during abstinence."

Reviewer Response: Again, given there is no direct comparison with non-drug reward, it is not clear to this reviewer that this conclusion can be made. In addition, this language does not clearly differentiate what is added by the current study (vs. the cited Nestor et al., 2011 study) in this area. It is this reviewer's opinion that this should clearly be stated as a limitation (vs. making a speculative statement not based in data). If there are data to support this contention, the case needs to be made more clearly.

New Author Response: We have discussed this as a limitation in the discussion as follows:

"Furthermore, we were not able to make a direct comparison regarding the neural correlates of drug (smoking) and non-drug reward anticipation and outcome processing, which may limit how our findings can inform the relative importance of reward alterations in nicotine addiction."

Figures 2 and 3

1. "Lateral OFC" activations appear to be more localized to anterior insula (Figs 2a,b; 3a,d). Anterior insula is an important brain region involved addiction, specifically in nicotine addiction (see Stoeckel et al., 2015 referenced earlier for further discussion and citations of other seminal work in this area). Figs should be modified with this in mind.

Reviewer Response: The authors' response is adequate.

Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses

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Abstract

Despite an increased understanding of nicotine addiction, there is a scarcity of research comparing the neural correlates of non-drug reward between smokers and ex-smokers. Long-term changes in reward-related brain functioning for non-drug incentives may elucidate patterns of functioning that potentially contribute to ongoing smoking behaviour in current smokers. Similarly, examining the effects of previous chronic nicotine exposure during a period of extended abstinence may reveal whether there are neural correlates responsible for non-drug reward processing that are different from current smokers. The current study, therefore, set out to examine the neural correlates of reward and loss anticipation, and their respective outcomes, in smokers, ex-smokers and matched controls using a monetary incentive delay task during functional MRI. Here we report that in the absence of any significant behavioural group differences, both smokers and ex-smokers showed a significantly greater activation change in the lateral orbitofrontal/anterior insular cortex compared to smokers when anticipating both potential monetary gains and losses. We further report that ex-smokers showed a significantly greater activation change in the ventral putamen compared to both controls and smokers, and in the caudate compared to controls during the anticipation of potential monetary losses only. The results suggest that smoking may sensitize striato-orbitofrontal circuitry subserving motivational processes for loss avoidance and reward gain in nicotine addiction.

Introduction

Nicotine and other drugs of addiction are conceived to commandeer some of the same neural substrates which have evolved to support beneficial forms of synaptic plasticity, such as learning and memory (Gerdeman et al., 2003). Laboratory studies in animals and humans have demonstrated nicotine-induced dopamine (DA) release within striatal regions of the brain (Domino et al., 2012), which is believed to underlie nicotine's reinforcing properties (Tuesta et al., 2011). Striatal regions are also critical neuroanatomical substrates for the processing of non-drug rewards in humans (Knutson et al., 2001; O'Doherty et al., 2006) and underlie incentive salience systems for goal-objects (Knutson et al., 2005; McClure et al., 2004). Therefore, drugs such as nicotine, capable of engaging striatal "reward circuitry", may potentially alter neural processing for non-drug rewards.

Disturbances in reward-related brain functioning for non-drug incentives may elucidate adaptations in neural circuitry that contribute to ongoing smoking behaviour in humans. The reward deficiency syndrome (RDS) (Blum et al., 2000) and the allostatic hypotheses (AH) (Koob et al., 2004), for example, view addiction as a deficit in DA motivational circuitry for non-drug incentives, such that only substances of abuse are able to normalize DA in fronto-striatal regions. The current, but limited, literature does suggest that chronic smokers have deficits in striatal DA integrity similar to other addiction populations (Fehr et al., 2008) and reduced reward-related neural activity for non-drug incentives (Rose et al., 2012), appearing to concur with an RDS view of nicotine addiction. Substance-dependent groups, however, have also been shown to exhibit both impulsive and reward-centred choice behaviours, particularly involving an increased preference for small immediate over

larger delayed non-drug rewards (Bechara et al., 2001), suggesting some type of mesolimbic reward hyperactivity (Bickel et al., 2007). Indeed, smokers (Martin et al., 2014), cannabis users (Filbey et al., 2013; Nestor et al., 2010), alcoholics (Gilman et al., 2015; Grodin et al., 2016) and even cocaine addicts in sustained abstinence (Balodis et al., 2016) have been reported to demonstrate hyperactivity in striatal regions during the pursuit of non-drug incentives. Previous and sustained substance use may also have a sensitizing effect in regions connected to, but outside, the striatum, such as the insular and orbitofrontal cortices, that represent motivational drive (Goldstein et al., 2007) and emotional and interoceptive states (Critchley et al., 2004; Terasawa et al., 2013). This would appear to oppose the notion of an RDS in some addiction populations, instead suggesting heightened and indiscriminate neural responses to cues that signal all forms of potential reward.

While the majority of cigarette smokers endorse the desire to quit, reported abstinence rates after twelve months are in the modest region of 5-17% (Hughes et al., 2008), with the vast majority relapsing to smoking within a week of cessation (Zhu et al., 2012). Executive functioning has been proposed to play a significant role in preventing relapse (Buhringer et al., 2008; Garavan et al., 2013), and indeed, research has reported that long-term abstinent ex-smokers demonstrate hyperactivity in lateral and medial prefrontal regions that sub-serve inhibitory control functioning (Kroenke et al., 2015; Nestor et al., 2011). This appears to suggest that the emergence of prefrontal cognitive neural substrates are necessary for successful abstinence in addiction; although this does not preclude the existence of other mechanisms that may explicate changes in neural and behavioural functioning that protect against relapse.

Therefore, in order to examine the effects of both current and previous nicotine exposure on the behavioural and fronto-striatal correlates of non-drug incentives, we compared current smokers, ex-smokers and demographically matched healthy controls using a monetary incentive delay task. Specifically, we were interested in exploring 1) whether smokers and ex-smokers demonstrate shared or dissociated differences from controls in the neural response to the anticipation, and receipt, of monetary gains and losses in fronto-striatal networks and 2) whether such differences imply signs of either an RDS or reward-centred correlate in these regions during the pursuit of non-drug incentives.

Material and Methods

Participants

15 current cigarette smokers, 10 ex-smokers and 15 controls completed the study. A semi-structured interview, as used in previous behavioural and functional imaging studies (Carey et al., 2015; Nestor et al., 2010) was conducted to screen participants for past or present histories of psychiatric or neurological illness. Information pertaining to any form of treatment (counselling, psychological, psychiatric), past or present, was carefully detailed, with any potential participant describing any major life-time psychiatric event or brain injury (e.g., head trauma resulting in a loss of consciousness, seizure or stroke) considered ineligible for the study. Participants were also considered ineligible if they reported any familial psychiatric history (i.e. sibling, parent or grandparent).

During the screening interview, all potential participants completed an inventory for drug use (questionnaire taken from the Addiction Severity index Lite-CF; see questionnaires section below) to screen for past or concurrent abuse of substances; participants were considered ineligible if they reported concurrent or past dependence on other drugs (e.g., alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, hallucinogens, MDMA and opiates). Information concerning alcohol and tobacco use in each participant was indexed in years (life-time) and recent (last 30 days). Other drug use information for each participant was indexed by the total number of separate occasions (life-time) and the total number of recent separate occasions (last 30 days).

Current smokers were required to have regularly consumed tobacco (≥10 cigarettes/day) for the previous 2 years in order to be eligible. Ex-smokers were required to have regularly consumed tobacco (≥10 cigarettes/day) for at least two years, but be nicotine abstinent for at least 12 months at the time of testing. Ex-smokers were considered eligible if they additionally reported no past or current use of products to facilitate nicotine abstinence (e.g., gum, patches, lozenges, nasal spray and inhalators). Control participants were required to have never smoked cigarettes. Smoking abstinence in ex-smokers and controls was confirmed by measuring expired carbon monoxide (CO) in parts per million (ppm) during the screening process. All participants in each of the three groups were required to provide a negative urine sample for various drugs of abuse on the day of testing, specifically screening for the presence of amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, MDMA, methadone, opiates and tricyclic antidepressants (Cozart RapiScan, UK).

Because previous research has shown that acute abstinence from cigarettes impairs concentration (Heishman, 1999; Newhouse et al., 2004) and increases BOLD activation changes during functional MRI (Azizian et al., 2010), cigarette smokers each smoked *ad lib* approximately 15 minutes prior to scanning in order to avoid the potential confounds of withdrawal and/or craving on MID task performance. Consequently, any differences in current smokers regarding task performance or BOLD activation changes could be attributable to the acute effects of their recent nicotine use. Given their frequent daily use, this is deemed desirable as it reveals the typical functioning of their neural systems.

All participants were right-handed as confirmed by the Edinburgh Handedness Inventory (Oldfield, 1971) during the screening process. All participants completing the study were neurologically normal (as confirmed by a registered radiologist who examined each structural MRI). All research participants provided informed consent and were financially compensated.

Questionnaires

The National Adult Reading Test (NART) (Nelson et al., 1978) was administered to all participants during the screening procedure to assess verbal intelligence, as was the Beck Depression Inventory (BDI) to assess mood (Beck et al., 1996). Information concerning alcohol and drug use (see Table 1) was obtained from all participants using a questionnaire taken from the Addiction Severity Index Lite-CF (McLellan et al., 1992). During the screening procedure, the Fagerström test of nicotine dependence (FTND) was administered to smokers. The FTND (Heatherton et al., 1991) is a 6-item questionnaire that measures the degree of

nicotine dependence in an individual smoker. We also administered the FTND to ex-smokers, retrospectively, in order to match their previous levels of nicotine dependence (when they were active smokers) to current smokers. The rationale for this was to eliminate previous dependency levels in ex-smokers as a potential contributing factor to neural differences with smokers.

The Shiffman-Jarvik smoking withdrawal questionnaire (SJWQ) and the urge to smoke (UTS) scale were administered to smokers prior to scanning. The 25-item SJWQ (Shiffman et al., 1976) asks individuals to respond to questions using a 7-point Likert-type scale that ranges from “very definitely” (7) to “very definitely not” (1) with respect to how they feel at that moment regarding separate withdrawal symptoms. These withdrawal symptoms are comprised of *craving*, *physical*, *psychological*, *sedation* and *appetite* constructs. Each construct is given a mean score, with the mean for each construct summed to provide an overall withdrawal score for an individual. The 10-item UTS scale (Jarvik et al., 2000) assesses responses to craving-related questions, using a 7-point Likert-type scale ranging from “very definitely” (7) to “very definitely not” (1).

Monetary Incentive Delay Task (MID)

We used a “monetary incentive delay task” (MID), which was based on that originally employed by Knutson (Knutson et al., 2001) and which we have previously used to assess the neural correlates of reward processing in cannabis users (Nestor et al., 2010). While being scanned participants performed the MID task, during which they anticipated potential monetary gain, loss or no potential monetary outcome. During each trial, participants viewed one of three coloured squares (cue)

that indicated the potential to gain fifty cent (green square), lose fifty cent (red square) or experience no financial outcome (blue square - here referred to as the neutral condition) following their response to an upcoming visual target. Each cue was presented for a variable duration (2-8 sec), after which participants made a button press response upon the presentation of a visual target (star located within a circle). Participants received feedback (1500 ms) following their response to the visual target, after which there was an end fixation period (2-8 sec) before the commencement of the next trial. Responses to the visual target falling within ("hits") or outside ("misses") a 400ms response deadline received feedback appropriate for that particular trial. We chose this 400 ms time frame in order to yield accuracy levels at ~50%, which would serve to maintain the participant's interest in the task. Therefore, participants had four hundred milliseconds to respond to the visual target in order to be successful on a gain, loss or neutral trial. There were a total of 27 trials in each condition (gain, loss, and neutral), with each trial lasting between six and eighteen seconds. The MID was composed of three runs, with each run lasting 340 seconds. The order of trials within each run was randomised. Dependent measures derived from the data included mean percentage accuracy and reaction time for the gain, loss and neutral conditions. The task was programmed and run using E-Prime version 1.1 (Psychology Software Tools, Pittsburgh, USA).

Functional MRI (fMRI) Data Acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected the visual display, which was projected onto a panel placed behind the participants' head outside the magnet. The mirror was mounted on the head coil in each participant's line of vision.

Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel sensitivity encoding (SENSE) approach with a reduction factor of 2 was utilised for all T1-weighted image acquisitions (Pruessmann et al., 1999). 180 high-resolution T1- weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size 0.9×0.9×0.9) were then acquired (total duration 325 s), to allow subsequent activation localization and spatial normalization. Functional data were acquired using a T2* weighted echo-planar imaging sequence collecting 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE=35 ms, TR=2000 ms, FOV 224 mm, 64×64 mm matrix size in Fourier space). Functional scans had a total duration of 340 s per run.

fMRI Data analyses

Data pre-processing and statistical analysis were conducted using FEAT (fMRI Expert Analysis Tool) from the FMRIB Software Library (FSL 4.1, www.fmrib.ox.ac.uk/fsl). Pre-statistical processing was as follows: motion correction utilizing FMRIB's Linear Image Registration Tool (MCFLIRT); non-brain matter removal using Brain Extraction Tool (BET); spatial smoothing with a 6-mm full-width half maximum Gaussian kernel; mean-based intensity normalization; nonlinear high-pass temporal filtering (Gaussian-weighted least squares straight line fit, with sigma = 25.0 sec).

For each participant, first level whole-brain mixed-effects analyses were performed by modelling the MID anticipation periods (i.e., gain, loss and neutral) as explanatory variables within the context of the general linear model on a voxel-by-voxel basis (variable boxcar functions for the anticipation period regressors were

convolved with the haemodynamic response function). The gain, loss and neutral outcome periods ("Hit" and "Miss") were also modelled (stick functions for the feedback period regressors were convolved with the haemodynamic response function). The end fixation period of the task served as the implicit baseline. Registration was conducted through a two-step procedure, whereby EPI images were first registered to the high-resolution T1 structural image, then into standard (Montreal Neurological Institute, MNI avg152 template) space, with 12-parameter affine transformations.

As striatal and orbitofrontal regions are critical neural substrates for the processing of non-drug rewards in humans (Knutson et al., 2001; O'Doherty et al., 2001), with evidence for both hypoactivity and hyperactivity to non-drug rewards in these regions in addiction populations, we took an *a priori* region of interest (ROI) approach, restricting our search for group differences in these regions. These regions were taken from the Harvard-Oxford cortical and subcortical structural atlas and grouped together into one ROI mask (see Supplementary Figure 1). Higher-level (between-group) analyses were conducted using FLAME (FMRIB's Local Analysis of Mixed Effects) on each of the gain, loss and neutral anticipation and outcome conditions. The end fixation period of the task served as the implicit baseline. Therefore, all reported differences between groups on the gain, loss and neutral conditions are for activation changes versus the baseline. Significant clusters in this ROI mask of *a priori* regions were determined by thresholding at $Z>2.3$ with a corrected (FWE) cluster significance threshold of $p<0.05$.

Other Statistics

For analyses conducted on the MID behavioural data, we performed three (Group: Control vs. Ex-smoker vs. Smoker) by three (Condition: Neutral vs. Loss vs. Gain) analyses of variance. For further group analyses performed on the mean BOLD signal change, we conducted one-way analyses of variance. These analyses were all conducted using the Statistical Package for the Social Sciences (SPSS Inc., Chicago).

Results

Demographics

Table 1 shows the demographic, smoking and alcohol use histories for the control, ex-smoker and smoker groups. The groups did not significantly differ on age, years of education, verbal intelligence, gender distribution or alcohol use history. The ex-smoker group had been abstinent from nicotine, on average, nearly 85 weeks (range: 52-180 weeks) at the time of testing.

-Insert Table 1 about here-

MID Performance

Figure 1a shows the mean MID accuracy (% “hits”) for the three conditions in the three groups. A three (Group: Control vs. Ex-smoker vs. Smoker) by three (Condition: Neutral vs. Loss vs. Gain) analysis of variance showed that there was a significant effect of condition (F=5.6; df=111, 2; p<0.01; neutral<loss, p<0.05; neutral<gain, p<0.01), but no group (F=0.5; df=111, 2; p=0.6) or condition x group interaction (F=0.09; df=111, 4; p=0.99). Figure 2b shows the mean MID reaction

time (milliseconds) for the three conditions in the three groups. There was a significant effect of condition (F=2.6; df=111, 2; p<0.05; loss<neutral, p<0.09; gain<neutral, p<0.05), no effect of group (F=1.4; df=111, 2; p=0.3) and no condition x group interaction (F=0.1; df=111, 4; p=1.0).

-Insert Figure 1 about here-

Functional MRI

We initially conducted mixed effects cluster-based one-way ANOVA analyses on the gain, loss and neutral conditions in the *priori* regions of interest (ROI) mask, but we were unable to find a group effect. Therefore, we combined the smoker and ex-smoker groups ("smokers") in order to increase sample size, and performed cluster-based independent t-test analyses (Control vs. "Smokers") to examine activation differences on the anticipation and outcome periods in the ROI mask. For the loss anticipation condition (Fig 2a) "smokers" showed significantly greater activation change in the right orbitofrontal/anterior insular cortex (OFC/AIC: 552 voxels; x=44; y=20; z=-6; Z=4.1; p<0.05); right putamen (756 voxels; x=20; y=18; z=-8; Z=3.65; p<0.01) and the left caudate (734 voxels; x=-10; y=8; z=4; Z=3.36; p<0.01) compared to controls. Similarly, for the gain anticipation condition (Fig 2b) "smokers" again showed significantly greater activation change in the right OFC/AIC (580 voxels; x=46; y=18; z=-10; Z=3.63; p=0.01); right caudate (646 voxels; x=10; y=12; z=-2; Z=3.94; p<0.01) and the left putamen (620 voxels; x=-16; y=8; z=-12; Z=3.47; p<0.01) compared to controls. There were no cluster-based analysis group differences that emerged for the neutral anticipation condition or on the gain, loss and neutral outcomes (hits and misses).

-Insert Figure 2 about here-

In order to assess whether the smoker and ex-smoker groups had independently contributed to the loss and gain anticipation group differences, we extracted the mean BOLD signal change from each of the t-test clusters and conducted one-way (Control vs. Ex-smoker vs. Smoker) analyses of variance. For the loss anticipation condition, there was a significant effect of group in the right OFC/AIC cluster (F=6.6; df=37, 2; p<0.01; ex-smoker>control, p<0.01; smoker>control, p<0.05; Fig 3a); the right putamen cluster (F=7.1; df=37, 2; p<0.01; ex-smoker>control, p<0.001; ex-smoker>smoker, p<0.05; Fig 3b) and left caudate cluster (F=3.6; df=37, 2; p<0.05; ex-smoker>control, p<0.05; Fig 3c). For the gain anticipation condition, there was a significant effect of group in only the right OFC/AIC cluster (F=4.4; df=37, 2; p<0.05; ex-smoker>control, p<0.05; smoker>control, p<0.05; Fig 3d).

Finally, we did not observe any differences in motion (mean absolute displacement in millimetres) between the groups (F=1.4; df=37, 2; p=0.3; control 0.21 ± 0.03; smoker 0.28 ± 0.05; ex-smoker 0.29 ± 0.03) when acquiring the MID images.

-Insert Figure 3 about here-

Correlations

We did not find any significant correlations between smoking demographics in smokers and ex-smokers and mean percentage BOLD change values within the clusters where we observed group differences.

Discussion

The present study examined loss and reward processing in cigarette smokers and demographically matched ex-smokers and controls using an MID task. Behaviourally, we observed no significant differences between the three groups with respect to mean accuracy or reaction time. The absence of performance differences, therefore, enable us to discount performance related effects (e.g., frustration) from confounding group comparisons with respect to the neural correlates of loss and gain anticipation. Furthermore, all three groups appeared to be equally incentivized to avoid losses and maximize gains, as revealed by the statistically significant differences in accuracy and response latencies compared to the neutral condition.

Greater OFC activation during loss and gain anticipation in ex-smokers and smokers

The current study reports that during the anticipation of potential monetary losses and gains, smokers, and to a greater degree ex-smokers, had greater activation in the lateral OFC. There is evidence that neural responses in the lateral OFC reflect both implicit motivational value (Rothkirch et al., 2012) and incentive salience (Walter et al., 2010), suggesting that these processes are heightened during both loss and gain anticipation in smokers and ex-smokers. These lateral

OFC clusters also partially covered a region of the anterior insular cortex. Importantly, the insular cortex is known to be involved in the evaluation of motivational states, reward, risk (Liu et al., 2011; Preuschoff et al., 2008; Samanez-Larkin et al., 2007), and addiction relapse (Naqvi et al., 2007; Paulus et al., 2005; Seo et al., 2013), with particular reference to its role in the awareness of interoceptive (i.e. bodily) states (Critchley et al., 2004). Activations originating in the anterior insular cortex (versus the lateral OFC) may further suggest that there is a disproportionate weighting of interoception in response to cues that signal non-drug rewards. This weighting may represent a sensitization of the lateral insular cortex by previous nicotine exposure, which through its connections with the OFC, represents a heightened motivational drive (Goldstein et al., 2007) and emotional and interoceptive state (Critchley et al., 2004; Terasawa et al., 2013) during non-drug reward expectancy.

The incentive-sensitization theory of addiction proposes that sensitized neural circuits function to attribute incentive salience to reward-related stimuli, allowing reward cues to trigger excessive “wanting” for the reward (Berridge et al., 1998). The focus of sensitized “wanting” in addiction, however, is believed to be primarily towards drug cues and drug rewards, rather than natural rewards (Robinson et al., 2001). Despite this contention, sensitization has been shown to enhance the pursuit of natural rewards in animals, where exposure to substances of abuse has been observed to significantly increase cue-elicited approach behaviour for non-drug rewards (Wyvell et al., 2001). Interestingly, a similar effect has also been observed in humans, where the neural correlates of reward and loss anticipation are greater in cannabis users (Filbey et al., 2013; Nestor et al., 2010), cigarette smokers (Martin et

al., 2014), alcoholics (Gilman et al., 2015; Grodin et al., 2016) and even cocaine addicts (Balodis et al., 2016). This may suggest that chronic exposure to nicotine through smoking sensitizes striato-orbitofrontal circuitry that subserves motivational processes for loss avoidance and reward gain. Alternatively, the hyperactivity observed in "smokers" may represent a trait-like effect that preceded smoking, but that has not "corrected" with abstinence in the ex-smoker group.

Greater striatal activation in ex-smokers during loss anticipation

We also report that ex-smokers demonstrated a greater activation change in the ventral putamen compared to smokers and controls, and compared to controls in the caudate during loss anticipation. Previous research has reported altered striatal activity for non-drug rewards in substance dependence (Buhler et al., 2010; Bustamante et al., 2014; Diekhof et al., 2008; Gradin et al., 2014; Peters et al., 2011; Wrase et al., 2007) with some evidence for a sustained striatal reward deficiency syndrome (Blum et al., 2000) in long-term substance abstinence. The current finding of increased ventral putamen and caudate activation in ex-smokers suggests that they have an increased motivational signal in a reward-motor network where preparatory responses might be optimized to avoid loss. Interestingly, this same sample of ex-smokers demonstrated reduced activation changes in the ventral striatum compared to smokers while viewing smoking stimuli (Nestor et al., 2011), suggesting a neural shift in the attribution of salience between drug and non-drug predictive cues in the striatum during abstinence. This should be tempered, however, with the fact that differences between ex-smokers and smokers were smaller, possibly suggesting some similarities within a network of regions that

function to integrate motivational drives (Goldstein et al., 2007) under conditions of loss avoidance.

Limitations of the current study involve small sample sizes, particularly in the ex-smoker group, and the low-moderate nicotine dependency range in current smokers. The levels of dependency, for example, may reflect the young age of the smoker and ex-smoker groups, where a modest exposure to nicotine may have a sensitizing effect on brain circuitry subserving motivational and reward processes. These relatively modest levels of dependency, therefore, may curtail the generalizability of the current findings to greater levels of nicotine dependence that are observed in older adults (i.e. the confound between age and nicotine dependence). There may also have been a limitation of retrospectively recording previous dependency in our ex-smoker sample, given the large range of abstinence, potentially contributing to some inaccuracies in remembering. Furthermore, we were not able to make a direct comparison regarding the neural correlates of drug (smoking) and non-drug reward anticipation and outcome processing, which may limit how our findings can inform the relative importance of reward alterations in nicotine addiction. Imperfections of standard coregistration and normalization algorithms in FSL may also mean that the group differences reported in the lateral OFC may also have included a contribution from the anterior insular cortex, which is implicated in addiction, particular relapse.

Conclusion

Despite the limitation of a small sample size (in particular, the ex-smoker group), and the low-moderate nicotine dependency, the current study has provided preliminary evidence for hyperresponsive OFC processing during cue-elicited approach behaviour for non-drug rewards in smokers and ex-smokers. This would appear to concur with the process of incentive-sensitization, as opposed to the RDS, in the current sample. Therefore, we tentatively propose that smokers and ex-smokers have a neural substrate for predicting potential monetary losses and gains that represents a sensitization of striato-orbitofrontal circuitry integrating motivational drives and incentive salience for goal objects.

Declaration of Conflicting Interests

The authors report no biomedical financial interests or potential conflicts of interest.

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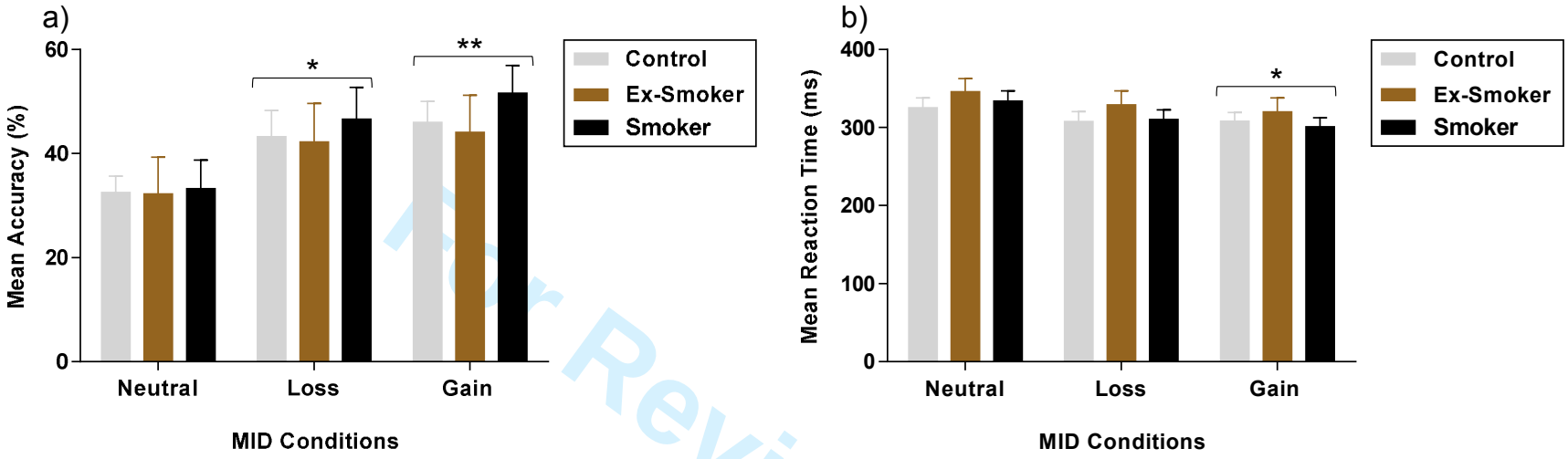


Figure 1. MID task performance in the control, ex-smoker and smoker groups for a) mean percentage accuracy (Loss>Neutral - *p<0.05; Gain>Neutral - **p<0.01) and b) mean reaction time (Gain<Neutral - **p<0.05). Data were analyzed using 3 (Group: Control vs. Ex-smoker vs. Smoker) by 3 (Condition: Neutral vs. Loss vs. Gain) analysis of variance. Data are expressed as means ± SEM.

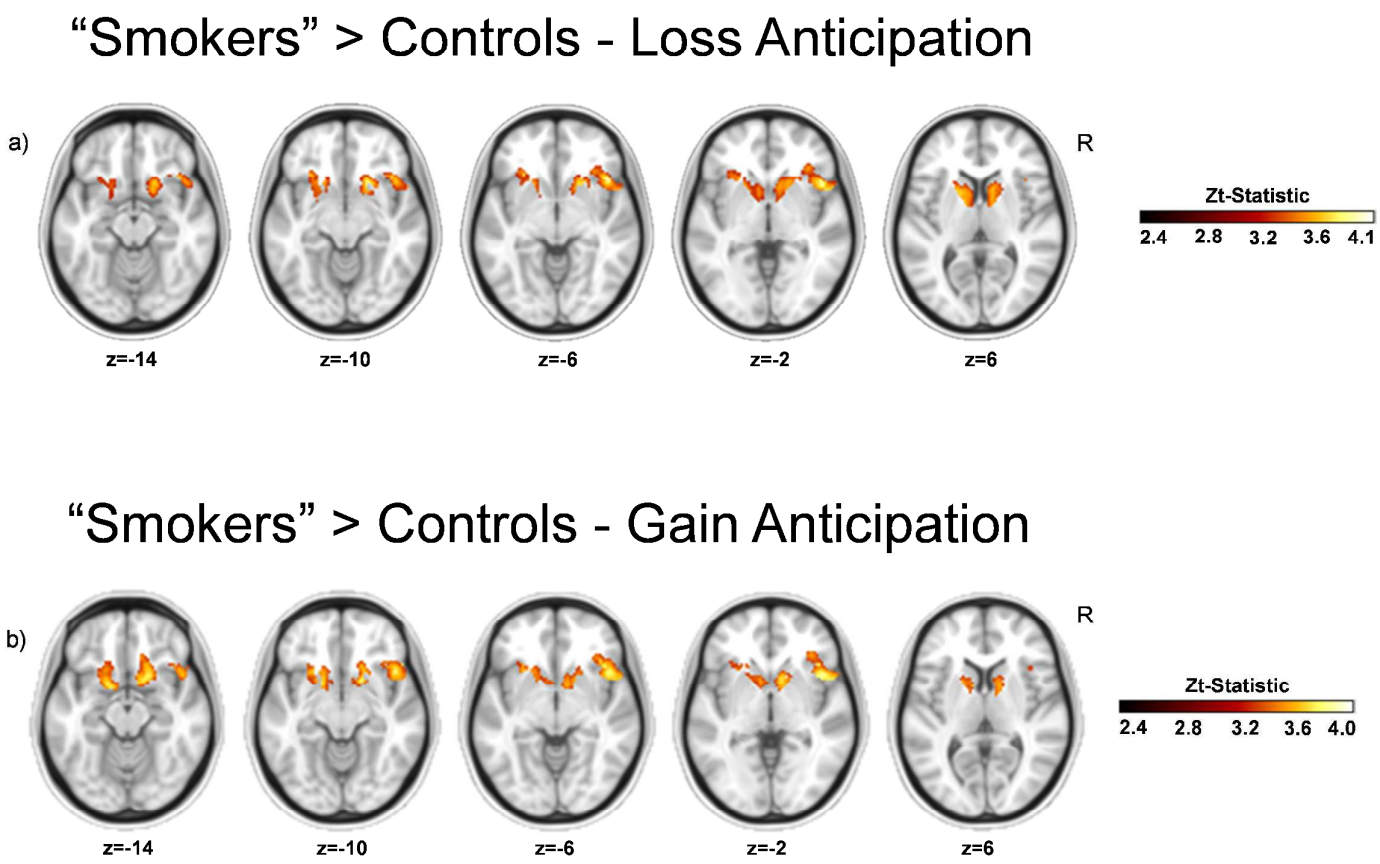


Figure 2. Initial zT-Statistical cluster maps generated by independent t-test analyses ("Smokers" vs. Controls) in the *a priori* regions of interest for a) loss anticipation and b) gain anticipation showing that "smokers" had significantly greater activation changes in orbitofrontal/anterior insular cortex and striatal regions compared to controls. Statistical images were first thresholded using clusters determined by $Z>2.3$ with a corrected (FWE) cluster significance level of $p<0.05$. The scale represents the colour (from dark to light yellow) of the cluster voxels corresponding to the increasing zT-statistic. Co-ordinates are represented in Montreal Neurological Institute (MNI) space.

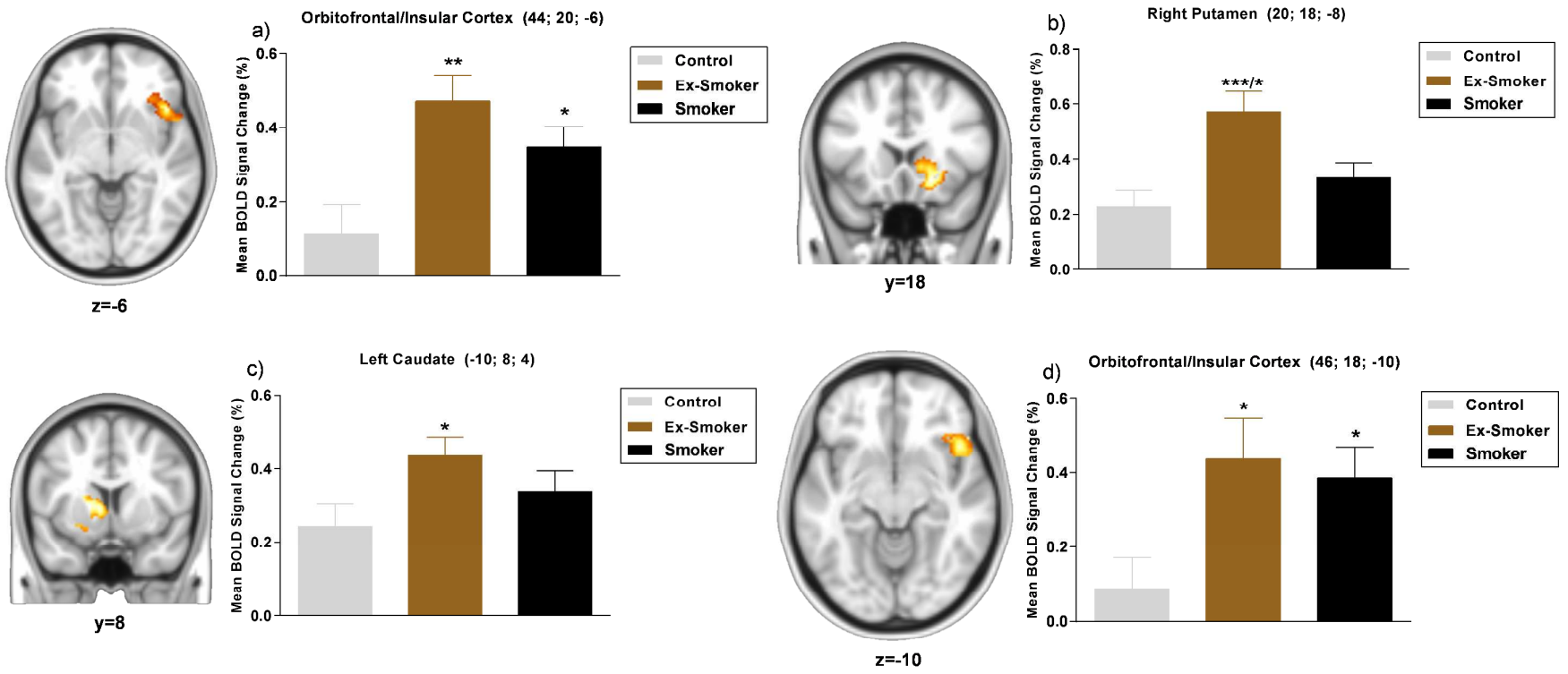
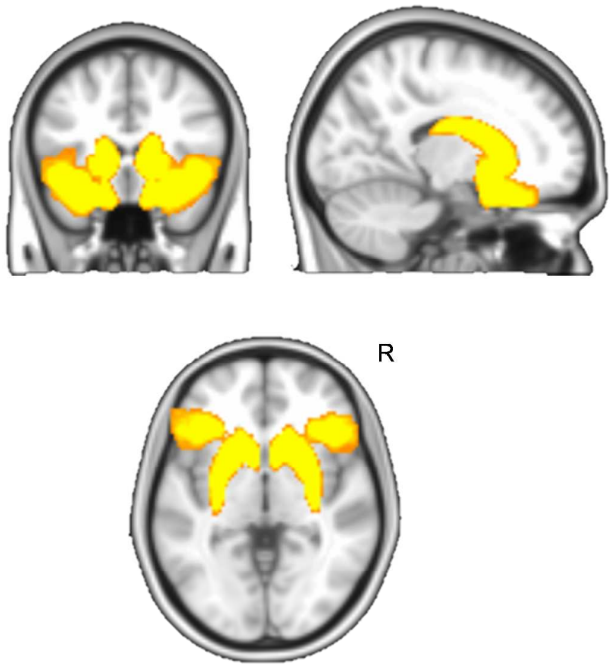


Figure 3. Mean BOLD signal change in the control, ex-smoker and smoker showing that a) ex-smokers (**p<0.01) and smokers (*p<0.05) had greater activation changes in the orbitofrontal/insular cortex compared to controls; b) ex-smokers had greater activation changes in the putamen compared to both controls (*p<0.001) and smokers (*p<0.05); c) ex-smokers (*p<0.05) had greater activation changes in the caudate compared to controls during the loss anticipation condition and d) ex-smokers and smokers had greater activation changes in the orbitofrontal/anterior insular cortex compared to controls (*p<0.05) during the gain anticipation condition. Data were analyzed using a one-way analysis of variance. Data are expressed as means ± SEM.

Table 1. Mean and SEM for the control, ex-smoker and smoker groups on demographic, smoking and alcohol use history ([†]denotes score prior to abstinence).

	Control (n=15)	**Ex-smoker** (n=10)	**Smoker** (n=15)
Age	23.8 ± 1.2	25.4 ± 1.6	23.3 ± 1.2
Years of Education	18.1 ± 0.4	17.9 ± 0.9	16.5 ± 0.5
Verbal Intelligence Score (NART)	123.4 ± 1.1	123.2 ± 1.0	121.5 ± 1.0
Females/Males	7/8	7/3	9/6
Years of Alcohol Use	7.4 ± 1.2	9.0 ± 1.5	7.6 ± 1.1
Alcohol Use in the Last Month (no. days)	8.1 ± 1.6	6.6 ± 1.6	8.3 ± 1.2
Alcohol Use Age Onset (Years)	15.5 ± 1.2	16.4 ± 0.6	15.8 ± 0.6
Years of Nicotine Use		7.1 ± 1.7	7.1 ± 1.3
Pack-Years		5.9 ± 1.5	6.2 ± 1.5
Number of Cigarettes/Day		16.0 ± 2.5[†]	16.0 ± 1.2
Nicotine Abstinence (wks)		84.8 ± 13.6	0.0 ± 0.0
Subscales of Shiffman/Javik Withdrawal Scale			
Craving		3.4 ± 0.2	3.5 ± 0.3
Physical Symptoms		2.0 ± 0.3	1.6 ± 0.2
Psychological Symptoms		3.4 ± 0.1	3.0 ± 0.2
Sedation		3.8 ± 0.2	3.0 ± 0.2
Appetite		2.0 ± 0.3	3.7 ± 0.4
Total Score		14.4 ± 0.7	14.9 ± 0.8
Fagerström Score		3.4 ± 0.1[†]	3.5 ± 0.2
Urge to Smoke Score		12.3 ± 1.1	39.4 ± 4.2
Expired Carbon Monoxide (ppm)	3.0 ± 0.0	3.0 ± 0.0	15.2 ± 0.8*



Supplementary figure 1. Striatal and orbitofrontal regions taken from the Harvard-Oxford cortical and subcortical structural atlas, grouped together into one ROI mask. Higher-level (between-group) analyses were conducted using FLAME (FMRIB's Local Analysis of Mixed Effects). Significant clusters in this ROI mask of *a priori* regions were determined by thresholding at $Z>2.3$ with a corrected (FWE) cluster significance threshold of $p<0.05$.