

Manuscript Number: BRES-D-17-00964R1

Title: Shared and divergent neural reactivity to non-drug operant response outcomes in current smokers and ex-smokers.

Article Type: Research paper

Section/Category: Systems Neuroscience and Behavior

Keywords: Reward; Addiction; fMRI

Corresponding Author: Dr. Liam Nestor, PhD

Corresponding Author's Institution: Imperial College London

First Author: Liam Nestor, PhD

Order of Authors: Liam Nestor, PhD; Ella McCabe; Jennifer Jones; Luke Clancy; Hugh Garavan

Manuscript Region of Origin: IRELAND

Abstract: Addiction to cigarettes presents with considerable health risks and induces high costs on healthcare resources. While the majority of cigarette smokers endorse the desire to quit, only a small percentage of quit attempts lead to full abstinence. Failure to achieve abstinence may arise from maladaptive reactivity in fronto-striatal regions that track positive and negative valence outcomes, thus biasing the choice to smoke in the presence of alternative, non-drug reinforcement. Alternatively, long-term nicotine abstinence may reveal neural substrates of adaptive valence outcome processing that promote and maintain smoking cessation. The present study set out to examine the neural correlates of operant response outcomes in current smokers, ex-smokers and matched controls using a monetary incentive delay task during functional MRI. Here we report that compared to controls, both current smokers and ex-smokers showed significantly less activation change in the left amygdala during positive response outcomes, and in the anterior cingulate cortex, during both positive and negative response outcomes. Ex-smokers, however, demonstrated significantly greater activation change compared to smokers and controls in the right amygdala during negative response outcomes. Activation change in the anterior cingulate cortex and middle frontal gyrus of smokers were significantly negatively correlated with nicotine dependence and cigarette pack-years. These results suggest a pattern of shared and divergent reactivity in current smokers and ex-smokers within corticolimbic regions that track both positive and negative operant response outcomes. Exaggerated adaptive processing in ex-smokers may promote long-term smoking cessation through amplified negative valence outcome monitoring.

Liam J. Nestor PhD

Senior Research Fellow
Neuropsychopharmacology Unit
Centre for Psychiatry
Division of Brain Sciences
Imperial College London
UK

Hugh P. Garavan PhD

Associate Professor
Department of Psychiatry
University of Vermont
USA

15 November 2017

Dear Dr Ravizza

Re: Shared and divergent neural reactivity to non-drug operant response outcomes in smokers and ex-smokers.

We are pleased to resubmit this manuscript for your consideration for publication in Brain Research. Hopefully, we have fully addressed the comments and suggestions made by you and the reviewers in order for the manuscript to be accepted.

We can confirm that this is an original volume of work and that we have not submitted this manuscript for consideration elsewhere. A complete declaration of interests is included in the manuscript.

We look forward to hearing from you.

Yours sincerely,

Liam Nestor and Hugh Garavan

Editor

Comment: First, I found the brackets indicating significance to be confusing in Figures 1a and 1b; namely, they seem to indicate group differences rather than condition differences. Perhaps bracketing the relevant comparison (e.g., gain vs neutral) would improve readability.

Response: We have now changed the bracket to a straight line, which hopefully indicates that it's the conditions rather the groups that show an effect. We have also changed the legend text related to figure 1 as follows:

Figure 1. MID task performance in the control, ex-smoker and current smoker groups showing A) mean percentage accuracy which was greater on the Loss compared to Neutral ($p < 0.05$) and Gain compared to Neutral (** $p < 0.01$) trials; and B) mean reaction time which was faster on the Gain compared to Neutral (* $p < 0.05$) trials only. Data were analyzed using a 3 (Condition: Neutral vs. Loss vs. Gain) $\times$ 3 (Group: Control vs. Ex-smoker vs. Smoker) analysis of variance. Data are expressed as means $\pm$ SEM.*

We have also changed the text related to figure 1 in the results as follows:

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 ($F = 5.6$; $df = 111, 2$; $p < 0.01$), with less accuracy on neutral compared to loss ($p < 0.05$) and gain ($p < 0.01$) trials. There was no effect of group ($F = 0.5$; $df = 111, 2$; $p = 0.6$) and no condition $\times$ group interaction ($F = 0.09$; $df = 111, 4$; $p = 0.99$). Figure 1b shows the mean MID reaction time (milliseconds) for the three conditions in the three groups. There was a significant effect of condition ($F = 2.6$; $df = 111, 2$; $p < 0.05$), with faster response reaction time on the gain compared to neutral ($p < 0.05$) trials only. There was no effect of group ($F = 1.4$; $df = 111, 2$; $p = 0.3$) and no condition $\times$ group interaction ($F = 0.1$; $df = 111, 4$; $p = 1.0$).

Comment: A more serious issue is the number of correlations performed and whether the correlations were corrected for multiple comparisons

Response: We did not correct for multiple comparisons with our correlation analyses. We did, however, only perform correlations between the smoking demographics of dependency (FTND) and dose (pack-years) and those regions where smokers had significantly less activation change on the MID task. This was a total of 20 correlations, which would have meant a Bonferonni correction of 0.0025. Our correlation p values exceeded this value, and therefore, would not have been significant using this correction.

Reviewer #1

Nestor and colleagues present results from a study comparing brain activity associated with reward-related outcomes in current cigarette smokers, former cigarette smokers, and controls without a history of smoking. The study addresses a topic that is both conceptually and clinically significant, as many important questions remain about the factors that contribute to successful smoking cessation and/or that change as a function of discontinuing cigarette use. In addition, the study uses an experimental task that has been widely used and is well characterized, increasing the potential interpretability and impact of the work. The manuscript is also generally well written and appropriately concise. Notwithstanding these strengths, the manuscript had some notable limitations, listed below, most of which are readily addressable.

Comment: The most significant limitation, in my opinion, is the small size of the groups included in the study. The field of human brain imaging (especially fMRI research) has been justifiably criticized in recent years for being replete with severely underpowered studies, which has implications for both the accuracy of parameter estimates within studies and the reliability of findings across studies. The ideal remedy for this issue would be to collect data from more participants, but I understand that doing so may not be feasible for various reasons. The authors do mention the small sample sizes as a limitation of the study, so I am not sure whether more can and should be done to address this issue.

Response: We do acknowledge the small sample sizes (especially in the ex-smoker group) that might curtail the generalizability of our results. Unfortunately, we are not in a position to collect any more data, which would be the ideal course of action to expand on our results. We further acknowledge this when referring to the ex-smoker results (under *Increased activation during negative operant response outcomes in ex-smokers*) as follows:

These results may be seen as tentatively pointing towards possible ex-smoker effects that need to be addressed in a larger sample, and in a hypothesis-driven manner.

Comment: I was also somewhat unsure about how to interpret the key findings of the study in light of the analytic approach used by the authors. The authors indicate that they collapsed across conditions (i.e., gain, loss, and neutral outcomes) for each of the "operant response outcome types" (i.e., hits and misses) because there were no group differences when the conditions were compared separately. If I understand correctly, this would have resulted in the collapsing of gain and neutral outcomes for hit trials (gaining a potential reward and avoiding a potential loss, respectively) and the collapsing of neutral and loss outcomes for miss trials (failing to gain a potential reward and incurring a potential loss, respectively). Given the psychological and subjective differences across these possibilities, as well as previous research indicating that they are associated with divergent patterns of brain activation, I was not sure what the implications of collapsing across these four possibilities would be in terms of making sense of the results. The authors recognize this issue and note the following in the Limitations section of the manuscript: "Our findings also represented group differences across the conditions (gain, loss and neutral), as we were unable to detect any group differences on the conditions independently. This may also necessitate a cautious interpretation of the findings, as we cannot unequivocally state how the positive and negative response outcomes on each of the conditions contributed to the group differences reported herein." It would be useful for the authors to further elaborate on the implications of the analysis strategy that they used and clarify more precisely how caution should be exercised when interpreting their findings.

Response: We have now further elaborated on the implications of our analysis strategy in the limitations section of the discussion as follows:

Our findings also represented group differences across the conditions (e.g., gain hit, loss hit, neutral hit), as we were unable to detect any group differences on the conditions independently. While the reported group differences do make it difficult to disentangle the possible differential effects of outcome, a conflation according to valence may still exploit similar processes during goal directed behaviour. There is evidence, for example, that successfully avoiding an aversive outcome is associated with a similar neural profile to being

rewarded (Kim et al., 2006; Seymour et al., 2005b), with stimuli that signal successful avoidance known to positively reinforce (Morris, 1975). This opponent process may likewise be attributed to negative valence, where the termination of a positive state would become aversive, and thus elicit a similar neural response. Our results, however, still necessitate a cautious interpretation, as we cannot unequivocally state how the valence outcomes on each condition individually contributed to our reported group differences.

Comment: The manuscript relies fairly heavily on what has been referred to as "reverse inference" (inferring psychological states/processes from patterns of fMRI activity) when interpreting the fMRI results. As I am sure the authors are aware, the limitations of this strategy have been highlighted in the literature in recent years. The manuscript would be strengthened by bolstering the validity of the reverse inferences that are made (e.g., by using a "quantitative reverse inference approach" in which they use a large database to derive information about the probability of the relevant terms being used across studies given activation of the regions of interest) or, if this is not possible, perhaps reducing their emphasis in the discussion.

Response: Investigators do tend to ascribe specific psychological significance to activations on the basis of prior reports that claim engagement of the associated brain region by a particular functional process. We have, therefore, reduced the use of reverse inferences in our discussion where possible.

Comment: The authors note the possibility that their results "represent disruptions to a dACC-amygdala functional network that tracks some form of prediction error signalling in both current smokers and ex-smokers." This is a very interesting possibility. It was not clear why this idea was not examined using connectivity analyses, as presumably it would have been possible to do so with the available data.

Response: The analyses presented (collapsing across valence type) were not suitable for capturing a modelled prediction error, as we did not probe the BOLD response related to fluctuating reward and loss predictions on a trial-by-trial basis. Therefore, we believe that this type of connectivity analysis would not be suitable for probing a dACC-amygdala functional network related to prediction error signalling for further investigation.

The highlights bullets may need to be revised slightly in light of the two preceding comments.

Comment: A fairly minor point: the goals of the study may be overstated slightly. The introduction states that "the present study set out to examine the effects of both current and previous nicotine exposure on corticolimbic correlates of operant response outcomes" and that one aim was to determine "whether divergent reactivity to positive and negative outcomes represents an adaptation in specific brain networks that might promote long-term smoking cessation through compensatory outcome monitoring." As the authors allude to at one point, the design of the study does not provide strong conclusions to be drawn regarding the extent to which observed group effects are caused by differences in smoking behavior, contributed to differences in smoking behavior, or something else (e.g., some combination or epiphenomenon). It may be useful to tweak the language in the introduction slightly, as well as perhaps specifically note this constraint in the limitations section.

Response: We have now amended the text related to the aims of the current study at the end of the introduction as follows:

Specifically, we aimed to elucidate whether 1) current smokers and ex-smokers demonstrate shared or divergent reactivity compared to controls in the neural substrates of tracking positive and negative operant response outcomes, and 2) whether divergent reactivity to positive and negative outcomes distinguishes ex-smokers from current smokers.

Reviewer #2

This paper examines the divergent neural processing of outcome in monetary incentive delay (MID) task between smokers, ex-smokers and non-smokers. The authors found lower activation in corticolimbic regions in current smokers and, to a lesser degree, in ex-smokers compared to non-smokers during outcome processing in both gain and loss conditions. During loss processing ex-smokers had higher activation in left amygdala compared to current smokers and non-smokers. Also, signal change in the medial frontal gyrus during gain processing reversely correlated with nicotine dependency and signal change in the anterior cingulate during loss processing reversely correlated with cigarette-pack-years. These findings are interesting and the study has the potential to contribute to the field. Below are the issues that should be addressed to maximize study impact.

Comment: 1st highlight point (Maladaptive neural reactivity to non-drug operant response outcomes may bias the choice to smoke) doesn't seem relevant since no evidence of how outcome processing may relate to decision choice is provided in the current work

Response: We have now changed this to:

Maladaptive neural reactivity to non-drug operant response outcomes may promote the continuation of addiction-related behaviours, such as smoking.

Comment: An introduction section seems underdeveloped and does not provide proper framing for the findings. Specifically:

1. Addictive properties of nicotine on motivational brain networks are mentioned, but neither networks nor addictive properties are specified.

Response: We have now changed this in the introduction to:

The reinforcing effects of nicotine (Brody et al., 2006; Domino et al., 2012; Tuesta et al., 2011), particularly in brain regions involved in motivation and reward processing (prefrontal cortex and striatum) in humans (Brody et al., 2004), are likely to be complicit in this failure to achieve abstinence.

Comment: 2. 'Maladaptive reactivity in brain networks that track the salience value of non-drug reward' is mentioned but not explained -the network and the direction of divergence are not specified.

Response: We have now changed this in the introduction to:

Recidivism in nicotine addiction may arise from maladaptive reactivity in fronto-striatal regions, whereby there are neural deficits when processing non-drug outcomes. For example, deprived smokers who exhibit the weakest ventral striatal responses to monetary reward are more likely to subsequently choose smoking over monetary reinforcement (Wilson et al., 2014). Similarly, fronto-striatal regions in nicotine-deprived smokers show a more pronounced response during smoking compared to monetary reward anticipation (Sweitzer et al., 2014). These disturbances in the reward processing may bias the choice to smoke in the presence of alternative, non-drug reinforcement.

Comment: 3. In the 2d paragraph in the introduction: 'Diminished responses during reward outcomes may trigger reactions that are characterized by a heightened motivation during the anticipation of rewards.' The motivation for this logic is unclear. Although citations are offered, they are not properly discussed to support this claim; perhaps the more detailed review of existing literature would clarify this.

Response: We have now reviewed some of this literature in more detail in the introduction as follows:

Indeed this dissociation has been observed in several populations. Cigarette smokers, for example, show increased ventromedial prefrontal cortex activation during loss anticipation of monetary incentives, but decreased activation in the inferior frontal gyrus during the receipt of monetary gains (Martin et al., 2014). Similar patterns of anticipation and outcome divergence have also been reported in binge-eating disorder patients, with increased fronto-striatal activation during loss and gain anticipation, but decreased activation to loss and reward notifications (Balodis et al., 2013). Pathological gamblers have also demonstrated similar differences in response to anticipation and outcomes, showing a hyper responsiveness in the ventral striatum during loss anticipation, but a hypo responsiveness during successful loss avoidance (Romanczuk-Seiferth et al., 2015). These findings, therefore, appear to suggest that in some addiction populations there is a divergence in reactivity between the anticipation and delivery of non-drug reinforcement in fronto-striatal regions, possibly due to deficits in valence outcome processing. Furthermore, for those who do achieve full abstinence, there may be adaptations in fronto-striatal regions that represent a neural substrate for promoting long-term cessation. These adaptations are represented by an amplified valence outcome monitoring that promotes the control over addiction-related behaviours (Garavan et al., 2005), even in the presence of heightened and sustained "wanting" of rewards.

Comment: 4. The authors discuss 'outcome salience' but it is not clear from the paper whether they just referring to outcome processing and use the word 'salience' referring to reward/loss value or specifically focus on salience processing. If the later, salience network should be discussed (Udin, 2014), if the earlier, than proper term should be used in order not to confuse the reader.

Response: We have now changed salience to valence throughout the manuscript, as we think this is a more appropriate term given that the outcomes were negative (loss) and positive (gain) in their valence.

Comment: 5. The 'adaptations in specific brain networks that represent a neural substrate for promoting long-term cessation through compensatory outcome monitoring that promotes the control over addictive behaviors' are discussed without naming the networks and neural components.

Response: We have now changed this in the introduction to:

Hyperactivity in lateral and medial prefrontal regions that sub-serve inhibitory control functioning and error monitoring have been reported in ex-smokers (Kroenke et al., 2015; Nestor et al., 2011). This may suggest that the emergence of amplified prefrontal cognitive functioning is necessary for successful abstinence in addiction. Therefore, similar adaptations that are represented by an amplified valence outcome monitoring may also promote the control over addiction-related behaviours (Garavan et al., 2005), even in the presence of heightened and sustained “wanting” of rewards.

Comment: The MID task description is unclear. Participants' goal, task flow (the outcome of each possible subject's action) are not defined. The reader is required to find the cited papers that describe the task in order to understand the reported experiment.

Response: We have now generated a supplementary figure showing the Monetary MID task structure. This will hopefully complement the description of the task in the methods section.

- Maladaptive neural reactivity to non-drug operant response outcomes may promote the continuation of addiction-related behaviours, such as smoking.
- Current *and* ex-smokers show less neural reactivity to positive and negative operant response outcomes.
- Ex-smokers show more amygdala reactivity to negative operant response outcomes.
- Exaggerated negative valence outcome processing in *ex-smokers* may promote long-term smoking cessation.

Shared and divergent neural reactivity to non-drug operant response outcomes in current smokers and ex-smokers.

Liam J Nestor^{1, 2}, Ella McCabe¹, Jennifer Jones¹, Luke Clancy³ Hugh Garavan^{1, 4}

¹*School of Psychology and Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland*

²*Neuropsychopharmacology Unit, Centre for Psychiatry, Imperial College London, UK*

³*TobaccoFree Research Institute Ireland, DIT, Dublin, Ireland*

⁴*Department of Psychiatry, University of Vermont, Burlington, Vermont, USA*

Abstract

Addiction to cigarettes presents with considerable health risks and induces high costs on healthcare resources. While the majority of cigarette smokers endorse the desire to quit, only a small percentage of quit attempts lead to full abstinence. Failure to achieve abstinence may arise from maladaptive reactivity in fronto-striatal regions that track positive and negative valence outcomes, thus biasing the choice to smoke in the presence of alternative, non-drug reinforcement. Alternatively, long-term nicotine abstinence may reveal neural substrates of adaptive valence outcome processing that promote and maintain smoking cessation. The present study set out to examine the neural correlates of operant response outcomes in current smokers, ex-smokers and matched controls using a monetary incentive delay task during functional MRI. Here we report that compared to controls, both current smokers *and* ex-smokers showed significantly less activation change in the left amygdala during positive response outcomes, and in the anterior cingulate cortex, during both positive and negative response outcomes. Ex-smokers, however, demonstrated significantly greater activation change compared to smokers *and* controls in the right amygdala during negative response outcomes. Activation change in the anterior cingulate cortex and middle frontal gyrus of smokers were significantly negatively correlated with nicotine dependence and cigarette pack-years. These results suggest a pattern of shared and divergent reactivity in current smokers and ex-smokers within corticolimbic regions that track both positive and negative operant response outcomes. Exaggerated adaptive processing in *ex-smokers* may promote long-term smoking cessation through amplified negative valence outcome monitoring.

Introduction

Addiction to cigarettes presents with considerable health risks (Bartal, 2001) and induces high costs on healthcare resources (Leistikow et al., 2000). 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).

This continued use in the face of adversity is a powerful testimony to the effects of nicotine dependence, demonstrating its reinforcing effects. The reinforcing effects of nicotine (Brody et al., 2006; Domino et al., 2012; Tuesta et al., 2011), particularly in brain regions involved in motivation and reward processing (prefrontal cortex and striatum) in humans (Brody et al., 2004), are likely to be complicit in this failure to achieve abstinence.

Recidivism in nicotine addiction may arise from maladaptive reactivity in fronto-striatal regions, whereby there are neural deficits when processing non-drug outcomes. For example, deprived smokers who exhibit the weakest ventral striatal responses to monetary reward are more likely to subsequently choose smoking over monetary reinforcement (Wilson et al., 2014). Similarly, fronto-striatal regions in nicotine-deprived smokers show a more pronounced response during smoking compared to monetary reward anticipation (Sweitzer et al., 2014). These disturbances in the reward processing may bias the choice to smoke in the presence of alternative, non-drug reinforcement.

We have previously reported that current smokers *and* ex-smokers, compared to controls, demonstrate heightened fronto-striatal activation during gain and loss anticipation (Nestor et al., 2016a), supporting previous research findings in

Formatted: Line spacing: Double

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: 12 pt, Not Italic

Formatted: Font: 12 pt, Not Italic

Formatted: Font: 12 pt, Not Italic

Formatted: Font: 12 pt, Not Italic

Formatted: Font: 12 pt, Not Italic

1 nicotine addiction (Martin et al., 2014). We proposed that this may represent a
2
3 sensitization by nicotine within this circuitry of smokers, that triggers the excessive
4
5 “wanting” of rewards in response to non-drug cues (Berridge et al., 1998). This
6
7 excessive “wanting” during anticipation may also arise from a dissociation from
8
9 consummation or receipt of rewards, where there is a diminished response in
10
11 regions that process incentive outcomes. Diminished responses during reward
12
13 outcomes may trigger reactions that are characterized by a heightened motivation
14
15 during the anticipation of rewards. Indeed this dissociation has been observed in
16
17 several populations. Cigarette smokers, for example, show increased ventromedial
18
19 prefrontal cortex activation during loss anticipation of monetary incentives, but
20
21 decreased activation in the inferior frontal gyrus during the receipt of monetary gains
22
23 (Martin et al., 2014). Similar patterns of anticipation and outcome divergence have
24
25 also been reported in binge-eating disorder patients, with increased fronto-striatal
26
27 activation during loss and gain anticipation, but decreased activation to loss and
28
29 reward notifications (Balodis et al., 2013). Pathological gamblers have also
30
31 demonstrated similar differences in response to anticipation and outcomes, showing
32
33 a hyper responsiveness in the ventral striatum during loss anticipation, but a hypo
34
35 responsiveness during successful loss avoidance (Romanczuk-Seiferth et al., 2015).
36
37 These findings, therefore, appear to suggest that in some addiction populations
38
39 there is a divergence in reactivity between the anticipation and delivery of non-drug
40
41 reinforcement in fronto-striatal regions, possibly due to deficits in valence outcome
42
43 processing. Hyperactivity in lateral and medial prefrontal regions that sub-serve
44
45 inhibitory control functioning and error monitoring have been reported in ex-smokers
46
47 (Kroenke et al., 2015; Nestor et al., 2011). This may suggest that the emergence of
48
49 amplified prefrontal cognitive functioning is necessary for successful abstinence in
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

addiction. Therefore, similar adaptations that are represented by an amplified valence outcome monitoring may also promote the control over addiction-related behaviours (Garavan et al., 2005), even in the presence of heightened and sustained “wanting” of rewards.

Therefore, the present study set out to examine the effects of both current and previous nicotine exposure on corticolimbic correlates of operant response outcomes. Here we compared current smokers, ex-smokers and demographically matched healthy controls using a monetary incentive delay task. Specifically, we aimed to elucidate whether 1) current smokers and ex-smokers demonstrate shared or divergent reactivity compared to controls in the neural substrates of tracking positive and negative operant response outcomes, and 2) whether divergent reactivity to positive and negative outcomes distinguishes ex-smokers from current smokers.

Formatted: Justified, Line spacing Double

Formatted: Font: 12 pt, Not Italic

Results

Demographics

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, 84.8 weeks (range: 52-180 weeks) at the time of testing (see Table 1 in supplementary materials for a more detailed description of group demographics).

Behaviour

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 ($F=5.6$; $df=111, 2$; $p<0.01$), with less accuracy on neutral compared to loss ($p<0.05$) and gain ($p<0.01$) trials. There was no effect of group ($F=0.5$; $df=111, 2$; $p=0.6$) and no condition x group interaction ($F=0.09$; $df=111, 4$; $p=0.99$). Figure 1b shows the mean MID reaction time (milliseconds) for the three conditions in the three groups. There was a significant effect of condition ($F=2.6$; $df=111, 2$; $p<0.05$), with faster response reaction time on the gain compared to neutral ($p<0.05$) trials only. There was 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 collapsed across conditions (gain, loss, neutral) for each of the operant response outcome types (“Hit” and “Miss”) separately, as we did not detect any significant group differences on the conditions independently.

Positive operant response outcomes

There were five clusters that showed a group effect in the ROI mask, comprising the anterior cingulate cortex (ACC: 733 voxels; $x=4$; $y=14$; $z=26$; $Z_{stat}=4.71$; $p<0.0001$; smoker<control, $p<0.0001_{Bonferroni}$; ex-smoker<control, $p<0.001_{Bonferroni}$); right amygdala (353 voxels; $x=20$; $y=-6$; $z=-20$; $Z_{stat}=3.37$; $p<0.001$; smoker<control, $p<0.001_{Bonferroni}$; smoker<ex-smoker, $p<0.0001_{Bonferroni}$ - Fig2A); frontal pole (347 voxels; $x=48$; $y=40$; $z=20$; $Z_{stat}=5.38$; $p<0.001$; smoker<ex-smoker, $p<0.05_{Bonferroni}$); middle frontal gyrus (MFG: 325 voxels; $x=36$; $y=20$; $z=40$; $Z_{stat}=7.23$; $p<0.001$; smoker<control, $p<0.05_{Bonferroni}$; smoker<ex-smoker, $p<0.001_{Bonferroni}$ - Fig2C) and the left amygdala (153 voxels; $x=-28$; $y=-10$; $z=-14$; $Z_{stat}=4.66$; $p<0.05$; smoker<control, $p<0.01_{Bonferroni}$; ex-smoker<control, $p<0.05_{Bonferroni}$ - Fig2B).

-Insert Figure 2 about here-

Negative operant response outcomes

There were also five clusters that showed a group effect, comprising the ACC (374 voxels; $x=2$; $y=-4$; $z=32$; $Z_{stat}=5.07$; $p<0.0001$; smoker<control, $p<0.001_{Bonferroni}$; ex-smoker<control, $p<0.001_{Bonferroni}$ - Fig3C); frontal pole (297 voxels; $x=48$; $y=40$; $z=20$; $Z_{stat}=4.12$; $p<0.0001$; smoker<ex-smoker, $p<0.001_{Bonferroni}$); right amygdala (194 voxels; $x=22$; $y=-4$; $z=-22$; $Z_{stat}=5.01$; $p<0.01$; ex-smoker>control, $p<0.05_{Bonferroni}$; ex-smoker>smoker, $p<0.001_{Bonferroni}$ - Fig3A); orbitofrontal cortex (OFC: 168 voxels; $x=-24$; $y=28$; $z=-18$; $Z_{stat}=4.37$; $p<0.01$; smoker<control, $p<0.05_{Bonferroni}$ - Fig3B) and the left inferior frontal gyrus (IFG: 157 voxels; $x=-34$; $y=26$; $z=20$; $Z_{stat}=5.43$; $p<0.01$; smoker<control, $p<0.0001_{Bonferroni}$; smoker<ex-smoker, $p<0.01_{Bonferroni}$).

-Insert Figure 3 about here-

Correlations

There were several significant negative correlations between smoking demographics in the smoker group, and their mean percentage BOLD change (collapsed across the gain, loss and neutral conditions) during the outcome types. FTND scores were negatively correlated with the mean BOLD signal change in the MFG ($r(13) = -.52$; $p < 0.05$ - Fig4A), with a trend for a negative correlation with ACC BOLD signal change ($r(13) = -.48$; $p < 0.07$), during positive operant response outcomes. Cigarette pack-years was negatively correlated with ACC BOLD signal change ($r(13) = -.61$; $p < 0.05$ - Fig4B), with a trend for a negative correlation with right amygdala BOLD signal change ($r(13) = -.49$; $p < 0.07$), during negative response outcomes. There were no significant correlations detected in the ex-smoker group.

-Insert Figure 4 about here-

Discussion

We compared activation changes elicited by operant response feedback during a monetary incentive delay task in demographically matched controls, current smokers and ex-smokers. Here we observed a pattern of shared and divergent reactivity in smokers and ex-smokers, compared to controls, within corticolimbic regions that track both positive and negative operant response outcome feedback. Activation differences in some of these regions were also found to negatively

correlate with nicotine dependence and cigarette use in current smokers. The group differences were also identified in the absence of group differences in behavioural performance, potentially discounting this as confounding the neural reactivity differences observed during the operant response outcomes.

Reduced activation change during operant response outcomes in current smokers

This study observed that current smokers had significantly reduced activation change in a number of corticolimbic regions during operant response outcomes. Notably, there was a reduced response in the right amygdala and middle frontal gyrus (MFG) during positive operant response outcomes compared to controls *and* ex-smokers. There is evidence that the amygdala encodes stimulus value (Jenison et al., 2011), responds to reward outcomes, (Ernst et al., 2005), and is involved in salience processing (Belova et al., 2007; Pessoa et al., 2010). Amygdala disturbances also play a role in addiction (Lesscher et al., 2012; See et al., 2003), with our findings possibly suggesting a disturbance in this region in current smokers. The MFG is also involved in representing and integrating reward information (Watanabe et al., 2007), with evidence for diminished activation in addiction populations (Ersche et al., 2005; Paulus et al., 2002), including smokers (Nestor et al., 2011). The current study additionally revealed MFG attenuation in response to positive operant response outcomes in current smokers that was also negatively correlated with the level of reported nicotine dependence.

The current study showed that current smokers showed a reduced activation change in the medial orbitofrontal cortex (OFC) compared to controls during negative response outcomes. We previously reported (Nestor et al., 2016a) that

1 there were *increased* responses in a region of the lateral OFC, compared to
2 controls, in the same sample of current smokers *and* ex-smokers during both loss
3 and gain (but not neutral) anticipation, suggesting some type of heightened
4 motivational drive during the expectancy of non-drug rewards. The present finding,
5 observed during negative operant outcomes, however, appears to suggest divergent
6 reactivity to anticipatory versus receipt phases of incentive processing in current
7 smokers - representing some type of disturbance in negative valence outcome
8 monitoring. Current smokers also showed a reduced activation change in the inferior
9 frontal gyrus (IFG) compared to both controls and ex-smokers during negative
10 response outcomes, pointing to disturbances in both ventral and dorsal cortical
11 regions in this group.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 *Reduced activation change during operant response outcomes in current smokers*
29 *and ex-smokers*
30
31

32 This study reports that during both positive and negative operant response
33 outcomes, ex-smokers, and to a greater degree, current smokers, showed less
34 activation change in the anterior cingulate cortex (ACC). Disturbances in ACC
35 functioning have been reported in addiction (Goldstein et al., 2002; Peoples, 2002;
36 Volkow et al., 2002), that have been shown to extend into abstinence (Nestor et al.,
37 2016b). Interestingly, these differences were observed in the dorsal ACC (dACC)
38 region, known to be involved in motivation (Kouneiher et al., 2009) and also
39 prediction error signalling (Wallis et al., 2010). We also observed a reduced
40 activation change in the left amygdala during positive operant response outcomes in
41 current smokers and ex-smokers. The amygdala and dACC, interestingly, are also
42 connected (Beckmann et al., 2009), with the possibility that our findings represent
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 disruptions in dACC-amygdala connectivity in both current smokers and ex-
3 smokers. Significantly, the level of reported cigarette use (pack-years) in smokers
4 was also found to significantly predict ACC attenuation during negative operant
5 response outcomes.
6
7
8
9

10 11 12 *Increased activation during negative operant response outcomes in ex-smokers* 13

14 The monitoring of one's behavioural outcomes during nicotine abstinence
15 may be especially important when there is a need to detect errors, particularly those
16 that might induce smoking relapse. The neural substrates of behavioural monitoring
17 in addiction may have implications for abstinence, as they may elucidate specific
18 adaptations that promote the control over addiction-related behaviours (Garavan et
19 al., 2005). Therefore, the ability of ex-smokers to remain abstinent may be related to
20 the amplified monitoring of performance errors. Here we report that in response to
21 negative operant response outcomes, ex-smokers demonstrated a significantly
22 greater activation change compared to smokers *and* controls in the right amygdala.
23 This was in contrast to the observed attenuation in the left amygdala during positive
24 operant response outcomes, where ex-smokers showed a similar effect to smokers.
25 This divergence in amygdala responding may suggest they exhibit a bias in the
26 tracking of negative valence outcomes. The amygdala does respond to negative
27 feedback (Klavrir et al., 2013) and is involved in prediction error signalling (Meder et
28 al., 2016; Seymour et al., 2005a), which may be amplified in ex-smokers. This
29 amplification following smoking cessation may potentially contribute to nicotine
30 abstinence through heightened error encoding. These results may be seen as
31 tentatively pointing towards possible ex-smoker effects that need to be addressed in
32 a larger sample, and in a hypothesis-driven manner.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6 Limitations of the current study involve small sample sizes, particularly in the ex-
7
8 smoker group, and the low-moderate nicotine dependency range in current
9
10 smokers. The relatively modest levels of dependency, for example, may curtail the
11
12 generalizability of the current findings to greater levels of nicotine dependence that
13
14 are reported in older adults. Our findings also represented group differences across
15
16 the conditions (e.g., gain hit, loss hit, neutral hit), as we were unable to detect any
17
18 group differences on the conditions independently. While the reported group
19
20 differences do make it difficult to disentangle the possible differential effects of
21
22 outcome, a conflation according to valence may still exploit similar processes during
23
24 goal directed behaviour. There is evidence, for example, that successfully avoiding
25
26 an aversive outcome is associated with a similar neural profile to being rewarded
27
28 (Kim et al., 2006; Seymour et al., 2005b), with stimuli that signal successful
29
30 avoidance known to positively reinforce (Morris, 1975). This opponent process may
31
32 likewise be attributed to negative valence, where the termination of a positive state
33
34 would become aversive, and thus elicit a similar neural response. Our results,
35
36 however, still necessitate a cautious interpretation, as we cannot unequivocally state
37
38 how the valence outcomes on each condition individually contributed to our reported
39
40 group differences.
41
42
43
44
45
46

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

47 Conclusion

48
49
50 Despite the limitation of a small sample size, and the low-moderate nicotine
51
52 dependency, this study has provided some preliminary evidence for a pattern of
53
54

1 shared and divergent reactivity in current smokers and ex-smokers within
2 corticolimbic regions that track positive and negative operant response outcomes.
3
4 We propose that exaggerated processing in ex-smokers may promote long-term
5
6 smoking cessation through amplified negative valence outcome monitoring, that
7
8 appears to remain disrupted in current smokers.
9
10
11
12
13
14

15 **Declaration of Conflicting Interests**

16
17 The authors report no biomedical financial interests or potential conflicts of interest.
18
19
20

21 **Acknowledgments**

22 This research was supported by a grant from the TobaccoFree Research Institute
23 (formerly the Research Institute for a Tobacco Free Society), Dublin, Ireland. The
24 assistance of Ms. Sheila Keogan (Research and Communication) and Ms. Vanessa
25 Clarke (Corporate Affairs) at the TobaccoFree Research Institute is gratefully
26 acknowledged.
27
28
29
30

31 **Methods and Materials**

32 *Participants*

33
34 For a more comprehensive description of the material and methods, please
35
36 see Nestor et al. (2016a), which used the same cohorts reported herein, to examine
37
38 differences in loss and reward anticipation. Briefly, 15 current cigarette smokers, 10
39
40 ex-smokers and 15 controls completed the study. A semi-structured interview, as
41
42 used in previous behavioural and functional imaging studies (Carey et al., 2015;
43
44 Nestor et al., 2016a) was conducted to screen participants for past or present
45
46 histories of psychiatric or neurological illness. Any potential participant describing
47
48 any major life-time psychiatric event or brain injury (e.g., head trauma resulting in a
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 loss of consciousness, seizure or stroke) or any familial psychiatric history (i.e.
3
4 sibling, parent or grandparent) was considered ineligible for the study.
5
6
7

8 Current smokers were required to have regularly consumed tobacco (≥ 10
9 cigarettes/day) for the previous 2 years in order to be eligible. Ex-smokers were
10 required to have regularly consumed tobacco (≥ 10 cigarettes/day) for at least two
11 years, but be nicotine abstinent for at least 12 months at the time of testing. Control
12 participants were required to have never smoked cigarettes. Smoking abstinence in
13 ex-smokers and controls was confirmed by measuring expired carbon monoxide
14 (CO) in parts per million (ppm) during the screening process. Cigarette smokers
15 each smoked *ad lib* approximately 15 minutes prior to scanning in order to avoid the
16 potential confounds of withdrawal and/or craving on MID task performance. All
17 participants were right-handed as confirmed by the Edinburgh Handedness
18 Inventory (Oldfield, 1971) during the screening process. All participants completing
19 the study were neurologically normal (as confirmed by a registered radiologist who
20 examined each structural MRI). All research participants provided informed consent
21 and were financially compensated.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 *Questionnaires*

45 The National Adult Reading Test (NART) (Nelson et al., 1978) was
46 administered to all participants during the screening procedure to assess verbal
47 intelligence, as was the Beck Depression Inventory (BDI) to assess mood (Beck et
48 al., 1996). Information concerning alcohol and drug use was obtained from all
49
50
51
52
53
54

1 participants using a questionnaire taken from the Addiction Severity Index Lite-CF
2 (McLellan et al., 1992). During the screening procedure, the Fagerström test of
3 nicotine (Heatherton et al., 1991) dependence (FTND) was administered to
4 smokers. The FTND was also administered to ex-smokers, retrospectively, in order
5 to match their previous levels of nicotine dependence (when they were active
6 smokers) to current smokers. The rationale for this was to eliminate previous
7 dependency levels in ex-smokers as a potential contributing factor to neural
8 differences with smokers. The Shiffman-Jarvik smoking withdrawal questionnaire
9 (SJWQ) (Shiffman et al., 1976) and the urge to smoke (UTS) scale (Jarvik et al.,
10 2000) were also administered to smokers prior to scanning to assess craving and
11 withdrawal.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

27 *Monetary Incentive Delay Task (MID)*

28 We used a “monetary incentive delay task” (MID), which was based on that
29 originally employed by Knutson (Knutson et al., 2001) and which we have previously
30 used to assess the neural correlates of reward processing in cannabis users (Nestor
31 et al., 2010) and smokers (Nestor et al., 2016a) – see Supplementary Figure 1.
32 While being scanned participants performed the MID task, during which they
33 anticipated potential monetary gain, loss or no potential monetary outcome. During
34 each trial, participants viewed one of three coloured squares (cue) that indicated the
35 potential to gain fifty cent (green square), lose fifty cent (red square) or experience
36 no financial outcome (blue square - here referred to as the neutral condition)
37 following their response to an upcoming visual target. Each cue was presented for a
38 variable duration (2-8 sec), after which participants made a button press response
39 upon the presentation of a visual target (star located within a circle). Participants
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 4.1.7, 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, separately, the gain, loss and neutral positive ("hit") operant response outcome periods, and the gain, loss and neutral negative ("miss") operant response outcome periods (stick functions for each of the feedback period regressors were convolved with the haemodynamic response function). First level whole-brain mixed-effects analyses were also 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 results of which have been previously reported (Nestor et al., 2016a). The end fixation period of the task served as the

1
2 implicit baseline. Therefore, all reported differences between groups are for
3
4 activation changes versus the baseline period. Registration was conducted through
5
6 a two-step procedure, whereby EPI images were first registered to the high-
7
8 resolution T1 structural image, then into standard (Montreal Neurological Institute,
9
10 MNI avg152 template) space, with 12-parameter affine transformations.
11
12
13

14 As corticolimbic regions are critical neural substrates for the processing of
15
16 non-drug rewards in humans (Knutson et al., 2001; O'Doherty et al., 2001), with
17
18 evidence for both hypoactivity and hyperactivity to non-drug rewards in these
19
20 regions in addiction populations, including current smokers (Martin et al., 2014;
21
22 Nestor et al., 2016a; Sweitzer et al., 2014; Wilson et al., 2008), we took an *a priori*
23
24 region of interest (ROI) approach in our search for group differences during the
25
26 operant response outcomes. Here we restricted this search to the nucleus
27
28 accumbens (NAcc), amygdala, caudate, putamen, insula, inferior frontal gyrus, and
29
30 the anterior cingulate, dorsolateral and orbitofrontal cortices, bilaterally. These
31
32 regions were taken from the Harvard-Oxford cortical and subcortical structural atlas
33
34 and grouped together into one ROI mask. Higher-level between-group analyses
35
36 (one-way ANOVAs) were conducted using FLAME (FMRIB's Local Analysis of
37
38 Mixed Effects) collapsing across conditions (gain, loss, neutral) for each of the
39
40 operant response outcome types ("hit" and "miss") separately. These analyses were
41
42 conducted as we did not detect any significant group differences on the conditions
43
44 independently. Significant clusters in this ROI mask of *a priori* regions were
45
46 determined by thresholding at $Z > 2.3$ with a corrected cluster significance threshold
47
48 of $p < 0.05$.
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 follow-up *post hoc* analyses on the mean BOLD signal change from the ANOVA group effect clusters, we conducted Bonferroni pairwise comparisons to examine which groups significantly differed from one another. Correlations between smoking demographics and mean BOLD signal change from the zF-statistic clusters were assessed using Spearman's rho correlations. All these analyses were conducted using the R statistical software package (2013).

References

- Balodis, I. M., Kober, H., Worhunsky, P. D., White, M. A., Stevens, M. C., Pearlson, G. D., Sinha, R., Grilo, C. M., & Potenza, M. N. (2013). Monetary reward processing in obese individuals with and without binge eating disorder. *Biol Psychiatry*, 73(9), 877-886. doi: 10.1016/j.biopsych.2013.01.014
- Bartal, M. (2001). Health effects of tobacco use and exposure. *Monaldi Arch Chest Dis*, 56(6), 545-554.
- Beck, A., Steer, R., & Brown, G. (1996). Beck Depression Inventory—Second Edition manual. *The Psychological Corporation, San Antonio, TX*.
- Beckmann, M., Johansen-Berg, H., & Rushworth, M. F. S. (2009). Connectivity-Based Parcellation of Human Cingulate Cortex and Its Relation to Functional Specialization. *Journal of Neuroscience*, 29(4), 1175-1190. doi: 10.1523/Jneurosci.3328-08.2009
- Belova, M. A., Paton, J. J., Morrison, S. E., & Salzman, C. D. (2007). Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. *Neuron*, 55(6), 970-984.
- Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*, 28(3), 309-369.
- Brody, A. L., Mandelkern, M. A., London, E. D., Olmstead, R. E., Farahi, J., Scheibal, D., Jou, J., Allen, V., Tiongson, E., Chefer, S. I., Koren, A. O., & Mukhin, A. G. (2006). Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Arch Gen Psychiatry*, 63(8), 907-915.
- Brody, A. L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., & Mandelkern, M. A. (2004). Smoking-induced ventral striatum dopamine release. *Am J Psychiatry*, 161(7), 1211-1218.

- Carey, S. E., Nestor, L., Jones, J., Garavan, H., & Hester, R. (2015). Impaired learning from errors in cannabis users: Dorsal anterior cingulate cortex and hippocampus hypoactivity. *Drug and Alcohol Dependence*, 155, 175-182. doi: 10.1016/j.drugalcdep.2015.07.671
- Domino, E. F., Evans, C. L., Ni, L., Guthrie, S. K., Koeppe, R. A., & Zubieta, J. K. (2012). Tobacco smoking produces greater striatal dopamine release in G-allele carriers with mu opioid receptor A118G polymorphism. *Prog Neuropsychopharmacol Biol Psychiatry*, 38(2), 236-240. doi: 10.1016/j.pnpbp.2012.04.003
- Ernst, M., Nelson, E. E., Jazbec, S., McClure, E. B., Monk, C. S., Leibenluft, E., Blair, J., & Pine, D. S. (2005). Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. *Neuroimage*, 25(4), 1279-1291. doi: 10.1016/j.neuroimage.2004.12.038
- Ersche, K. D., Fletcher, P. C., Lewis, S. J. G., Clark, L., Stocks-Gee, G., London, M., Deakin, J. B., Robbins, T. W., & Sahakian, B. J. (2005). Abnormal frontal activations related to decision-making in current and former amphetamine and opiate dependent individuals. *Psychopharmacology*, 180(4), 612-623. doi: 10.1007/s00213-005-2205-7
- Garavan, H., & Stout, J. C. (2005). Neurocognitive insights into substance abuse. *Trends Cogn Sci*, 9(4), 195-201.
- Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry*, 159(10), 1642-1652.
- Heatherston, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerstrom, K. O. (1991). The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*, 86(9), 1119-1127.
- Hughes, J. R., Peters, E. N., & Naud, S. (2008). Relapse to smoking after 1 year of abstinence: a meta-analysis. *Addict Behav*, 33(12), 1516-1520.
- Jarvik, M. E., Madsen, D. C., Olmstead, R. E., Iwamoto-Schaap, P. N., Elins, J. L., & Benowitz, N. L. (2000). Nicotine blood levels and subjective craving for cigarettes. *Pharmacol Biochem Behav*, 66(3), 553-558.
- Jenison, R. L., Rangel, A., Oya, H., Kawasaki, H., & Howard, M. A. (2011). Value encoding in single neurons in the human amygdala during decision making. *J Neurosci*, 31(1), 331-338. doi: 10.1523/JNEUROSCI.4461-10.2011
- Kim, H., Shimojo, S., & O'Doherty, J. P. (2006). Is avoiding an aversive outcome rewarding? Neural substrates of avoidance learning in the human brain. *PLoS Biol*, 4(8), e233.
- Klavir, O., Genuit-Gabai, R., & Paz, R. (2013). Functional Connectivity between Amygdala and Cingulate Cortex for Adaptive Aversive Learning. *Neuron*, 80(5), 1290-1300. doi: 10.1016/j.neuron.2013.09.035
- Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci*, 21(16), RC159.
- Kouneiher, F., Charron, S., & Koechlin, E. (2009). Motivation and cognitive control in the human prefrontal cortex. *Nat Neurosci*, 12(7), 939-945. doi: 10.1038/nn.2321
- Kroenke, K. M., Wolff, M., Benz, A., & Goschke, T. (2015). Successful smoking cessation is associated with prefrontal cortical function during a Stroop task: A preliminary study. *Psychiatry Research-Neuroimaging*, 234(1), 52-56. doi: 10.1016/j.psychresns.2015.08.005

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
 - 61
 - 62
 - 63
 - 64
 - 65
- Leistikow, B. N., Martin, D. C., & Milano, C. E. (2000). Fire injuries, disasters, and costs from cigarettes and cigarette lights: a global overview. *Prev Med*, 31(2 Pt 1), 91-99.
- Lesscher, H. M. B., & Vanderschuren, L. J. M. J. (2012). Compulsive drug use and its neural substrates. *Reviews in the Neurosciences*, 23(5-6), 731-745. doi: 10.1515/revneuro-2012-0066
- Martin, L. E., Cox, L. S., Brooks, W. M., & Savage, C. R. (2014). Winning and losing: differences in reward and punishment sensitivity between smokers and nonsmokers. *Brain and Behavior*, 4(6), 915-924. doi: 10.1002/brb3.285
- McLellan, A. T., Kushner, H., Metzger, D., Peters, R., Smith, I., Grissom, G., Pettinati, H., & Argeriou, M. (1992). The fifth edition of the Addiction Severity Index. *J. Subst. Abuse Treat*, 9, 199-213.
- Meder, D., Madsen, K. H., Hulme, O., & Siebner, H. R. (2016). Chasing probabilities - Signaling negative and positive prediction errors across domains. *Neuroimage*, 134, 180-191. doi: 10.1016/j.neuroimage.2016.04.019
- Nelson, H., & O'Connell, A. (1978). Dementia: The estimation of pre-morbid intelligence levels using the new adult reading test. *Cortex*, 14((2)), 234-244.
- Nestor, L., Hester, R., & Garavan, H. (2010). Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage*, 49(1), 1133-1143. doi: 10.1016/j.neuroimage.2009.07.022
- Nestor, L., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2011). Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage*, 56(4), 2258-2275. doi: 10.1016/j.neuroimage.2011.03.054
- Nestor, L. J., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2016a). Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses. *Addict Biol*. doi: 10.1111/adb.12484
- Nestor, L. J., Murphy, A., McGonigle, J., Orban, C., Reed, L., Taylor, E., Flechais, R., Paterson, L. M., Smith, D., Bullmore, E. T., Ersche, K. D., Suckling, J., Tait, R., Elliott, R., Deakin, B., Rabiner, I., Lingford-Hughes, A., Nutt, D. J., Sahakian, B., Robbins, T. W., & Consortium, I. (2016b). Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations during a monetary incentive delay task. *Addiction Biology*, n/a-n/a. doi: 10.1111/adb.12444
- O'Doherty, J., Kringelbach, M. L., Rolls, E. T., Hornak, J., & Andrews, C. (2001). Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat Neurosci*, 4(1), 95-102.
- Oldfield, R. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, 9, 97-113.
- Paulus, M. P., Hozack, N. E., Zauscher, B. E., Frank, L., Brown, G. G., Braff, D. L., & Schuckit, M. A. (2002). Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. *Neuropsychopharmacology*, 26(1), 53-63. doi: S0893133X01003347 [pii] 10.1016/S0893-133X(01)00334-7
- Peoples, L. L. (2002). Will, anterior cingulate cortex, and addiction. *Science*, 296, 1623-1624.
- Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. *Nature Reviews Neuroscience*, 11(11), 773-782. doi: 10.1038/nrn2920

- 1 Pruessmann, K. P., Weiger, M., Scheidegger, M. B., & Boesiger, P. (1999). SENSE:
2 sensitivity encoding for fast MRI. *Magn Reson Med*, 42(5), 952-962.
- 3 Romanczuk-Seiferth, N., Koehler, S., Dreesen, C., Wustenberg, T., & Heinz, A.
4 (2015). Pathological gambling and alcohol dependence: neural disturbances
5 in reward and loss avoidance processing. *Addict Biol*, 20(3), 557-569. doi:
6 10.1111/adb.12144
- 7 See, R. E., Fuchs, R. A., Ledford, C. C., & McLaughlin, J. (2003). Drug addiction,
8 relapse, and the amygdala. *Amygdala in Brain Function: Basic and Clinical*
9 *Approaches*, 985, 294-307.
- 10 Seymour, B., O'Doherty, J. P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston,
11 K., & Dolan, R. (2005a). Opponent appetitive-aversive neural processes
12 underlie predictive learning of pain relief. *Nat Neurosci*, 8(9), 1234-1240.
- 13 Seymour, B., O'Doherty, J. P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston,
14 K. J., & Dolan, R. (2005b). Opponent appetitive-aversive neural processes
15 underlie predictive learning of pain relief. *Nature Neuroscience*, 8(9), 1234-
16 1240. doi: 10.1038/nn1527
- 17 Shiffman, S., & Jarvik, M. (1976). Smoking withdrawal symptoms in two weeks of
18 abstinence. *Psychopharmacology (Berl)*, 50(1), 35-39.
- 19 Sweitzer, M. M., Geier, C. F., Joel, D. L., McGurrin, P., Denlinger, R. L., Forbes, E.
20 E., & Donny, E. C. (2014). Dissociated Effects of Anticipating Smoking versus
21 Monetary Reward in the Caudate as a Function of Smoking Abstinence.
22 *Biological Psychiatry*, 76(9), 681-688. doi: 10.1016/j.biopsych.2013.11.013
- 23 Tuesta, L. M., Fowler, C. D., & Kenny, P. J. (2011). Recent advances in
24 understanding nicotinic receptor signaling mechanisms that regulate drug
25 self-administration behavior. *Biochemical Pharmacology*, 82(8), 984-995. doi:
26 10.1016/j.bcp.2011.06.026
- 27 Volkow, N., Fowler, J., Wang, G., & Goldstein, R. (2002). Role of dopamine, the
28 frontal cortex and memory circuits in drug addiction: insight from imaging
29 studies. *Neurobiol Learn Mem*, 78(3), 610-624.
- 30 Wallis, J. D., & Kennerley, S. W. (2010). Heterogeneous reward signals in prefrontal
31 cortex. *Current Opinion in Neurobiology*, 20(2), 191-198. doi:
32 10.1016/j.conb.2010.02.009
- 33 Watanabe, M., & Sakagami, M. (2007). Integration of cognitive and motivational
34 context information in the primate prefrontal cortex. *Cereb Cortex*, 17 Suppl
35 1, i101-109. doi: 10.1093/cercor/bhm067
- 36 Wilson, S. J., Delgado, M. R., McKee, S. A., Grigson, P. S., MacLean, R. R.,
37 Nichols, T. T., & Henry, S. L. (2014). Weak ventral striatal responses to
38 monetary outcomes predict an unwillingness to resist cigarette smoking.
39 *Cogn Affect Behav Neurosci*, 14(4), 1196-1207. doi: 10.3758/s13415-014-
40 0285-8
- 41 Wilson, S. J., Sayette, M. A., Delgado, M. R., & Fiez, J. A. (2008). Effect of smoking
42 opportunity on responses to monetary gain and loss in the caudate nucleus. *J*
43 *Abnorm Psychol*, 117(2), 428-434. doi: 10.1037/0021-843X.117.2.428
- 44 Zhu, S. H., Lee, M., Zhuang, Y. L., Gamst, A., & Wolfson, T. (2012). Interventions to
45 increase smoking cessation at the population level: how much progress has
46 been made in the last two decades? *Tob Control*, 21(2), 110-118.
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65



Formatted: Indent: Left: 0 cm,
First line: 0 cm

Shared and divergent neural reactivity to non-drug operant response outcomes in current smokers and ex-smokers.

Liam J Nestor^{1, 2}, Ella McCabe¹, Jennifer Jones¹, Luke Clancy³ Hugh Garavan^{1, 4}

¹*School of Psychology and Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland*

²*Neuropsychopharmacology Unit, Centre for Psychiatry, Imperial College London, UK*

³*TobaccoFree Research Institute Ireland, DIT, Dublin, Ireland*

⁴*Department of Psychiatry, University of Vermont, Burlington, Vermont, USA*

Abstract

Addiction to cigarettes presents with considerable health risks and induces high costs on healthcare resources. While the majority of cigarette smokers endorse the desire to quit, only a small percentage of quit attempts lead to full abstinence. Failure to achieve abstinence may arise from maladaptive reactivity in fronto-striatal regions that track positive and negative valence outcomes, thus biasing the choice to smoke in the presence of alternative, non-drug reinforcement. Alternatively, long-term nicotine abstinence may reveal neural substrates of adaptive valence outcome processing that promote and maintain smoking cessation. The present study set out to examine the neural correlates of operant response outcomes in current smokers, ex-smokers and matched controls using a monetary incentive delay task during functional MRI. Here we report that compared to controls, both current smokers *and* ex-smokers showed significantly less activation change in the left amygdala during positive response outcomes, and in the anterior cingulate cortex, during both positive and negative response outcomes. Ex-smokers, however, demonstrated significantly greater activation change compared to smokers *and* controls in the right amygdala during negative response outcomes. Activation change in the anterior cingulate cortex and middle frontal gyrus of smokers were significantly negatively correlated with nicotine dependence and cigarette pack-years. These results suggest a pattern of shared and divergent reactivity in current smokers and ex-smokers within corticolimbic regions that track both positive and negative operant response outcomes. Exaggerated adaptive processing in *ex-smokers* may promote long-term smoking cessation through amplified negative valence outcome monitoring.

Introduction

Addiction to cigarettes presents with considerable health risks (Bartal, 2001) and induces high costs on healthcare resources (Leistikow et al., 2000). 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). This continued use in the face of adversity is a powerful testimony to the effects of nicotine dependence, demonstrating its reinforcing effects. The reinforcing effects of nicotine (Brody et al., 2006; Domino et al., 2012; Tuesta et al., 2011), particularly in brain regions involved in motivation and reward processing (prefrontal cortex and striatum) in humans (Brody et al., 2004), are likely to be complicit in this failure to achieve abstinence. Recidivism in nicotine addiction may arise from maladaptive reactivity in fronto-striatal regions, whereby there are neural deficits when processing non-drug outcomes. For example, deprived smokers who exhibit the weakest ventral striatal responses to monetary reward are more likely to subsequently choose smoking over monetary reinforcement (Wilson et al., 2014). Similarly, fronto-striatal regions in nicotine-deprived smokers show a more pronounced response during smoking compared to monetary reward anticipation (Sweitzer et al., 2014). These disturbances in the reward processing may bias the choice to smoke in the presence of alternative, non-drug reinforcement.

We have previously reported that current smokers *and* ex-smokers, compared to controls, demonstrate heightened fronto-striatal activation during gain and loss anticipation (Nestor et al., 2016a), supporting previous research findings in nicotine addiction (Martin et al., 2014). We proposed that this may represent a

sensitization by nicotine within this circuitry of smokers, that triggers the excessive “wanting” of rewards in response to non-drug cues (Berridge et al., 1998). This excessive “wanting” during anticipation may also arise from a dissociation from consummation or receipt of rewards, where there is a diminished response in regions that process incentive outcomes. Diminished responses during reward outcomes may trigger reactions that are characterized by a heightened motivation during the anticipation of rewards. Indeed this dissociation has been observed in several populations. Cigarette smokers, for example, show increased ventromedial prefrontal cortex activation during loss anticipation of monetary incentives, but decreased activation in the inferior frontal gyrus during the receipt of monetary gains (Martin et al., 2014). Similar patterns of anticipation and outcome divergence have also been reported in binge-eating disorder patients, with increased fronto-striatal activation during loss and gain anticipation, but decreased activation to loss and reward notifications (Balodis et al., 2013). Pathological gamblers have also demonstrated similar differences in response to anticipation and outcomes, showing a hyper responsiveness in the ventral striatum during loss anticipation, but a hypo responsiveness during successful loss avoidance (Romanczuk-Seiferth et al., 2015). These findings, therefore, appear to suggest that in some addiction populations there is a divergence in reactivity between the anticipation and delivery of non-drug reinforcement in fronto-striatal regions, possibly due to deficits in valence outcome processing. Hyperactivity in lateral and medial prefrontal regions that sub-serve inhibitory control functioning and error monitoring have been reported in ex-smokers (Kroenke et al., 2015; Nestor et al., 2011). This may suggest that the emergence of amplified prefrontal cognitive functioning is necessary for successful abstinence in addiction. Therefore, similar adaptations that are represented by an amplified

valence outcome monitoring may also promote the control over addiction-related behaviours (Garavan et al., 2005), even in the presence of heightened and sustained “wanting” of rewards.

Therefore, the present study set out to examine the effects of both current and previous nicotine exposure on corticolimbic correlates of operant response outcomes. Here we compared current smokers, ex-smokers and demographically matched healthy controls using a monetary incentive delay task. Specifically, we aimed to elucidate whether 1) current smokers and ex-smokers demonstrate shared or divergent reactivity compared to controls in the neural substrates of tracking positive and negative operant response outcomes, and 2) whether divergent reactivity to positive and negative outcomes distinguishes ex-smokers from current smokers.

Results

Demographics

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, 84.8 weeks (range: 52-180 weeks) at the time of testing (see Table 1 in supplementary materials for a more detailed description of group demographics).

Behaviour

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 ($F=5.6$; $df=111, 2$; $p<0.01$), with greater accuracy on loss compared to neutral ($p<0.05$) and gain compared to neutral ($p<0.01$) trials. There was no effect of group ($F=0.5$; $df=111, 2$; $p=0.6$) and no condition $\times$ group interaction ($F=0.09$; $df=111, 4$; $p=0.99$). Figure 1b shows the mean MID reaction time (milliseconds) for the three conditions in the three groups. There was a significant effect of condition ($F=2.6$; $df=111, 2$; $p<0.05$), with faster reaction time on the gain compared to neutral ($p<0.05$) trials only. There was no effect of group ($F=1.4$; $df=111, 2$; $p=0.3$) and no condition $\times$ group interaction ($F=0.1$; $df=111, 4$; $p=1.0$).

-Insert Figure 1 about here-

Functional MRI

We collapsed across conditions (gain, loss, neutral) for each of the operant response outcome types (“Hit” and “Miss”) separately, as we did not detect any significant group differences on the conditions independently.

Positive operant response outcomes

There were five clusters that showed a group effect in the ROI mask, comprising the anterior cingulate cortex (ACC: 733 voxels; $x=4$; $y=14$; $z=26$;

$Z_{stat}=4.71$; $p<0.0001$; smoker<control, $p<0.0001_{Bonferroni}$; ex-smoker<control, $p<0.001_{Bonferroni}$); right amygdala (353 voxels; $x=20$; $y=-6$; $z=-20$; $Z_{stat}=3.37$; $p<0.001$; smoker<control, $p<0.001_{Bonferroni}$; smoker<ex-smoker, $p<0.0001_{Bonferroni}$ - Fig2A); frontal pole (347 voxels; $x=48$; $y=40$; $z=20$; $Z_{stat}=5.38$; $p<0.001$; smoker<ex-smoker, $p<0.05_{Bonferroni}$); middle frontal gyrus (MFG: 325 voxels; $x=36$; $y=20$; $z=40$; $Z_{stat}=7.23$; $p<0.001$; smoker<control, $p<0.05_{Bonferroni}$; smoker<ex-smoker, $p<0.001_{Bonferroni}$ - Fig2C) and the left amygdala (153 voxels; $x=-28$; $y=-10$; $z=-14$; $Z_{stat}=4.66$; $p<0.05$; smoker<control, $p<0.01_{Bonferroni}$; ex-smoker<control, $p<0.05_{Bonferroni}$ - Fig2B).

-Insert Figure 2 about here-

Negative operant response outcomes

There were also five clusters that showed a group effect, comprising the ACC (374 voxels; $x=2$; $y=-4$; $z=32$; $Z_{stat}=5.07$; $p<0.0001$; smoker<control, $p<0.001_{Bonferroni}$; ex-smoker<control, $p<0.001_{Bonferroni}$ - Fig3C); frontal pole (297 voxels; $x=48$; $y=40$; $z=20$; $Z_{stat}=4.12$; $p<0.0001$; smoker<ex-smoker, $p<0.001_{Bonferroni}$); right amygdala (194 voxels; $x=22$; $y=-4$; $z=-22$; $Z_{stat}=5.01$; $p<0.01$; ex-smoker>control, $p<0.05_{Bonferroni}$; ex-smoker>smoker, $p<0.001_{Bonferroni}$ - Fig3A); orbitofrontal cortex (OFC: 168 voxels; $x=-24$; $y=28$; $z=-18$; $Z_{stat}=4.37$; $p<0.01$; smoker<control, $p<0.05_{Bonferroni}$ - Fig3B) and the left inferior frontal gyrus (IFG: 157 voxels; $x=-34$; $y=26$; $z=20$; $Z_{stat}=5.43$; $p<0.01$; smoker<control, $p<0.0001_{Bonferroni}$; smoker<ex-smoker, $p<0.01_{Bonferroni}$).

-Insert Figure 3 about here-

Correlations

There were several significant negative correlations between smoking demographics in the smoker group, and their mean percentage BOLD change (collapsed across the gain, loss and neutral conditions) during the outcome types. FTND scores were negatively correlated with the mean BOLD signal change in the MFG ($r(13) = -.52$; $p < 0.05$ - Fig4A), with a trend for a negative correlation with ACC BOLD signal change ($r(13) = -.48$; $p < 0.07$), during positive operant response outcomes. Cigarette pack-years was negatively correlated with ACC BOLD signal change ($r(13) = -.61$; $p < 0.05$ - Fig4B), with a trend for a negative correlation with right amygdala BOLD signal change ($r(13) = -.49$; $p < 0.07$), during negative response outcomes. There were no significant correlations detected in the ex-smoker group.

-Insert Figure 4 about here-

Discussion

We compared activation changes elicited by operant response feedback during a monetary incentive delay task in demographically matched controls, current smokers and ex-smokers. Here we observed a pattern of shared and divergent reactivity in smokers and ex-smokers, compared to controls, within corticolimbic regions that track both positive and negative operant response outcome feedback. Activation differences in some of these regions were also found to negatively correlate with nicotine dependence and cigarette use in current smokers. The group differences were also identified in the absence of group differences in behavioural

performance, potentially discounting this as confounding the neural reactivity differences observed during the operant response outcomes.

Reduced activation change during operant response outcomes in current smokers

This study observed that current smokers had significantly reduced activation change in a number of corticolimbic regions during operant response outcomes. Notably, there was a reduced response in the right amygdala and middle frontal gyrus (MFG) during positive operant response outcomes compared to controls *and* ex-smokers. There is evidence that the amygdala encodes stimulus value (Jenison et al., 2011), responds to reward outcomes, (Ernst et al., 2005), and is involved in salience processing (Belova et al., 2007; Pessoa et al., 2010). Amygdala disturbances also play a role in addiction (Lesscher et al., 2012; See et al., 2003), with our findings possibly suggesting a disturbance in this region in current smokers. The MFG is also involved in representing and integrating reward information (Watanabe et al., 2007), with evidence for diminished activation in addiction populations (Ersche et al., 2005; Paulus et al., 2002), including smokers (Nestor et al., 2011). The current study additionally revealed MFG attenuation in response to positive operant response outcomes in current smokers that was also negatively correlated with the level of reported nicotine dependence.

The current study showed that current smokers showed a reduced activation change in the medial orbitofrontal cortex (OFC) compared to controls during negative response outcomes. We previously reported (Nestor et al., 2016a) that there were *increased* responses in a region of the lateral OFC, compared to controls, in the same sample of current smokers *and* ex-smokers during both loss

1 and gain (but not neutral) anticipation, suggesting some type of heightened
2 motivational drive during the expectancy of non-drug rewards. The present finding,
3
4 observed during negative operant outcomes, however, appears to suggest divergent
5
6 reactivity to anticipatory versus receipt phases of incentive processing in current
7
8 smokers - representing some type of disturbance in negative valence outcome
9
10 monitoring. Current smokers also showed a reduced activation change in the inferior
11
12 frontal gyrus (IFG) compared to both controls and ex-smokers during negative
13
14 response outcomes, pointing to disturbances in both ventral and dorsal cortical
15
16 regions in this group.
17
18
19
20
21
22
23
24

25 *Reduced activation change during operant response outcomes in current smokers*
26
27 *and ex-smokers*
28

29
30 This study reports that during both positive and negative operant response
31
32 outcomes, ex-smokers, and to a greater degree, current smokers, showed less
33
34 activation change in the anterior cingulate cortex (ACC). Disturbances in ACC
35
36 functioning have been reported in addiction (Goldstein et al., 2002; Peoples, 2002;
37
38 Volkow et al., 2002), that have been shown to extend into abstinence (Nestor et al.,
39
40 2016b). Interestingly, these differences were observed in the dorsal ACC (dACC)
41
42 region, known to be involved in motivation (Kouneiher et al., 2009) and also
43
44 prediction error signalling (Wallis et al., 2010). We also observed a reduced
45
46 activation change in the left amygdala during positive operant response outcomes in
47
48 current smokers and ex-smokers. The amygdala and dACC, interestingly, are also
49
50 connected (Beckmann et al., 2009), with the possibility that our findings represent
51
52 disruptions in dACC-amygdala connectivity in both current smokers and ex-
53
54 smokers. Significantly, the level of reported cigarette use (pack-years) in smokers
55
56
57
58
59
60
61
62
63
64
65

was also found to significantly predict ACC attenuation during negative operant response outcomes.

Increased activation during negative operant response outcomes in ex-smokers

The monitoring of one's behavioural outcomes during nicotine abstinence may be especially important when there is a need to detect errors, particularly those that might induce smoking relapse. The neural substrates of behavioural monitoring in addiction may have implications for abstinence, as they may elucidate specific adaptations that promote the control over addiction-related behaviours (Garavan et al., 2005). Therefore, the ability of ex-smokers to remain abstinent may be related to the amplified monitoring of performance errors. Here we report that in response to negative operant response outcomes, ex-smokers demonstrated a significantly greater activation change compared to smokers *and* controls in the right amygdala. This was in contrast to the observed attenuation in the left amygdala during positive operant response outcomes, where ex-smokers showed a similar effect to smokers. This divergence in amygdala responding may suggest they exhibit a bias in the tracking of negative valance outcomes. The amygdala does respond to negative feedback (Klavir et al., 2013) and is involved in prediction error signalling (Meder et al., 2016; Seymour et al., 2005a), which may be amplified in ex-smokers. This amplification following smoking cessation may potentially contribute to nicotine abstinence through heightened error encoding. These results may be seen as tentatively pointing towards possible ex-smoker effects that need to be addressed in a larger sample, and in a hypothesis-driven manner.

1 Limitations of the current study involve small sample sizes, particularly in the
2 ex-smoker group, and the low-moderate nicotine dependency range in current
3 smokers. The relatively modest levels of dependency, for example, may curtail the
4 generalizability of the current findings to greater levels of nicotine dependence that
5 are reported in older adults. Our findings also represented group differences across
6 the conditions (e.g., gain hit, loss hit, neutral hit), as we were unable to detect any
7 group differences on the conditions independently. While the reported group
8 differences do make it difficult to disentangle the possible differential effects of
9 outcome in the conditions, a conflation according to valence may still exploit similar
10 processes during goal directed behaviour. There is evidence, for example, that
11 successfully avoiding an aversive outcome is associated with a similar neural profile
12 to being rewarded (Kim et al., 2006; Seymour et al., 2005b), with stimuli that signal
13 successful avoidance known to positively reinforce (Morris, 1975). This opponent
14 process may likewise be attributed to negative valence outcomes, where the
15 termination of a positive state would become aversive, and thus elicit a similar
16 neural response. Our results, however, still necessitate a cautious interpretation, as
17 we cannot unequivocally state how the valence outcomes on each condition
18 individually contributed to our reported group differences.

46 *Conclusion*

48 Despite the limitation of a small sample size, and the low-moderate nicotine
49 dependency, this study has provided some preliminary evidence for a pattern of
50 shared and divergent reactivity in current smokers and ex-smokers within
51 corticolimbic regions that track positive and negative operant response outcomes.
52 We propose that exaggerated processing in ex-smokers may promote long-term

1 smoking cessation through amplified negative valence outcome monitoring, that
2 appears to remain disrupted in current smokers.
3
4
5
6
7

8 **Declaration of Conflicting Interests**

9

10 The authors report no biomedical financial interests or potential conflicts of interest.
11
12
13
14

15 **Acknowledgments**

16

17 This research was supported by a grant from the TobaccoFree Research Institute
18 (formerly the Research Institute for a Tobacco Free Society), Dublin, Ireland. The
19 assistance of Ms. Sheila Keogan (Research and Communication) and Ms. Vanessa
20 Clarke (Corporate Affairs) at the TobaccoFree Research Institute is gratefully
21 acknowledged.
22
23
24
25
26

27 **Methods and Materials**

28

29 *Participants*

30

31 For a more comprehensive description of the material and methods, please
32 see Nestor et al. (2016a), which used the same cohorts reported herein, to examine
33 differences in loss and reward anticipation. Briefly, 15 current cigarette smokers, 10
34 ex-smokers and 15 controls completed the study. A semi-structured interview, as
35 used in previous behavioural and functional imaging studies (Carey et al., 2015;
36 Nestor et al., 2016a) was conducted to screen participants for past or present
37 histories of psychiatric or neurological illness. Any potential participant describing
38 any major life-time psychiatric event or brain injury (e.g., head trauma resulting in a
39 loss of consciousness, seizure or stroke) or any familial psychiatric history (i.e.
40 sibling, parent or grandparent) was considered ineligible for the study.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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. 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. 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. 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 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 (Heatherton et al., 1991) dependence (FTND) was administered to

1 smokers. The FTND was also administered to ex-smokers, retrospectively, in order
2 to match their previous levels of nicotine dependence (when they were active
3 smokers) to current smokers. The rationale for this was to eliminate previous
4 dependency levels in ex-smokers as a potential contributing factor to neural
5 differences with smokers. The Shiffman-Jarvik smoking withdrawal questionnaire
6 (SJWQ) (Shiffman et al., 1976) and the urge to smoke (UTS) scale (Jarvik et al.,
7 2000) were also administered to smokers prior to scanning to assess craving and
8 withdrawal.

21 *Monetary Incentive Delay Task (MID)*

24 We used a “monetary incentive delay task” (MID), which was based on that
25 originally employed by Knutson (Knutson et al., 2001) and which we have previously
26 used to assess the neural correlates of reward processing in cannabis users (Nestor
27 et al., 2010) and smokers (Nestor et al., 2016a) – see Supplementary Figure 1.
28 While being scanned participants performed the MID task, during which they
29 anticipated potential monetary gain, loss or no potential monetary outcome. During
30 each trial, participants viewed one of three coloured squares (cue) that indicated the
31 potential to gain fifty cent (green square), lose fifty cent (red square) or experience
32 no financial outcome (blue square - here referred to as the neutral condition)
33 following their response to an upcoming visual target. Each cue was presented for a
34 variable duration (2-8 sec), after which participants made a button press response
35 upon the presentation of a visual target (star located within a circle). Participants
36 received feedback (1500 ms) following their response to the visual target, after
37 which there was an end fixation period (2-8 sec) before the commencement of the
38 next trial. Responses to the visual target falling within (“hits”) or outside (“misses”) a

1 400ms response deadline received feedback appropriate for that particular trial. We
2 chose this 400 ms time frame in order to yield accuracy levels at ~50%, which would
3
4 serve to maintain the participant's interest in the task. Therefore, participants had
5
6 four hundred milliseconds to respond to the visual target in order to be successful on
7
8 a gain, loss or neutral trial. There were a total of 27 trials in each condition (gain,
9
10 loss, and neutral), with each trial lasting between six and eighteen seconds. The
11
12 MID was composed of three runs, with each run lasting 340 seconds. The order of
13
14 trials within each run was randomised. Dependent measures derived from the data
15
16 included mean percentage accuracy and reaction time for the gain, loss and neutral
17
18 conditions. The task was programmed and run using E-Prime version 1.1
19
20 (Psychology Software Tools, Pittsburgh, USA).
21
22
23
24
25
26
27
28
29

30 *Functional MRI (fMRI) Data Acquisition*

31 All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system
32
33 (Best, The Netherlands) equipped with a mirror that reflected the visual display,
34
35 which was projected onto a panel placed behind the participants' head outside the
36
37 magnet. The mirror was mounted on the head coil in each participant's line of vision.
38
39 Each scanning sequence began with a reference scan to resolve sensitivity
40
41 variations. A parallel sensitivity encoding (SENSE) approach with a reduction factor
42
43 of 2 was utilised for all T1-weighted image acquisitions (Pruessmann et al., 1999).
44
45 180 high-resolution T1- weighted anatomic MPRAGE axial images (FOV 230 mm,
46
47 thickness 0.9 mm, voxel size 0.9×0.9×0.9) were then acquired (total duration 325 s),
48
49 to allow subsequent activation localization and spatial normalization. Functional data
50
51 were acquired using a T2* weighted echo-planar imaging sequence collecting 32
52
53 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE=35 ms,
54
55
56
57
58
59
60
61
62
63
64
65

1 TR=2000 ms, FOV 224 mm, 64×64 mm matrix size in Fourier space). Functional
2 scans had a total duration of 340 s per run.
3
4
5
6

7 *fMRI Data analyses*

8

9 Data pre-processing and statistical analysis were conducted using FEAT
10 (fMRI Expert Analysis Tool) from the FMRIB Software Library (FSL 4.1.7,
11 www.fmrib.ox.ac.uk/fsl). Pre-statistical processing was as follows: motion correction
12 utilizing FMRIB's Linear Image Registration Tool (MCFLIRT); non-brain matter
13 removal using Brain Extraction Tool (BET); spatial smoothing with a 6-mm full-width
14 half maximum Gaussian kernel; mean-based intensity normalization; nonlinear high-
15 pass temporal filtering (Gaussian-weighted least squares straight line fit, with sigma
16 = 25.0 sec). For each participant, first level whole-brain mixed-effects analyses were
17 performed by modelling, separately, the gain, loss and neutral positive ("hit") operant
18 response outcome periods, and the gain, loss and neutral negative ("miss") operant
19 response outcome periods (stick functions for each of the feedback period
20 regressors were convolved with the haemodynamic response function). First level
21 whole-brain mixed-effects analyses were also performed by modelling the MID
22 anticipation periods (i.e., gain, loss and neutral) as explanatory variables within the
23 context of the general linear model on a voxel-by-voxel basis (variable boxcar
24 functions for the anticipation period regressors were convolved with the
25 haemodynamic response function), the results of which have been previously
26 reported (Nestor et al., 2016a). The end fixation period of the task served as the
27 implicit baseline. Therefore, all reported differences between groups are for
28 activation changes versus the baseline period. Registration was conducted through
29 a two-step procedure, whereby EPI images were first registered to the high-
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 resolution T1 structural image, then into standard (Montreal Neurological Institute,
2 MNI avg152 template) space, with 12-parameter affine transformations.
3
4
5
6

7 As corticolimbic regions are critical neural substrates for the processing of
8 non-drug rewards in humans (Knutson et al., 2001; O'Doherty et al., 2001), with
9 evidence for both hypoactivity and hyperactivity to non-drug rewards in these
10 regions in addiction populations, including current smokers (Martin et al., 2014;
11 Nestor et al., 2016a; Sweitzer et al., 2014; Wilson et al., 2008), we took an *a priori*
12 region of interest (ROI) approach in our search for group differences during the
13 operant response outcomes. Here we restricted this search to the nucleus
14 accumbens (NAcc), amygdala, caudate, putamen, insula, inferior frontal gyrus, and
15 the anterior cingulate, dorsolateral and orbitofrontal cortices, bilaterally. These
16 regions were taken from the Harvard-Oxford cortical and subcortical structural atlas
17 and grouped together into one ROI mask. Higher-level between-group analyses
18 (one-way ANOVAs) were conducted using FLAME (FMRIB's Local Analysis of
19 Mixed Effects) collapsing across conditions (gain, loss, neutral) for each of the
20 operant response outcome types ("hit" and "miss") separately. These analyses were
21 conducted as we did not detect any significant group differences on the conditions
22 independently. Significant clusters in this ROI mask of *a priori* regions were
23 determined by thresholding at $Z > 2.3$ with a corrected cluster significance threshold
24 of $p < 0.05$.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 *Other Statistics*

54 For analyses conducted on the MID behavioural data, we performed three
55 (Group: Control vs. Ex-smoker vs. Smoker) by three (Condition: Neutral vs. Loss vs.
56
57
58
59
60
61
62
63
64
65

Gain) analyses of variance. For follow-up *post hoc* analyses on the mean BOLD signal change from the ANOVA group effect clusters, we conducted Bonferroni pairwise comparisons to examine which groups significantly differed from one another. Correlations between smoking demographics and mean BOLD signal change from the zF-statistic clusters were assessed using Spearman's rho correlations. All these analyses were conducted using the R statistical software package (2013).

References

- Balodis, I. M., Kober, H., Worhunsky, P. D., White, M. A., Stevens, M. C., Pearlson, G. D., Sinha, R., Grilo, C. M., & Potenza, M. N. (2013). Monetary reward processing in obese individuals with and without binge eating disorder. *Biol Psychiatry*, 73(9), 877-886. doi: 10.1016/j.biopsych.2013.01.014
- Bartal, M. (2001). Health effects of tobacco use and exposure. *Monaldi Arch Chest Dis*, 56(6), 545-554.
- Beck, A., Steer, R., & Brown, G. (1996). Beck Depression Inventory—Second Edition manual. *The Psychological Corporation, San Antonio, TX*.
- Beckmann, M., Johansen-Berg, H., & Rushworth, M. F. S. (2009). Connectivity-Based Parcellation of Human Cingulate Cortex and Its Relation to Functional Specialization. *Journal of Neuroscience*, 29(4), 1175-1190. doi: 10.1523/Jneurosci.3328-08.2009
- Belova, M. A., Paton, J. J., Morrison, S. E., & Salzman, C. D. (2007). Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. *Neuron*, 55(6), 970-984.
- Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*, 28(3), 309-369.
- Brody, A. L., Mandelkern, M. A., London, E. D., Olmstead, R. E., Farahi, J., Scheibal, D., Jou, J., Allen, V., Tiongson, E., Chefer, S. I., Koren, A. O., & Mukhin, A. G. (2006). Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Arch Gen Psychiatry*, 63(8), 907-915.
- Brody, A. L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., & Mandelkern, M. A. (2004). Smoking-induced ventral striatum dopamine release. *Am J Psychiatry*, 161(7), 1211-1218.
- Carey, S. E., Nestor, L., Jones, J., Garavan, H., & Hester, R. (2015). Impaired learning from errors in cannabis users: Dorsal anterior cingulate cortex and hippocampus hypoactivity. *Drug and Alcohol Dependence*, 155, 175-182. doi: 10.1016/j.drugalcdep.2015.07.671
- Domino, E. F., Evans, C. L., Ni, L., Guthrie, S. K., Koeppe, R. A., & Zubieta, J. K. (2012). Tobacco smoking produces greater striatal dopamine release in G-

- allele carriers with mu opioid receptor A118G polymorphism. *Prog Neuropsychopharmacol Biol Psychiatry*, 38(2), 236-240. doi: 10.1016/j.pnpbp.2012.04.003
- Ernst, M., Nelson, E. E., Jazbec, S., McClure, E. B., Monk, C. S., Leibenluft, E., Blair, J., & Pine, D. S. (2005). Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. *Neuroimage*, 25(4), 1279-1291. doi: 10.1016/j.neuroimage.2004.12.038
- Ersche, K. D., Fletcher, P. C., Lewis, S. J. G., Clark, L., Stocks-Gee, G., London, M., Deakin, J. B., Robbins, T. W., & Sahakian, B. J. (2005). Abnormal frontal activations related to decision-making in current and former amphetamine and opiate dependent individuals. *Psychopharmacology*, 180(4), 612-623. doi: 10.1007/s00213-005-2205-7
- Garavan, H., & Stout, J. C. (2005). Neurocognitive insights into substance abuse. *Trends Cogn Sci*, 9(4), 195-201.
- Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry*, 159(10), 1642-1652.
- Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerstrom, K. O. (1991). The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*, 86(9), 1119-1127.
- Hughes, J. R., Peters, E. N., & Naud, S. (2008). Relapse to smoking after 1 year of abstinence: a meta-analysis. *Addict Behav*, 33(12), 1516-1520.
- Jarvik, M. E., Madsen, D. C., Olmstead, R. E., Iwamoto-Schaap, P. N., Elins, J. L., & Benowitz, N. L. (2000). Nicotine blood levels and subjective craving for cigarettes. *Pharmacol Biochem Behav*, 66(3), 553-558.
- Jenison, R. L., Rangel, A., Oya, H., Kawasaki, H., & Howard, M. A. (2011). Value encoding in single neurons in the human amygdala during decision making. *J Neurosci*, 31(1), 331-338. doi: 10.1523/JNEUROSCI.4461-10.2011
- Kim, H., Shimojo, S., & O'Doherty, J. P. (2006). Is avoiding an aversive outcome rewarding? Neural substrates of avoidance learning in the human brain. *PLoS Biol*, 4(8), e233.
- Klavir, O., Genud-Gabai, R., & Paz, R. (2013). Functional Connectivity between Amygdala and Cingulate Cortex for Adaptive Aversive Learning. *Neuron*, 80(5), 1290-1300. doi: 10.1016/j.neuron.2013.09.035
- Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci*, 21(16), RC159.
- Kouneiher, F., Charron, S., & Koechlin, E. (2009). Motivation and cognitive control in the human prefrontal cortex. *Nat Neurosci*, 12(7), 939-945. doi: 10.1038/nn.2321
- Kroenke, K. M., Wolff, M., Benz, A., & Goschke, T. (2015). Successful smoking cessation is associated with prefrontal cortical function during a Stroop task: A preliminary study. *Psychiatry Research-Neuroimaging*, 234(1), 52-56. doi: 10.1016/j.psychresns.2015.08.005
- Leistikow, B. N., Martin, D. C., & Milano, C. E. (2000). Fire injuries, disasters, and costs from cigarettes and cigarette lights: a global overview. *Prev Med*, 31(2 Pt 1), 91-99.
- Lesscher, H. M. B., & Vanderschuren, L. J. M. J. (2012). Compulsive drug use and its neural substrates. *Reviews in the Neurosciences*, 23(5-6), 731-745. doi: 10.1515/revneuro-2012-0066

- Martin, L. E., Cox, L. S., Brooks, W. M., & Savage, C. R. (2014). Winning and losing: differences in reward and punishment sensitivity between smokers and nonsmokers. *Brain and Behavior*, 4(6), 915-924. doi: 10.1002/brb3.285
- McLellan, A. T., Kushner, H., Metzger, D., Peters, R., Smith, I., Grissom, G., Pettinati, H., & Argeriou, M. (1992). The fifth edition of the Addiction Severity Index. *J. Subst. Abuse Treat*, 9, 199-213.
- Meder, D., Madsen, K. H., Hulme, O., & Siebner, H. R. (2016). Chasing probabilities - Signaling negative and positive prediction errors across domains. *Neuroimage*, 134, 180-191. doi: 10.1016/j.neuroimage.2016.04.019
- Nelson, H., & O'Connell, A. (1978). Dementia: The estimation of pre-morbid intelligence levels using the new adult reading test. *Cortex*, 14((2)), 234-244.
- Nestor, L., Hester, R., & Garavan, H. (2010). Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage*, 49(1), 1133-1143. doi: 10.1016/j.neuroimage.2009.07.022
- Nestor, L., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2011). Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage*, 56(4), 2258-2275. doi: 10.1016/j.neuroimage.2011.03.054
- Nestor, L. J., McCabe, E., Jones, J., Clancy, L., & Garavan, H. (2016a). Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses. *Addict Biol*. doi: 10.1111/adb.12484
- Nestor, L. J., Murphy, A., McGonigle, J., Orban, C., Reed, L., Taylor, E., Flechais, R., Paterson, L. M., Smith, D., Bullmore, E. T., Ersche, K. D., Suckling, J., Tait, R., Elliott, R., Deakin, B., Rabiner, I., Lingford-Hughes, A., Nutt, D. J., Sahakian, B., Robbins, T. W., & Consortium, I. (2016b). Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations during a monetary incentive delay task. *Addiction Biology*, n/a-n/a. doi: 10.1111/adb.12444
- O'Doherty, J., Kringelbach, M. L., Rolls, E. T., Hornak, J., & Andrews, C. (2001). Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat Neurosci*, 4(1), 95-102.
- Oldfield, R. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, 97-113.
- Paulus, M. P., Hozack, N. E., Zauscher, B. E., Frank, L., Brown, G. G., Braff, D. L., & Schuckit, M. A. (2002). Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. *Neuropsychopharmacology*, 26(1), 53-63. doi: S0893133X01003347 [pii] 10.1016/S0893-133X(01)00334-7
- Peoples, L. L. (2002). Will, anterior cingulate cortex, and addiction. *Science*, 296, 1623-1624.
- Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. *Nature Reviews Neuroscience*, 11(11), 773-782. doi: 10.1038/nrn2920
- Pruessmann, K. P., Weiger, M., Scheidegger, M. B., & Boesiger, P. (1999). SENSE: sensitivity encoding for fast MRI. *Magn Reson Med*, 42(5), 952-962.
- Romanczuk-Seiferth, N., Koehler, S., Dreesen, C., Wustenberg, T., & Heinz, A. (2015). Pathological gambling and alcohol dependence: neural disturbances in reward and loss avoidance processing. *Addict Biol*, 20(3), 557-569. doi: 10.1111/adb.12144

- 1 See, R. E., Fuchs, R. A., Ledford, C. C., & McLaughlin, J. (2003). Drug addiction,
2 relapse, and the amygdala. *Amygdala in Brain Function: Basic and Clinical*
3 *Approaches*, 985, 294-307.
- 4 Seymour, B., O'Doherty, J. P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston,
5 K., & Dolan, R. (2005a). Opponent appetitive-aversive neural processes
6 underlie predictive learning of pain relief. *Nat Neurosci*, 8(9), 1234-1240.
- 7 Seymour, B., O'Doherty, J. P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston,
8 K. J., & Dolan, R. (2005b). Opponent appetitive-aversive neural processes
9 underlie predictive learning of pain relief. *Nature Neuroscience*, 8(9), 1234-
10 1240. doi: 10.1038/nn1527
- 11 Shiffman, S., & Jarvik, M. (1976). Smoking withdrawal symptoms in two weeks of
12 abstinence. *Psychopharmacology (Berl)*, 50(1), 35-39.
- 13 Sweitzer, M. M., Geier, C. F., Joel, D. L., McGurrin, P., Denlinger, R. L., Forbes, E.
14 E., & Donny, E. C. (2014). Dissociated Effects of Anticipating Smoking versus
15 Monetary Reward in the Caudate as a Function of Smoking Abstinence.
16 *Biological Psychiatry*, 76(9), 681-688. doi: 10.1016/j.biopsych.2013.11.013
- 17 Tuesta, L. M., Fowler, C. D., & Kenny, P. J. (2011). Recent advances in
18 understanding nicotinic receptor signaling mechanisms that regulate drug
19 self-administration behavior. *Biochemical Pharmacology*, 82(8), 984-995. doi:
20 10.1016/j.bcp.2011.06.026
- 21 Volkow, N., Fowler, J., Wang, G., & Goldstein, R. (2002). Role of dopamine, the
22 frontal cortex and memory circuits in drug addiction: insight from imaging
23 studies. *Neurobiol Learn Mem*, 78(3), 610-624.
- 24 Wallis, J. D., & Kennerley, S. W. (2010). Heterogeneous reward signals in prefrontal
25 cortex. *Current Opinion in Neurobiology*, 20(2), 191-198. doi:
26 10.1016/j.conb.2010.02.009
- 27 Watanabe, M., & Sakagami, M. (2007). Integration of cognitive and motivational
28 context information in the primate prefrontal cortex. *Cereb Cortex*, 17 Suppl
29 1, i101-109. doi: 10.1093/cercor/bhm067
- 30 Wilson, S. J., Delgado, M. R., McKee, S. A., Grigson, P. S., MacLean, R. R.,
31 Nichols, T. T., & Henry, S. L. (2014). Weak ventral striatal responses to
32 monetary outcomes predict an unwillingness to resist cigarette smoking.
33 *Cogn Affect Behav Neurosci*, 14(4), 1196-1207. doi: 10.3758/s13415-014-
34 0285-8
- 35 Wilson, S. J., Sayette, M. A., Delgado, M. R., & Fiez, J. A. (2008). Effect of smoking
36 opportunity on responses to monetary gain and loss in the caudate nucleus. *J*
37 *Abnorm Psychol*, 117(2), 428-434. doi: 10.1037/0021-843X.117.2.428
- 38 Zhu, S. H., Lee, M., Zhuang, Y. L., Gamst, A., & Wolfson, T. (2012). Interventions to
39 increase smoking cessation at the population level: how much progress has
40 been made in the last two decades? *Tob Control*, 21(2), 110-118.

Figure 1
[Click here to download Figure: Figure 1.docx](#)

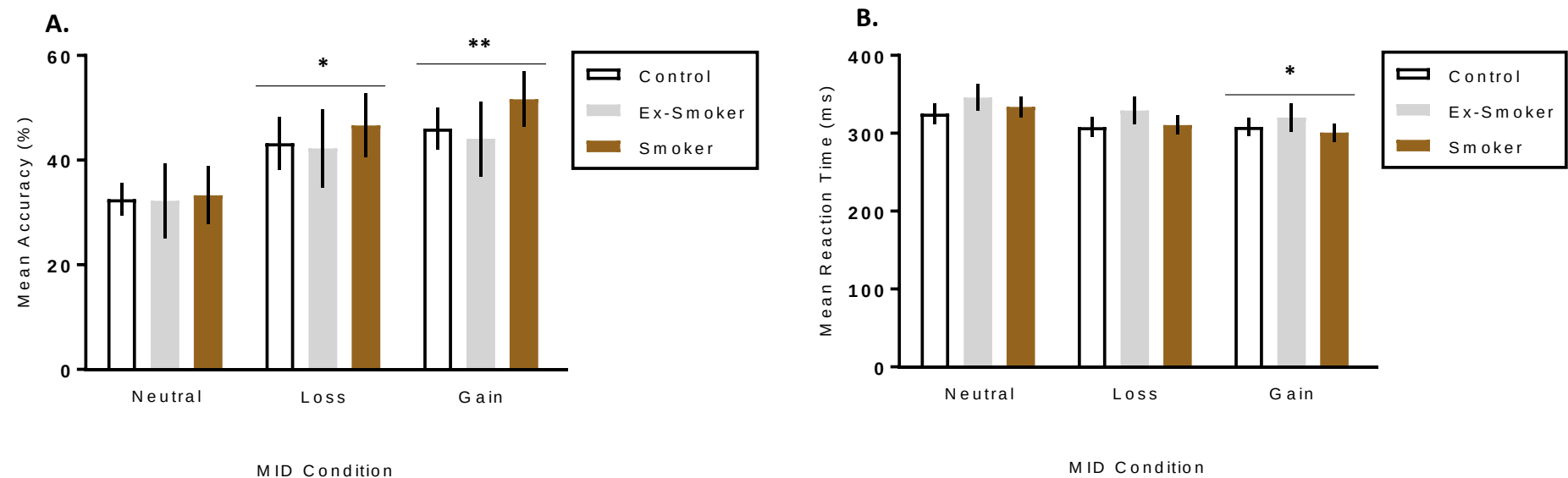


Figure 1. MID task performance in the control, ex-smoker and current smoker groups showing A) mean percentage accuracy which was greater on the Loss compared to Neutral ($p<0.05$) and Gain compared to Neutral ($p<0.01$) trials; and B) mean reaction time which was faster on the Gain compared to Neutral ($p<0.05$) trials only. Data were analyzed using a 3 (Condition: Neutral vs. Loss vs. Gain) $\times$ 3 (Group: Control vs. Ex-smoker vs. Smoker) analysis of variance. Data are expressed as means $\pm$ SEM.

Figure 2
[Click here to download Figure: Figure 2.docx](#)

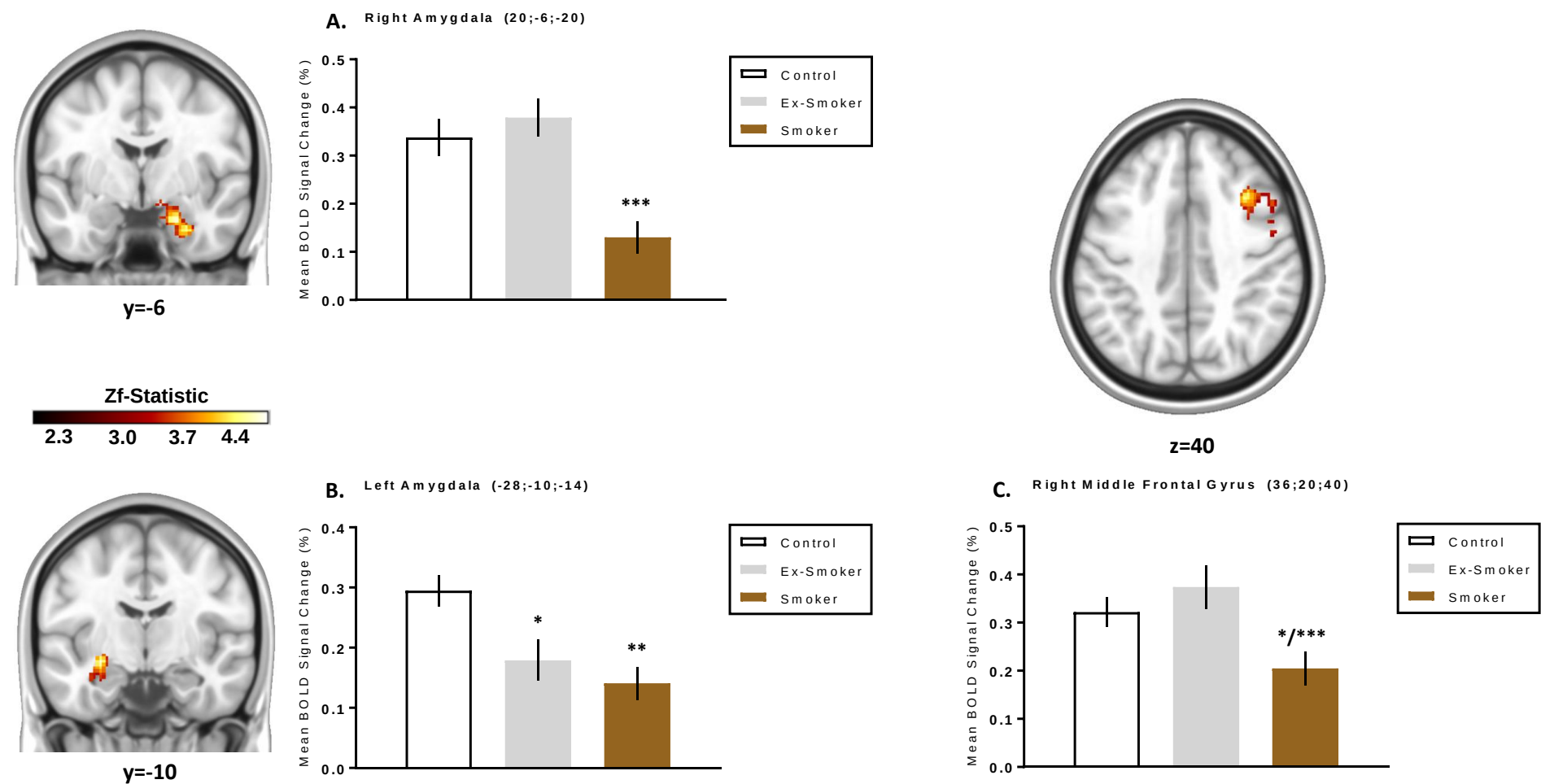


Figure 2. Follow-up Bonferroni pairwise comparisons on the mean BOLD signal change scores within the group ANOVA zF-statistic clusters for positive operant response outcomes in A) the right amygdala (** $p < 0.001$ - smoker<control & smoker<ex-smoker; B) the left amygdala (* $p < 0.05$ - ex-smoker<control; ** $p < 0.01$ - smoker<control); and C) the right middle frontal gyrus (* $p < 0.05$ - smoker<control; *** $p < 0.001$ - smoker<ex-smoker). The scale represents the colour (from dark to light yellow) of the cluster voxels corresponding to the increasing zF-statistic that came from the initial mixed effects one-way ANOVA. Data are expressed as the mean of the gain, loss, neutral conditions $\pm$ SEM.

Figure 3
[Click here to download Figure: Figure 3.docx](#)

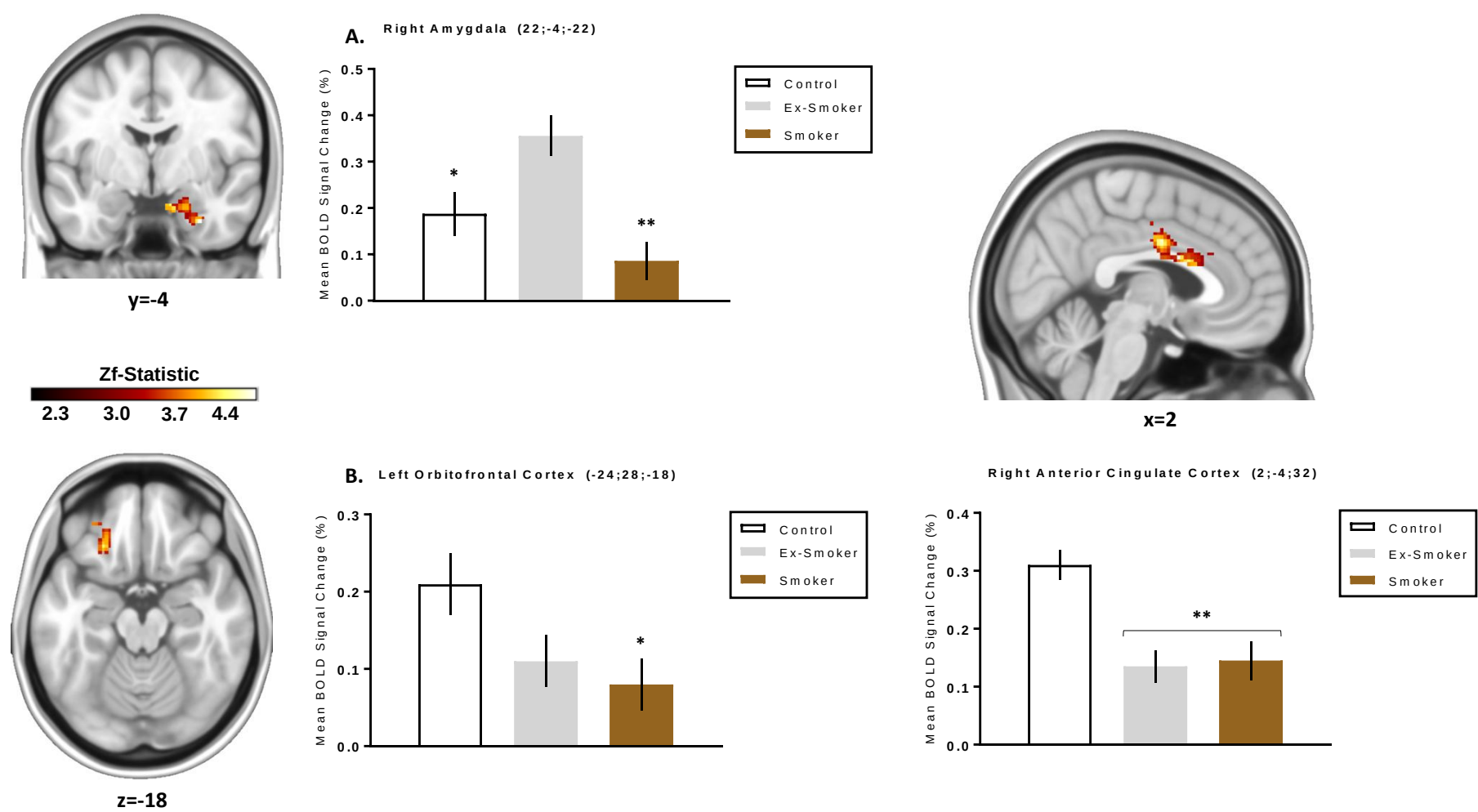


Figure 3. Follow-up Bonferroni pairwise comparisons on the mean BOLD signal change scores within the group ANOVA zF-statistic clusters for negative operant outcomes in A) the right amygdala ($*p<0.05$ - control<ex-smoker; $**p<0.01$ - smoker<ex-smoker); B) the left orbitofrontal cortex ($*p<0.05$ - smoker<control); and C) the right anterior cingulate cortex ($**p<0.01$ – smoker<control & ex-smoker<control). The scale represents the colour (from dark to light yellow) of the cluster voxels corresponding to the increasing zF-statistic that came from the initial mixed effects one-way ANOVA. Co-ordinates are represented in Montreal Neurological Institute (MNI) space. Data are expressed as means of the gain, loss, neutral conditions $\pm$ SEM.

Figure 4

[Click here to download Figure: Figure 4.docx](#)

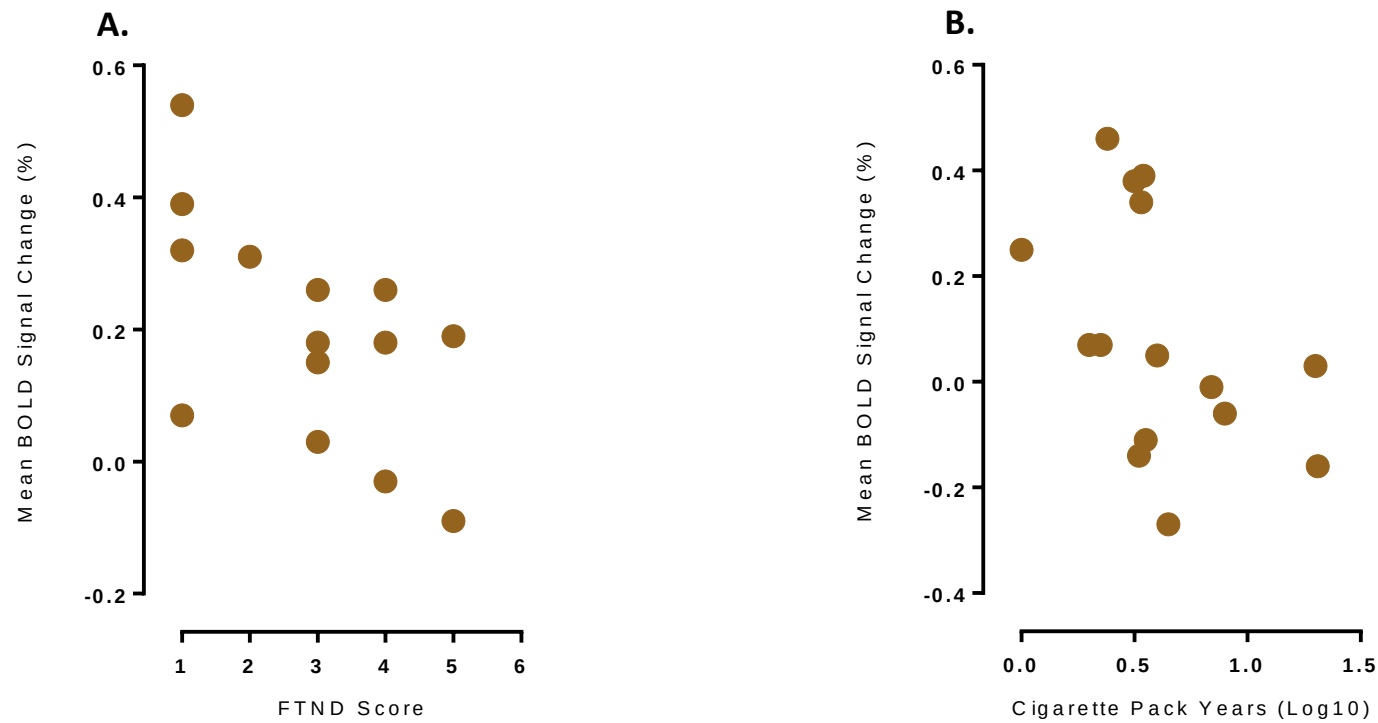


Figure 4. Showing negative correlations in the current smoker group between A) Fagerström Test of Nicotine Dependence (FTND) and right middle frontal gyrus mean BOLD signal change for positive operant response outcomes ($r(13) = -.52$, $p < 0.05$); and B) Cigarette Pack Years (Log10) and right anterior cingulate cortex mean BOLD signal change for negative operant response outcomes ($r(13) = -.61$, $p < 0.05$).

Electronic Supplementary Material (online publication only)

[Click here to download Electronic Supplementary Material \(online publication only\): Supplementary Figure 1.docx](#)

