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Effects of 7.5% Carbon Dioxide Inhalation on Anxiety and Mood in Cigarette Smokers

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Abstract

Cigarette smoking is associated with elevated risk of anxiety and mood disorder. Using the 7.5% carbon dioxide (CO_2) inhalation model of anxiety induction, we examined the effects of smoking status and abstinence from smoking on anxiety responses.

Physiological and subjective responses to CO_2 and medical air were compared in smokers and non-smokers (Experiment One) and in overnight abstinent and non-abstinent smokers (Experiment Two).

CO_2 induced greater increases in blood pressure in non-smokers compared with smokers (ps < 0.043), and greater increases in anxiety ($p = 0.005$) and negative affect ($p = 0.054$) in non-abstinent compared with abstinent smokers.

CO_2 increased physiological and subjective indices of anxiety. There were differences across smoking groups indicating that the CO_2 inhalation model is a useful tool for examining the relationship between smoking and anxiety. The findings suggested that both acute smoking and acute abstinence may protect against anxious responding. Further investigation is needed in long-term heavy smokers.

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Introduction

Cigarette smoking is strongly associated with elevated risk of anxiety and depression, and there is growing evidence that the relationship is bidirectional [Paperwalla *et al.*, 2004, Munafo *et al.*, 2008, Cosci *et al.*, 2010, Munafo *et al.*, 2010, Lewis *et al.*, 2011]. Smokers often report smoking in times of low mood and anxiety, and identify anxiety as a trigger of cigarette seeking and relapse. The belief that smoking reduces anxiety is strong among smokers, but there is little empirical evidence of anxiolytic effects in non-deprived smokers. In contrast, acute withdrawal from smoking increases feelings of anxiety [Hughes, 2007]. Therefore, while cigarette smokers report smoking to alleviate anxiety, they may in fact simply be smoking to alleviate abstinence-induced anxiety, in particular given the fast action and clearance of nicotine.

While the link between cigarette smoking and anxiety is well described, the causal nature of the relationship remains unclear. Epidemiological studies that prospectively examine this relationship have identified anxiety as a risk factor for smoking initiation, nicotine dependence and relapse [Patton *et al.*, 1998, Park *et al.*, 2009, Mckenzie *et al.*, 2010]. The proposed mechanisms for this include increased peer influence (particularly in relation to initiation of smoking) [Patton *et al.*, 1998] and smoking as anxiolytic self-medication [Park *et al.*, 2009]. However, smoking has also been shown to predict the incidence of depression [Johnson *et al.*, 2000, Mojtabai *et al.*, 2013, Moylan *et al.*, 2013] generalised anxiety and panic disorder [Zvolensky *et al.*, 2005, Moylan *et al.*, 2012].

A number of explanations have been offered to account for the elevated risk of anxiety and anxiety disorders in smokers, and these vary depending on the specific anxiety disorder considered. The relationship between panic and smoking has been attributed to smoking-induced respiratory abnormalities [Caldirola *et al.*, 2004], which may increase sensitivity to respiratory signals that induce or maintain panic symptoms [Klein, 1993]. Zvolensky [2003] proposes a pathoplasty model in which smoking modifies panic disorder by inducing acute physical changes akin to fearful bodily sensations (such as autonomic effects, noradrenaline release). Alongside these changes, the act of smoking also negatively impacts on perception of general health and can produce actual medical disturbance that further contribute to panic symptomology [Zvolensky *et al.*, 2003].

There is also general consensus that repeated tobacco exposure can directly alter the stress systems of the body [Bruijnzeel, 2012], which may explain more generalised changes in anxiety and anxiety sensitivity. Nicotine, the main psychoactive constituent of tobacco, acts primarily at the nicotinic acetylcholine receptors (nAChRs), which are widespread throughout the brain [Picciotto *et al.*, 2002]. Their activation affects many neurotransmitter systems and neural regions involved in the stress response, including the hippocampus which is a key output centre regulating hypothalamic-pituitary-adrenal axis (HPA) function [Jacobson *et al.*, 1991]. Acutely, cigarette smoking activates the HPA axis and increases adrenocorticotropic hormone (ACTH) and cortisol levels [Kirschbaum *et al.*, 1992, Steptoe *et al.*, 2006], and repeated smoking results in desensitisation and upregulation of nAChRs [Dani *et al.*, 1996] and changes basal activity of the HPA [Rohleder *et al.*, 2006].

These changes provide a biological explanation for the smoking-related vulnerability towards anxiety, and the differences in reported anxiety between smokers and non-smokers. Despite

smokers reporting anxiolytic effects of smoking [Nichter *et al.*, 1997, Fidler *et al.*, 2009], they often show higher levels of anxiety and anxious arousal than non-smokers [Parrott, 1999, Collins *et al.*, 2009, Ameringer *et al.*, 2010]. Acute smoking abstinence is associated with a negative affective comprising low mood, irritability, anxiety and craving [Hughes *et al.*, 1986, Perkins *et al.*, 1992, Benowitz, 2008], and smoking relieves these symptoms. Therefore, smokers may come to believe that smoking reduces anxiety, when in fact it simply alleviates withdrawal-related anxiety [Hughes *et al.*, 1984]. In contrast, long-term abstinence from smoking is associated with marked reductions in anxiety [Mcdermott *et al.*, 2013]. Understanding the true nature of the smoking-anxiety interaction is important as a misperception of anxiolytic effects of smoking and anxiogenic effects of smoking cessation may promote smoking maintenance and negate quit attempts. In order to investigate these relationships, it is logical to stimulate anxiety in a controlled research setting, and a variety of anxiogenic models have been used in laboratory settings. Childs and de Wit [2010] exposed non-deprived smokers to a psychosocial stressor (Trier Social Stress Test) and found that cigarette craving and pleasure from first cigarette, but not smoking, were increased in response to the stressor compared to a non-stressful control task. Increases in actual smoking behaviour have been observed in response to other "stressful" manipulations including loud noise exposure [Cherek, 1985] and stage performances [Rose *et al.*, 1983]. These findings indicate that anxiety-provoking situations incite craving and increased smoking in some instances, but subjective anxiety levels were not measured directly, which limits the interpretation of these findings. Furthermore, psychological models of anxiety induction are often limited in terms of their external validity, practicality and the degree of individual variation in responding.

To circumvent some of these issues, respiratory challenges that involve breathing air with increased levels of carbon dioxide have been used [Zvolensky *et al.*, 2001]. This can be achieved in a number of ways, one of which is by individuals rebreathing air in a closed system. Using this method Abrams [2008] found decreased respiratory rate but increased report of panic symptoms in a group of nicotine withdrawn heavy smokers compared with non-smokers. In a follow up study comparing abstinent and non-abstinent smokers, Abrams [2011] did not find an effect of smoking status on response to a 5-minute rebreathing procedure, although pre-challenge withdrawal symptoms were associated with stronger subjective responses to the challenge. A limitation of this technique is that the level of CO$_2$ inhaled gradually increases over time and therefore inhalation techniques that deliver fixed levels of CO$_2$-enriched air afford the researcher greater control of the anxiety response.

Another respiratory challenge involves controlled inhalation of air mixtures that have higher CO$_2$ concentrations than normal air. Like other CO$_2$ challenges, these inhalations induce physiological and psychological symptoms of anxious arousal. The effects of CO$_2$ appear to be dose dependent with higher doses producing panic-like symptoms and lower doses producing less intense generalised anxiety [Bailey *et al.*, 2007]. In support, single breath of 35% CO$_2$ concentration, but not lower doses, reliably stimulates ACTH and cortisol release [Kaye *et al.*, 2004]. However, some individuals experience panic attacks and panic-like symptoms at lower doses [Zvolensky *et al.*, 2001]. Due to the high prevalence of smoking among panic disorder patients [Mccabe *et al.*, 2004], several studies have examined the effects of higher CO$_2$ concentrations (around $20\% - 35\%$). Cosci et al. [2006] investigated the effect of transdermal nicotine (via skin patch) on reactivity to 35% CO$_2$ challenge in non-smokers. Although nicotine patch increased baseline autonomic activation compared to placebo patch, there was no effect of patch type on CO$_2$ reactivity. Using 20% challenge,

Zvolensky et al. [2001] were able to differentiate between smokers who able and unable to maintain quit attempts for seven days, with evidence of increase reactivity in those who reported being unable do so. The effects of lower CO$_2$ concentration (i.e., $5\% - 10\%$) have been largely unexplored in smoking research. The effects of withdrawal on stress reactivity have been explored using 10% CO$_2$ challenge and these studies have shown that higher levels of withdrawal are associated with greater stress reactivity in individuals with panic disorder [Leyro *et al.*, 2013] and high anxiety sensitivity [Vujanovic *et al.*, 2009].

The current study extends previous research by exploring the relationship between smoking and anxiety using a lower 7.5% CO$_2$ concentration. The effects of this dose in healthy volunteers are now well described [Bailey *et al.*, 2005]; compared with the inhalation of air, this produces marked increases in heart rate and blood pressure, and corresponding increases in subjective ratings of anxiety, fear and tension, with decreased subjective ratings of feeling relaxed and happy. These effects are sustained throughout the 20 minute inhalation, with rapid recovery when inhalation of normal air is resumed. A single dose of the benzodiazepines lorazepam, alprazolam and zolpidem reduces the subjective effects of 7.5% CO$_2$ [Bailey *et al.*, 2007, Bailey *et al.*, 2009], as does 3-week treatment with the serotonin reuptake inhibitor paroxetine [Bailey *et al.*, 2007]. Furthermore, this challenge induces cognitive biases characteristic of anxiety [Garner *et al.*, 2011, Cooper *et al.*, 2012].

Based on previous findings, we hypothesised that smokers would show greater stress reactivity than non-smokers (Experiment One) and abstinent smokers would show greater anxiogenic response and higher craving compared to non-abstinent smokers (Experiment Two). These studies also informed the validity of the 7.5% CO$_2$ model as a tool for future smoking research.

Experiment One

Methods

Design and overview: The experiment consisted of a repeated-measures design, with gas (air, 7.5% CO_2) as the within-subjects factor and smoking status (smoker, non-smoker) as a between-subjects factor. The order of gas inhalation was counterbalanced across participants.

Participants: Twelve smokers (smoke at least 5 cigarettes per day and within one hour of waking) and twelve non-smokers (current non-smokers and have smoked no more than 100 cigarettes in lifetime) were recruited from the staff and students of the University of Bristol and from a participant database. Exclusion criteria were current or a history of psychiatric disorder or drug or alcohol dependence (excluding caffeine), assessed using a structured interview by a trained researcher, based on the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Blood pressure (<140/90 mmHg), heart rate (50–90 bpm) and body mass index (BMI; 18–30 kg/m2) were required to be within the normal range. Prior to the study, participants were asked to refrain from alcohol for 36 hours, and from caffeine after midnight. Participants were reimbursed £20 for their time at the end of testing. The study was reviewed and approved by the University of Bristol Faculty of Science Research Ethics Committee.

Gas mixtures: The gas mixtures used were CO_2 7.5%/O_2 21%/N 71.5% and medical air (O_2 21%; BOC Ltd.). These were administered to participants using a oro-nasal mask (Hans

Rudolph, Kansas City, MO, USA), which was attached to a 500 L bag with tubing. For safety reasons, the gas was administered single-blind.

Physiological assessment: Participants were tested for alcohol and carbon monoxide levels in exhaled breath using the Alcohawk PT400 (Q3 Innovations, Independence, IA, USA) and the Bedfont Micro Smokerlyser (Bedfont Scientific Ltd, Maidstone, UK), respectively. Blood pressure was recorded using the OMRON M6 Comfort Digital Blood Pressure Monitor (OMRON Healthcare Ltd, Milton Keynes, UK). Fresh urine samples were provided to test for the presence of barbiturates, benzodiazepines, opiates, tetrahydrocannabinol (THC), methamphetamine, amphetamine and cocaine, and to test for pregnancy in female participants (Surescreen Diagnostics Ltd, Derby, UK).

Psychological assessment: Participants completed the Spielberger State-Trait Anxiety Inventory State (STAI-S) and Trait (STAI-T) subscales (Spielberger, 1983), Anxiety Sensitivity Inventory (ASI) [Reiss *et al.*, 1986], and the Positive and Negative Affect Schedule positive (PANAS-P) and negative (PANAS-N) subscales [Watson *et al.*, 1988]. Cigarette craving was measures using the Questionnaire on Smoking Urges (QSU) [Tiffany *et al.*, 1991].

Procedure: Participants completed an initial telephone screening before attending the testing session, in order to ascertain likely eligibility for the study. Smokers were required to be abstinent from smoking overnight (i.e., at least 12 hours), but were asked to smoke one of their own cigarettes before entering the laboratory in order to keep recent smoking levels consistent within the smoker group. On the day of testing, participants provided signed informed consent, and then completed further screening procedures, including exhaled breath

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and urine testing, assessment of blood pressure, heart rate, height and weight, and completion of the psychiatric screening interview. Eligible participants then completed the STAI-S, STAI-T, ASI, PANAS-P, and PANAS-N measures prior to the first inhalation. Inhalations of air and CO_2 each lasted for twenty minutes. Inhalations periods were separated by 40 minutes of rest, and the order of air and CO_2 was counterbalanced across participants. Throughout the study, an experimenter remained within close proximity of the gas cylinders to ensure the bag was adequately full at all times. Participants sat with their back to the gas cylinders. Immediately after each inhalation, blood pressure and heart rate were recorded while the participant remained seated, and the STAI-S, PANAS and QSU (smokers only) were then completed. While anxiety induced by the inhalation is sustained during inhalation, it declines very rapidly on inhalation of normal air. Participants were therefore asked to indicate how they felt during the inhalation when the effects of the gas were "at their strongest" when completing the questionnaire measures at the end of the inhalation. Participants were then required to stay in the laboratory with the researcher for a further 30 minutes (post end of last inhalation) to allow for recovery (as assessed through heart rate and blood pressure response and participant self-report).

Statistical analysis: Data were analysed by repeated measures ANOVA, with gas (air, CO_2) as a within-subjects factor, and smoking status (smoker, non-smoker) as a between-subjects factor. For effects of gas on craving, t-tests were run on craving scores after CO_2 and air in smoking group only. To explore the relationship between subjective anxiety and craving Pearson's correlations were conducted on post-CO_2 SSAI and QSU scores. All analyses were performed using PASW Statistics version 18 (SPSS Inc., Chicago IL, USA). Precise *p*-values are reported throughout.

Previous research has indicated that inhalation of 7.5% CO_2 increases state anxiety by approximately 19 points (SD 8.5 points) compared to inhalation of air. Our sample size therefore provided 99% power to detect an effect of inhalation of 7.5% CO_2 versus inhalation of air on subjective anxiety, and 80% power to detect a difference of 10 points between smokers and non-smokers in the increase in anxiety induced by inhalation of 7.5% CO_2, assuming an alpha level of 5%. This effect is comparable to that observed for the effects of anxiolytic medications on response to inhalation of 7.5% CO_2 (Bailey et al., 2007).

Results

Characteristics of participants: Participant characteristics ($n = 24$; 50% female) at baseline are shown in Table 1, and did not differ between smokers and non-smokers ($ps > 0.48$).

Insert Table 1 about here.

Cardiovascular measures: CO_2 inhalation increased systolic blood pressure (F [1, 22] = 28.48, $p < 0.001$, $\eta^2 = 0.56$), diastolic blood pressure (F [1, 22] = 4.89, $p = 0.038$, $\eta^2 = 0.18$), and heart rate (F [1, 22] = 13.31, $p = 0.001$, $\eta^2 = 0.38$) compared with air inhalation (Table 2). For systolic and diastolic blood pressure, the gas × smoking status interaction terms indicated that these effects were stronger in non-smokers than smokers ($ps < 0.043$). This was not observed for heart rate ($p = 0.89$).

Anxiety and mood: CO_2 inhalation increased state anxiety (F [1, 22] = 18.72, $p < 0.001$, $\eta^2 = 0.46$) and negative affect (F [1, 22] = 11.98, $p = 0.002$, $\eta^2 = 0.35$), compared with air inhalation (Table 2). There was no main effect on positive affect ($p = 0.12$), and the gas ×

smoking status interaction terms indicated that there was no evidence that these effects differed by smoking status (ps > 0.10). These results are shown in Figure 1 (panel a).

Insert Figure 1 about here.

Cigarette craving: There was no difference in craving among smokers after CO$_2$ (M = 32.9, SD = 12.0) compared to air (M = 30.3, SD = 9.8) (t = .91, df = 11, p = .38). Post-CO$_2$ craving did not correlate with post-CO$_2$ anxiety (p = .67).

Discussion

CO$_2$ inhalation consistently increased cardiovascular parameters and self-reported anxiety and negative affect compared with air. Smokers showed reduced physiological responses to CO$_2$ inhalation than non-smokers, with lesser increases in blood pressure. There was no interaction between gas and smoking status for self-reported anxiety and negative affect. The lesser physiological response in the smokers may be due to an anxiolytic effect of smoking, which may be a conditioned response (i.e., learned association between smoking and anxiolysis that may be withdrawal related) or due to a pharmacological anxiolytic effect of nicotine. The smokers in this study smoked before entering the laboratory. While these findings suggest no exacerbated anxiety responses to this challenge in non-abstinent smokers, it remains unclear how abstinence may explain these responses. In regular smokers, abstinence from smoking results in a range of physiological and subjective symptoms, including increased anxiety, and previous research has reported increased panic symptoms in withdrawn smokers following a rebreathing procedure [Abrams *et al.*, 2008]. Furthermore, it is widely accepted that anxiety is a risk factor for smoking and smoking relapse [Parrott, 1998, Albertsen *et al.*, 2006]. Therefore, smokers may be particularly vulnerable to anxiety

when in a withdrawn state, and this was investigated in Experiment Two, which compared reactivity to the CO$_2$ challenge in a group of abstinent and non-abstinent daily smokers.

Experiment Two

Methods

Design and overview: The experimental design and study procedures were identical to those described in Experiment One, with the exception that only smokers were recruited. Participants were randomised to abstain from smoking for 12 hours prior to testing, or smoke as usual (with their last cigarette being smoked just prior to entering the laboratory). A balanced randomisation schedule (i.e., equal numbers per group) was produced using random number generation software. Gas order was not counterbalanced (the air inhalation was administered first). Participants were told that they would complete two inhalations, one of (normal) air and one of CO$_2$–enriched air, but they were not told the order in which they would receive these mixtures. The fixed order schedule was utilised due to increases in anxiety during the first inhalation (regardless of gas content), which may have inflated the anxiolytic effect of the gas by interacting with the CO$_2$-induced anxiety. To create a more conservative test, medical air was administered first throughout.

Participants: Twenty-four smokers (smoke at least 5 cigarettes per day and within one hour of waking) were recruited from the staff and students of the University of Bristol and from a participant database, and randomised to either abstinent or non-abstinent conditions (twelve participants per group). All inclusion and exclusion criteria were identical to those described

in Experiment One. None of the participants recruited had taken part in Experiment One. Abstinence status on the day of testing was confirmed by exhaled CO monitoring (< 7 ppm).

Statistical analysis: Data were analysed by repeated measures ANOVA, with gas (air, CO_2) as a within-subjects factor, and smoking abstinence (abstinent, non-abstinent) as a between-subjects factor. Analyses were performed with and without adjustment for changes in craving between gas conditions. To explore the relationship between subjective anxiety and craving Pearson's correlations were conducted on post-CO_2 SSAI and QSU scores. All analyses were performed using PASW Statistics version 18 (SPSS Inc., Chicago IL, USA). Precise *p*-values are reported throughout.

Results

Characteristics of participants: Participant characteristics ($n = 24$; 46% female) at baseline are shown in Table 1. There were baseline differences in craving ($t = 4.31$, df = 22, $p < 0.001$) and heart rate ($t = 2.81$, df = 22, $p = 0.010$) with higher craving ($M = 42.9$, $SD = 12.8$ versus $M = 22.3$, $SD = 10.5$) and lower heart rate ($M = 68.6$, $SD = 9.8$ versus $M = 79.8$, $SD = 9.8$) in abstinent versus non-abstinent smokers. There were no other differences between abstinence groups ($ps > 0.10$).

Cardiovascular measures: CO_2 inhalation increased systolic blood pressure ($F [1, 22] = 4.89$, $p = 0.038$, $\eta^2 = 0.18$), compared with air inhalation (Table 2). There were no main effects on diastolic blood pressure ($p = 0.13$) or heart rate ($p = 0.65$), and the gas $\times$ abstinence interaction terms indicated that there was no evidence that these effects differed by

abstinence status (ps > 0.36). These results were not altered when change in craving was included as a covariate.

Anxiety and mood: CO_2 inhalation increased state anxiety as measured by SSAI (F [1, 22] = 52.13, $p < 0.001$, $\eta^2 = 0.70$) and negative affect (F [1, 22] = 21.76, $p < 0.001$, $\eta^2 = 0.50$), and decreased positive affect (F [1, 22] = 5.24, $p = 0.032$, $\eta^2 = 0.19$) compared with air inhalation (Table 2). For state anxiety, the gas × abstinence interaction term indicated that this effect was stronger in non-abstinent smokers than abstinent smokers ($p = 0.005$). A similar pattern was observed more weakly for negative affect ($p = 0.054$), but not positive affect ($p = 0.27$). These results are shown in Figure 1 (panel b), and were not altered when change in craving was included as a covariate.

Craving: There was no effect of gas on craving ($p = .82$), but craving was higher in the abstinent group ($M = 40.4$, $SD = 12.5$) than non-abstinent group ($M = 29.5$, $SD = 13.1$) (F [1, 22] = 4.37, $p = 0.048$, $\eta^2 = 0.17$). There was a gas × abstinence interaction (F [1, 22] = 5.67, $p = 0.026$, $\eta^2 = 0.21$), with non-abstinent smokers showing higher craving after CO_2 compared to air ($t = 2.24$, df = 11, $p = 0.046$), but no difference between CO_2 and air in abstinent smokers ($p = 0.21$) (Figure 1, panel b). Post-CO_2 craving did not correlate with post-CO_2 anxiety across the collective data set ($p = .998$), but based on the gas × abstinence interaction correlations were conducted separately for each abstinent group. There was evidence of a correlation in the non-abstinent group ($r = .53$, n = 12, $p = 0.075$) but not the abstinent group ($r = -.35$, n = 12, $p = 0.26$).

Discussion

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CO_2 inhalation increased subjective anxiety and systolic blood pressure, and induced negative changes in mood. Overnight abstinent smokers showed lesser response to CO_2 than did non-abstinent smokers. Furthermore, abstinent smokers did not show increases in craving after CO_2 compared to air as was observed in non-abstinent smokers. These findings suggest that abstinence from smoking may have beneficial effects on anxious responding in smokers [Parrott, 1995, West *et al.*, 1997]. The relationship between smoking and anxiety is undoubtedly complex. Paradoxically, smokers often assert that smoking is anxiolytic, yet at a population level, smokers report higher levels of anxiety than do non-smokers [Parrott, 1998]. Anxiolytic effects reported by smokers may actually be due to reversal of an anxiogenic smoking withdrawal state that develops with chronic smoking, and as a result smokers may come to associate the act of smoking with reductions in anxiety and smoke in times of stress. Although our findings did not indicate heightened reactivity to CO_2 in abstinent smokers, it is noteworthy that this group did not differ from the non-abstinent group on state measures of anxiety or positive or negative affect, as would be expected in individuals experiencing acute nicotine withdrawal. This lack of withdrawal symptoms suggests that our sample were not strongly tobacco dependent. We recruited individuals who smoked at least five cigarettes per day and who smoked within one hour of waking in the morning (which are considered reliable indicators or smoking dependence). There were group differences in baseline levels of craving with higher craving in abstinent smokers as would be expected for smokers who usually smoke upon waking, but as noted above this craving difference was independent of other withdrawal symptoms such as negative affect. We did not record the number of years smoked. Given the young age of many of our participants, it is unlikely that many have sustained the regular pattern of smoking for very long, and therefore may not have experienced the physiological changes associated with long-term regular smoking. These data indicate a dissociation between craving and negative affect in our

sample of abstinent smokers. Future research should replicate this study in a sample of heavier smokers with a longer smoking history, who would be expected to show withdrawal syndrome upon acute abstinence from smoking.

General Discussion

Inhalation of CO$_2$-enriched air increased physiological and subjective indices of anxiety and negative affect in both experiments, providing validation of its use as a model of anxiety induction in experimental studies. Furthermore, gas by smoking group interactions were observed in both experiments, indicating that responses to the gas are influenced by smoking-related variables. Lower reactivity to CO$_2$ was observed in smokers compared with non-smokers (Experiment One) and in abstinent compared with non-abstinent smokers (Experiment Two).

These findings suggest that during periods of smoking, smokers do not show substantial increases in anxious responding, as demonstrated by lesser reactivity to CO$_2$ than non-smokers (Experiment One). Despite this, there was also evidence that acute abstinence from smoking may be beneficial, reducing anxious responding beyond that of non-abstinent smokers (Experiment Two). It is important to note that there was little evidence of negative withdrawal symptoms in our overnight abstinent sample, and therefore these findings may only generalise to abstinence in the absence of marked withdrawal symptoms.

Interactions between gas and smoking group were observed in both experiments, yet the dependent variable for which the interaction was obtained differed across studies. These were systolic and diastolic blood pressure in Experiment One and state anxiety and negative affect

in Experiment Two. While it is possible that different smoking variables have differential effects across anxiety parameters, it seems that there were more general patterns of reactivity change that simply did not reach statistical significance on all dependent measures. For example, in Experiment One, in addition to reduced blood pressure response to the gas, smokers also showed a pattern consistent with reduced subjective anxiety and negative (see Figures 1 and two, panels A). However, the smoking groups in Experiments One and Two differed in relation to gas-related craving. The non-abstinent group in Experiment Two reported higher craving after CO$_2$ compared to air but this effect was not observed in Experiment One (where the smokers had the same abstinence profile). This suggests possible differences in the smokers between the two studies, despite use of the same inclusion criteria. This variation again may be partly due to the inclusion of some smokers with short smoking histories and low dependence. Both groups reported increases in anxiety after CO$_2$ suggesting a partial dissociation between anxiety and craving.

Taken together, these findings support an acute anxiolytic effect of smoking as has been reported elsewhere [Jarvik *et al.*, 1989, Cohen *et al.*, 2009]. However, it has been suggested that this effect is relatively short-lived [Szyndler *et al.*, 2001], and that chronic smoking induces physiological changes [Rohleder *et al.*, 2006] that may increase basal levels anxiety in smokers compared to non-smokers [Sheahan *et al.*, 2006]. The absence of withdrawal symptoms (i.e., negative affect) in the abstinent group suggests low levels of dependency. Instead of the expected higher reactivity to the gas in abstinent smokers, we observed lower anxiety and craving in this group. These findings imply that abstinence may be beneficial when not accompanied by a pronounced withdrawal syndrome which supports the contention that long term abstinence (i.e., post-withdrawal) from smoking has beneficial effects on anxiety and anxious responding [Mcdermott *et al.*, 2013]. However, Leyro et al. [2013]

investigated panic responses to 10% CO_2 challenge in heavier smokers, and found that high nicotine withdrawal was only associated with greater panic attack symptoms in individuals with panic disorder. In contrast, healthy controls had lower symptomology when heavily withdrawn from smoking, suggesting that the withdrawal may not be strongly associated with stress reactivity. However, this study examined more extreme (panic-related) outcomes and future research should examine withdrawal effects in heavy long-term smokers on subjective anxiety.

The relationship between smoking and anxiety is complex and may vary depending on the smoking variable under investigation [Leventhal *et al.*, 2013] and also as a function of individual differences. There are also reports of gender differences with females showing greater increases in negative affect (including anxiety) during smoking abstinence [Pang *et al.*, 2013]. In a qualitative review of the smoking-affect literature, Ameringer and Leventhal [2010] identified associations with negative affect and emotional arousal with smoking status but not smoking heaviness. The current studies assessed changes in phasic craving as a function of anxiety-induction but future research should consider a wider range of smoking variables and individual differences. Furthermore, due to the nature of the manipulation and sample, it is difficult to directly extrapolate from the effect sizes we observed to clinical significance. However, the study was powered to detect effects comparable in magnitude to studies of the effects of anxiolytic medications on response to inhalation of 7.5% CO_2 [Bailey *et al.*, 2007].

There are some limitations of the current studies which should be acknowledged. First, the sample was predominately students from the University of Bristol. This may limit the generalisability of findings to the wider population. Second, while the studies were

sufficiently powered to demonstrate effects of the CO$_2$ inhalation on anxious outcomes, the small sample size may underlie the relatively weak statistical evidence for the key interaction effects. Therefore, these findings should be replicated using a larger sample. Third, a number of personal and situational variables may affect smoking and anxiety outcomes, including fitness, sleep levels and menstrual cycle, which were not controlled for in the current studies.

These experiments are the first to use the 7.5% CO$_2$ anxiety induction model to investigate the relationship between smoking and anxiety. The model consistently increased physiological and subjective indices of anxiety across all experiments. Furthermore, we observed differences between smoking groups in anxious responding in both experiments, thereby validating the use of this model as a tool to be used in future smoking research. Given the important role that anxiety is believed to play particularly in the maintenance and relapse to smoking, developing novel and valid tools is an important advancement in the field. As well as investigating the effects of smoking variables, such as smoking status and abstinence, future research should strive to further elucidate the mechanisms underlying the smoking-anxiety relationship. Habitual smoking induces changes in a number of biological systems; however, other cognitive and psychological factors, including emotional dysregulation, outcome expectancies and smoking motives all warrant future investigation. The relationship between smoking and anxiety in undoubtedly complex, and while further research is required, these studies provide an important first step in the use of physiological anxiety manipulation techniques to understand smoking behaviour.

Acknowledgements

Conflict of Interest Statement

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Figure 1. Effects of CO_2 vs. air on anxiety (SSAI), positive and negative affect (PANAS) and craving (QSU) (Experiment Two only) in smokers vs. non-smokers (Experiment One) and abstinent vs. non-abstinent smokers (Experiment Two)

Figure 2. Effects of CO_2 vs. air on systolic blood pressure, diastolic blood pressure and heart rate in smokers vs. non-smokers (Experiment One) and abstinent vs. non-abstinent smokers (Experiment Two)