

# Functional neuroimaging study in identical twin pairs discordant for regular cigarette smoking

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## ABSTRACT

Despite the tremendous public health and financial burden of cigarette smoking, relatively little is understood about brain mechanisms that subserve smoking behavior. This study investigated the effect of lifetime regular smoking on brain processing in a reward guessing task using functional magnetic resonance imaging and a co-twin control study design in monozygotic (MZ) twin pairs that maximally controls for genetic and family background factors. Young adult (24–34 years) MZ female twin pairs ( $n = 15$  pairs), discordant for regular smoking defined using Centers for Disease Control criteria as having smoked  $\geq 100$  cigarettes in their lifetime, were recruited from an ongoing genetic epidemiological longitudinal study of substance use and psychopathology. We applied hypothesis-driven region of interest (ROI) and whole-brain analyses to investigate the effect of regular smoking on reward processing. Reduced response to reward and punishment in regular compared with never-regular smokers was seen in hypothesis-driven ROI analysis of bilateral ventral striatum. Whole-brain analysis identified bilateral reward-processing regions that showed activation differences in response to winning or losing money but no effect of regular smoking; and frontal/parietal regions, predominantly in the right hemisphere, that showed robust effect of regular smoking but no effect of winning or losing money. Altogether, using a study design that maximally controls for group differences, we found that regular smoking had modest effects on striatal reward processing regions but robust effects on cognitive control/attentional systems.

**Keywords** Cigarette smoking, co-twin control, cognitive control, discordant, fMRI, reward.

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## INTRODUCTION

Reward processing is a common mechanism of action for all drugs of abuse (Goodman 2008; Koob & Le Moal 2008). Investigation of monetary brain reward processing in relation to cigarette smoking behavior has consistently shown decreased response in the striatum in smokers compared with controls. Early positron emission tomography studies showed no activation in the striatum in smokers but robust activation in non-smokers (Martin-Solch *et al.* 2001, 2003). However, these conclusions were qualitative as smokers and non-smokers were not statistically compared. More recently, functional magnetic resonance imaging (fMRI) studies have shown decreased activity in the striatum in smokers compared with non-smokers in response to the anticipation of

monetary reward (van Hell *et al.* 2010; Luo *et al.* 2011). Decreased anticipatory reward-related activity in the striatum and frontal and cingulate cortex has also been shown in dependent compared with non-dependent smokers (Buhler *et al.* 2010), implicating blunted activation to monetary reward anticipation as a mechanism of nicotine dependence. However, in a large sample of 14-year-olds who had varying but overall low levels of smoking exposure, with the majority not meeting criteria for nicotine dependence, lower activation to anticipation of monetary reward was also seen in the ventral striatum in smokers relative to never smoking controls (Peters *et al.* 2011). The authors surmised that the blunted ventral striatum activation 'may reflect a risk factor for the development of early substance use' (Peters *et al.* 2011, p. 547).

A co-twin control study design is ideally suited to deal with the potential confound of predisposing factors on group differences by its ability to provide tight control of predisposing factors; thus, decoupling, as much as possible, the influence of predisposing factors from cigarette exposure itself. We investigated reward processing in monozygotic (MZ) twin pairs discordant for lifetime smoking behavior (but concordant for ever having tried smoking cigarettes). MZ twins are essentially genetically identical, share early family environmental factors and commonly try smoking their first cigarettes on the same occasion (Pergadia *et al.* 2006). Thus, in an MZ co-twin control study, within-pair differences in brain reward processing could be more readily attributed to within-pair differences in smoking exposure than to differences in genetic or environmental risk for smoking.

## METHODS

### Sample

MZ twin pairs were recruited from a prospective, general population-representative study of a birth cohort of female like-sex twin pairs born in Missouri 1975–1985 (Heath *et al.* 2002). Twins were first targeted for assessment in adolescence, at mean age 15 years (baseline), with up to five follow-up psychiatric interview assessments and an ongoing sixth round of assessments (2011–2014). Polydiagnostic interview assessments were adapted from the Semi-Structured Assessment for the Genetics of Alcoholism (Hesselbrock *et al.* 1982; Bucholz *et al.* 1994) and focused on *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)* substance abuse and dependence and major axis one disorders. The smoking section was modified from the Composite International Diagnostic Interview (Robins *et al.* 1988; Cottler, Robins & Helzer 1989).

Smoking behavior reported as part of the diagnostic interviews at waves 4 (2001–2005) and 5 (2005–2008) was used to identify a sample of MZ twin pairs who were matched for exposure to cigarettes (i.e. both twins of each pair had tried smoking cigarettes, at least ‘a puff’) to control for exposure effects, but differed in their cumulative lifetime exposure to cigarettes; one twin had smoked  $\geq 100$  cigarettes [regular smoker (RS)], while the co-twin had smoked  $< 100$  cigarettes [never-regular smoker (NRS)]. Having smoked  $\geq 100$  cigarettes in one’s life defines a smoker according to the Centers for Disease Control and Prevention (CDC 2002) and is used to define smokers in large national surveys. There is also a nearly one-to-one correspondence ( $r = 0.96$ ) in individuals self-identifying as smokers and having smoked  $\geq 100$  cigarettes in a lifetime (A. Heath, pers. comm.). Furthermore, this measure reflects a substantially increased risk for

continued smoking, other drug use and psychopathology (Supporting Information Table S1, Supplemental Results). Altogether, the 100 cigarette phenotype reflects an important transitional stage in smoking behavior, yet it is a measure that captures smokers of varying smoking histories allowing investigation of brain mechanisms associated with transitions to more severe smoking stages.

### Subject recruitment and eligibility

The study protocol was approved by the Institutional Review Board of Washington University and was carried out using ethical principles for medical research involving human subjects in accordance with the Declaration of Helsinki. The study included a screening interview over the telephone to determine study eligibility and a neuro-imaging appointment. Exclusion criteria were: (1) only one twin from a pair agreed/was eligible to participate; (2) current or past 12-month heavy alcohol ( $> 4$  drinks/day) or illicit drug use ( $> 1$  use/week for cannabis;  $> 1$  use/month for other drugs); (3) pregnancy; (4) history of significant neurological diagnosis; (5) claustrophobia; or (6) presence of any metal in the body. Individuals with lifetime history of psychopathology or substance dependence as well as current diagnosis of tobacco dependence were *not* excluded. Current or past 12-month use of psychotropic medication was an exclusion criterion at the start of the study. However, this criterion significantly limited our ability to recruit twins because antidepressant medication use was common. Consequently, this initial exclusion criterion was dropped. As a result, three of the NRSs were using antidepressants (selective serotonin reuptake inhibitors), two RSs were taking topiramate for migraines and one of them was also using cyclobenzaprine for muscle spasms on as-needed basis, and one NRS was using cetirizine for seasonal allergies.

### Behavioral assessment

At the neuro-imaging appointment, subjects provided signed informed consent and completed a questionnaire assessing the past 4-week (1) frequency and quantity of caffeine, tobacco, alcohol and illicit drug use; (2) physical activity; (3) second-hand smoke exposure; (4) nicotine withdrawal using the Minnesota Nicotine Withdrawal Scale (Hughes & Hatsukami 1986); (5) mood using the 20-item positive and negative affect schedule (Watson, Clark & Tellegen 1988); (6) past 2-week depressive symptoms using the 21-item Beck Depression Inventory [BDI; (Beck *et al.* 1961)]; and (7) current anxiety using the 20-item State Trait Anxiety Inventory (Spielberger *et al.* 1983) Participants completed two subtests (vocabulary and matrix reasoning) of the Wechsler Abbreviated Scale of Intelligence to estimate IQ. Prior to magnetic

resonance imaging scanning, current RSs ( $n = 9$ ) were given opportunity to smoke a cigarette ( $n = 8$ ) to minimize the experience of nicotine withdrawal while in the scanner. Time between cigarette smoking and entrance into the scanner was about 15 minutes, which included measurement of breath carbon monoxide (CO) (~2 minutes), assessment of mood in the past hour (~2 minutes), reading directions for task performance (~3 minutes) and setup of the subject on the scanner table (~8 minutes).

### Cognitive task

We adapted the card-guessing task (Delgado *et al.* 2000, 2003) by eliminating the card cue, because of its potential association with gambling, and implementing a rapid event-related fMRI design. In our modified 'number-guessing task', subjects saw a white question mark in the middle of a black screen (Supporting Information Fig. S1). Subjects were told that there is a number behind the question mark that could range from 1 to 9. Subjects had to guess whether the number behind the question mark was smaller or larger than 5 by pressing a left or right button on a button box. Button mapping was the same within twin pairs but counter-balanced across twin pairs. Subjects won \$1 for correct guesses (reward condition) and lost \$0.50 for incorrect guesses (punishment condition). No money was won or lost when the number 5 was behind the question mark (neutral condition).

Each run consisted of 20 reward trials, 20 punishment trials, 20 neutral trials, 60 fixation trials (for jittering), in addition to three fixation trials at the beginning and nine fixation trials at the end. Each trial was 2-seconds long. For calculation of event-related responses, reward, punishment and neutral trials were pseudorandomly jittered such that 50% of the time two task trials could follow each other without fixation between them, 25% of the time, one fixation separated task trials and 25% of the time two fixations separated task trials, to allow extraction of signal associated with each event-related response (Miezin *et al.* 2000). The trial type sequence was predetermined; if the trial type was 'reward', subjects won money regardless of their guess (i.e. if they guessed above 5, the outcome was above 5). The sequence of trials was different for each task run, and the order of the four runs was randomized within and across twin pairs. The total possible number of different orders of the four runs was 24 (four factorial).

The number-guessing task was controlled by scripts compiled using PsyScopeX (Carnegie Mellon, Pittsburgh, PA, USA) (Cohen *et al.* 1993). Visual stimuli were projected (Boxlight CP730e, 832 × 624 pixels, Boxlight, Poulosbo, WA, USA) to a magnet compatible polacoat rear-projection diffusion screen (Da-Lite Screen Company,

Inc., Warsaw, IN, USA) viewable by the subjects through a mirror mounted on the head coil (usable visual field = 24° wide × 14° high).

### Image acquisition

All images were obtained with a Siemens MAGNETOM Trio 3 Tesla scanner (Erlangen, Germany) using Food and Drug Administration-approved sequences and a 12-channel head matrix coil. A high-resolution T1-weighted sagittal magnetization-prepared rapid gradient-echo (MP-RAGE) structural image was obtained (TE = 3.08 ms, TR (partition) = 2.4 second, TI = 1000 ms, flip angle = 8°, 128 slices with 1 × 1 × 1.25 mm voxels) (Mugler & Brookeman 1990). A rapid low-resolution (4 × 2 × 2 mm) three-dimensional anatomical MP-RAGE volumetric image (Mugler & Brookeman 1990) was also acquired and warped to a target MP-RAGE data set that represents the Talairach atlas (Talairach & Tournoux 1988). The alignment parameters were then used to adjust the scanner such that functional images were acquired parallel to the anterior–posterior commissure plane.

Functional images were obtained using a blood oxygenation level-dependent (BOLD) contrast sensitive gradient echo echo-planar imaging (EPI) sequence (TE = 27 ms, TR = 2.0 second, flip angle = 90°, in-plane resolution 4 × 4 mm). Whole-brain coverage was obtained with 32 contiguous interleaved 4 mm axial slices. Each of four runs of functional imaging consisted of 132 consecutive frames of whole-brain imaging.

### Data analysis

#### *Image pre-processing and estimation of event-related response*

Analysis methods used tools developed in-house for image pre-preprocessing and visualization, and for statistical analysis implemented in a software program called FIDL and based in the interactive data language (ITT Visual Information Solutions, Boulder, CO, USA) (Miezin *et al.* 2000; Ollinger, Corbetta & Shulman 2001; Ollinger, Shulman & Corbetta 2001). Image pre-processing involved frame alignment and debanding to correct for asynchronous and interleaved slice acquisition, image realignment to correct for movement, and intensity normalization that scaled each functional run to a mode value of 1000. EPI images were registered to each subject's T2-weighted structural volumes, which were registered to each subject's T1-weighted MP-RAGE volumes, which in turn were transformed to Talairach atlas space (Talairach & Tournoux 1988).

After pre-processing, a fixed effects general linear model estimated the effect magnitude of each trial type for each subject, using an unassumed hemodynamic

response function to model the BOLD response shape, yielding individual-specific estimates of the intercept, BOLD signal related to each of the reward, punishment and neutral conditions, as well as error trials where subjects failed to press the button during the allotted 1 second, with estimates for each of nine timepoints (to model the time-course of the hemodynamic response function over 18 seconds). This approach has been successfully implemented by others (Jimura, Locke & Braver 2010; Padmala & Pessoa 2010).

#### A priori region of interest (ROI) analysis

Because of findings in the literature of blunted reward-related activation in smokers in the ventral striatum, we conducted hypothesis-driven a priori analysis of bilateral ventral striatum. We examined ventral striatum ROIs based on coordinates with peak activation from the main effect of time image (Talairach x, y and z: -21, 3, -6 and 19, 3, -6 for left and right ventral striatum, respectively). The BOLD time series in the 10 mm spheres around these coordinates were subjected to a random effects three-way analysis of variance (ANOVA) with Condition (reward, punishment and neutral), Group (smokers and NRS) and Timepoint (nine TR frames) modeled as within-subject factors. The effect of primary interest was the three-way Condition  $\times$  Group  $\times$  Timepoint interaction testing differences in the BOLD response (i.e. evolution of the hemodynamic response function over the nine TR frames) to reward or punishment between RSs and NRSs. Significant effects were followed by *post hoc* pairwise comparisons using paired *t*-tests.

#### Whole-brain analysis

A random effects three-way ANOVA was conducted with Condition, Group and Timepoint as within-subject effects.

The effects of interest were differences in the time-course of the BOLD response across conditions (Condition  $\times$  Timepoint interaction), across smoking history (Group  $\times$  Timepoint interaction), and across both condition and smoking history (Condition  $\times$  Group  $\times$  Timepoint interaction).

For each interaction effect image, ROIs with activation reaching brain-wide significance  $P < 0.05$  after multiple comparisons correction using Monte Carlo simulation were extracted. ROIs were defined using a peak detection procedure followed by a region growing procedure (Church *et al.* 2008). Images were first smoothed with a 4 mm radius hard sphere kernel. A peak search algorithm was used to identify peaks with a  $z$  threshold  $3.5 < z < -3.5$  in the smoothed image and a cluster size of  $\geq 24$  voxels. Peaks separated by less than 10 mm were consolidated via coordinate averaging.

## RESULTS

### Sample characteristics

A total of 80 individuals from 47 twin pairs were screened for study eligibility. Of these, both twins from 22 pairs were eligible for participation and 16 pairs underwent the neuro-imaging protocol. Data from one twin from one of these pairs were not usable due to excessive movement, leaving data from 15 twin pairs ( $n = 30$  individuals) for analysis. Sample demographic and behavioral characteristics are shown in Table 1. Based on birth record information, two of the pairs were African American and all others were of European American descent. Based on self-report on the Edinburgh Handedness Inventory (Oldfield 1971), 10 pairs were right-handed; in four pairs one twin was right-handed and the co-twin was ambidextrous; and in one pair, one twin was left-handed

**Table 1** Personal and behavioral characteristics of the 15 MZ twin pairs comprised of lifetime regular smokers and their never-regular smoking twin sisters.

| Characteristics                      | RS ( $n = 15$ ) |           | NRS ( $n = 15$ ) |           |
|--------------------------------------|-----------------|-----------|------------------|-----------|
|                                      | Mean (SD)       | Range     | Mean (SD)        | Range     |
| Weight (lb)                          | 162.2 (43.4)    | 114–272   | 167.9 (50.9)     | 110–285   |
| IQ (vocabulary + matrix reasoning)   | 102.2 (11.7)    | 77–120    | 104.3 (15.6)     | 71–127    |
| Age first tried smoking cigarettes   | 13.20 (3.19)    | 8–17      | 14.27 (3.10)     | 8–18      |
| BDI (total score)                    | 5.67 (5.02)     | 0–13      | 2.87 (3.27)*     | 0–9       |
| PANAS (positive affect total score)  | 28.47 (7.29)    | 12–42     | 32.93 (9.53)     | 10–47     |
| PANAS (negative affect total score)  | 15.53 (3.72)    | 10–21     | 15.47 (4.75)     | 10–25     |
| STAI (positive symptoms total score) | 28.00 (5.69)    | 17–36     | 30.93 (3.61)     | 24–36     |
| STAI (negative symptoms total score) | 14.47 (4.34)    | 11–26     | 14.27 (4.79)     | 11–27     |
| Carbon monoxide (p.p.m.)             | 12.67 (12.9)    | 2–43      | 2.13 (1.13)*     | 1–5       |
| Movement (root mean square)          | 0.22 (0.11)     | 0.07–0.52 | 0.15 (0.07)*     | 0.08–0.32 |

\* $P < 0.05$ . BDI = Beck Depression Inventory; NRS = never-regular smoker; PANAS = Positive Affect and Negative Affect Schedule; p.p.m. = parts per million; RS = regular smoker; SD = standard deviation; STAI = State Trait Anxiety Inventory.



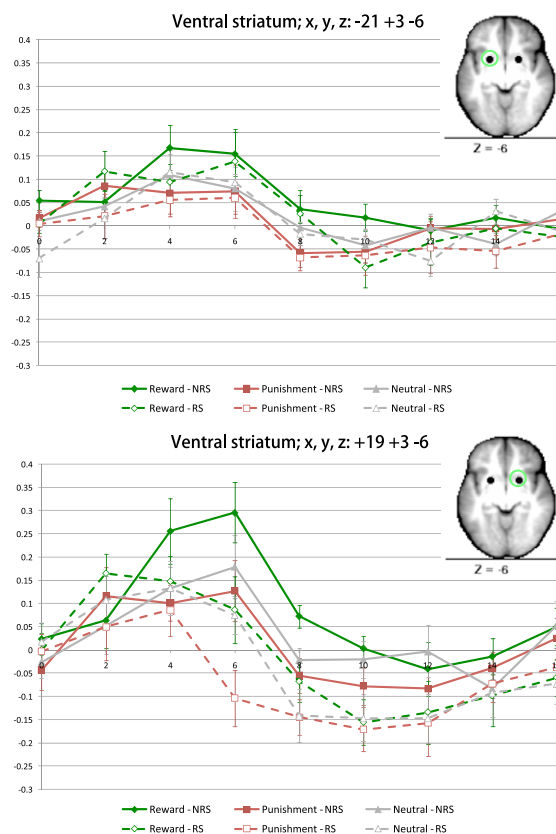
and the co-twin was ambidextrous. The sample average age was 28.7 years (SD = 3.27; range 24–34 years). Co-twins differed in BDI and pre-scan CO where RSs had significantly higher levels (paired *t*-tests,  $P < 0.05$ ). Mean CO levels of the eight RSs who smoked a cigarette prior to neuro-imaging was 21.3 parts per million (SD = 12.3; range 9–43). Movement was low in both groups but significantly higher in RSs ( $P < 0.05$ ). There were no significant group differences in median reaction times; a three-way ANOVA, with Condition, Group and Hand (left or right) as factors, showed no significant interactions. Missing BDI data for one NRS and missing CO data for another were substituted based on mean sample values (Supplemental Methods in Supporting Information). Within-pair differences in BDI scores and CO levels remained significant when missing data were excluded from analysis.

Data collected as part of the questionnaire survey at the time of the neuro-imaging appointment showed that the RSs were overall ‘light’ smokers (Supporting Information Table S2, Supplemental Results) though 46.7% met lifetime DSM-IV criteria for tobacco dependence (Supporting Information Table S3, Supplemental Results). Based on existing psychiatric interview data, within-pair comparison of other drug use and psychiatric history showed that significantly more of the RSs had used marijuana and met criteria for DSM-IV major depressive disorder (Supporting Information Table S3, Supplemental Results).

## fMRI data

### A priori ROI analysis

The Condition  $\times$  Group  $\times$  Timepoint interaction was significant for the left ventral striatum ( $P = 0.017$ ) and near significant for the right ventral striatum ( $P = 0.055$ ) (Fig. 1). Supporting Information Table S4 shows *P* values of effect size estimations for all pairwise comparisons. *Post hoc* paired *t*-tests at peak response (average of 4 and 6 seconds post-stimulus) in the left ventral striatum showed significantly greater activation to reward relative to punishment and neutral feedback in the NRSs and no significant effects of condition in the RSs. There were no significant group differences in response to reward, punishment or neutral feedback. In the right ventral striatum, the RSs showed significantly blunted response to reward and punishment compared with their MZ NRS co-twins. Further, the NRSs had significantly greater activation to reward relative to punishment and neutral feedback, which did not differ from each other, and the RSs had significantly blunted response to punishment relative to both reward and neutral feedback, which did not differ from each other. As shown in Supporting Information Table S4, effects of medium to large size (Cohen’s  $d \sim 0.5$ – $0.8$ ) were statistically significant ( $P < 0.05$ ).



**Figure 1** Time-courses from a priori regions of interest in left (top) and right (bottom) ventral striatum (circled). The x-axis shows 16 seconds of blood oxygenation level-dependent (BOLD) signal after stimulus onset in 2-second intervals, and the y-axis represents percent BOLD change. Bars are standard error of the mean. RS = regular smoker; dashed lines; NRS = never-regular smoker; solid lines. Green = time-course in response to winning money; Red = time-course in response to losing money; Grey = time-course in response to neutral feedback

Thus, NRSs had similar effects in the left and right ventral striata with relatively greater activation to reward than to both punishment and neutral feedback, while their MZ RS co-twins showed attenuated response to punishment in the right ventral striatum.

### Whole-brain analysis

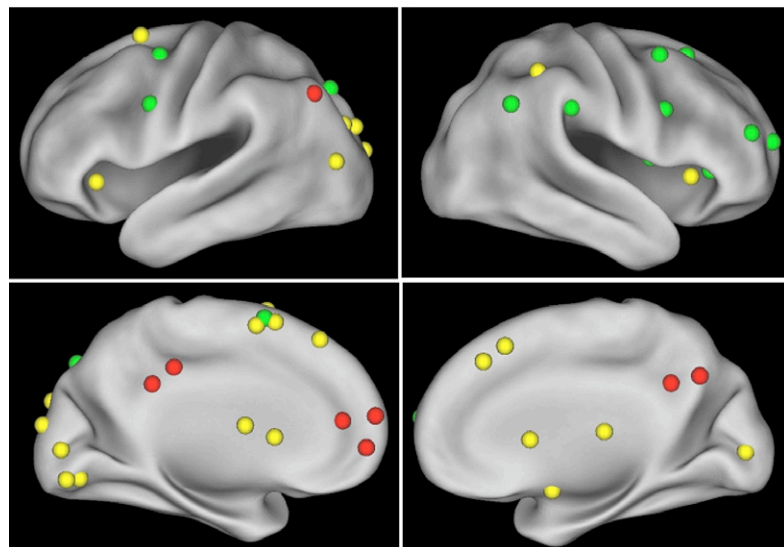
Differences in the time-course of the BOLD response across conditions were found in subcortical, medial cortical and occipital regions (significant Condition  $\times$  Timepoint interaction) (Table 2, Fig. 2). Some of these regions had positive time-courses (Fig. 2 yellow spheres, Table 2). Most of the regions with negative time-courses (Fig. 2 red spheres, Table 2) are recognized as part of the default mode network (posterior cingulate cortex (PCC), medial prefrontal cortex, medial precuneus and left angular gyrus) (Raichle *et al.* 2001).

**Table 2** Regions identified from the Condition  $\times$  Timepoint interaction.

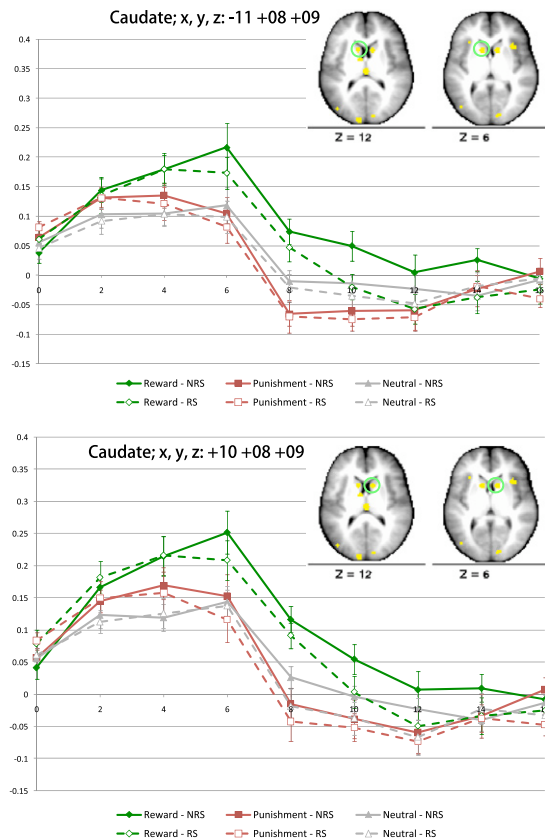
| Condition $\times$ Timepoint regions | Hemisphere | Talairach coordinates |     |     | Z value | No voxels |
|--------------------------------------|------------|-----------------------|-----|-----|---------|-----------|
|                                      |            | x                     | y   | z   |         |           |
| Positive time-courses                |            |                       |     |     |         |           |
| Superior frontal gyrus               | L          | -22                   | 3   | 62  | 3.74    | 26        |
| Medial frontal gyrus                 | L          | -4                    | 8   | 58  | 3.66    | 34        |
| Medial frontal gyrus                 | L          | -7                    | -3  | 57  | 4.64    | 62        |
| Medial frontal gyrus                 | L          | -1                    | 29  | 53  | 4.07    | 46        |
| Medial frontal gyrus                 |            | 0                     | 19  | 49  | 4.46    | 62        |
| Medial frontal gyrus                 |            | 0                     | 29  | 41  | 5.01    | 61        |
| Caudate                              | L          | -8                    | -5  | 15  | 4.82    | 56        |
| Caudate                              | L          | -11                   | 8   | 9   | 8.15    | 66        |
| Caudate                              | R          | 10                    | 8   | 9   | 7.38    | 65        |
| Putamen/globus pallidus              | R          | 16                    | 3   | -3  | 3.94    | 33        |
| Thalamus                             | R          | 1                     | -23 | 14  | 4.70    | 73        |
| Anterior insula                      | R          | 33                    | 14  | 6   | 4.33    | 44        |
| Anterior insula                      | L          | -31                   | 19  | 2   | 4.07    | 43        |
| Inferior parietal lobule             | R          | 44                    | -48 | 49  | 3.69    | 29        |
| Cuneus                               | L          | -10                   | -95 | 12  | 5.63    | 57        |
| Cuneus                               | R          | 11                    | -89 | 8   | 3.62    | 36        |
| Lingual gyrus                        | L          | -4                    | -88 | -1  | 5.29    | 66        |
| Lingual gyrus                        | L          | -11                   | -80 | -10 | 5.09    | 64        |
| Middle occipital                     | L          | -30                   | -79 | 19  | 4.53    | 64        |
| Middle occipital                     | L          | -41                   | -77 | 9   | 3.70    | 32        |
| Middle occipital                     | L          | -23                   | -88 | 22  | 5.00    | 69        |
| Cerebellum                           | L          | -1                    | -84 | -21 | 3.77    | 38        |
| Negative time-courses                |            |                       |     |     |         |           |
| Medial aPFC                          | L          | -3                    | 56  | 12  | 4.67    | 57        |
| Medial aPFC                          | L          | -1                    | 47  | 3   | 4.97    | 65        |
| ACC                                  | L          | -4                    | 41  | 14  | 5.50    | 67        |
| PCC                                  | L          | -2                    | -37 | 40  | 4.71    | 64        |
| PCC                                  | R          | 9                     | -51 | 31  | 3.98    | 50        |
| Precuneus                            | L          | -2                    | -47 | 33  | 4.80    | 64        |
| Precuneus                            |            | 0                     | -72 | 34  | 4.42    | 66        |
| Angular gyrus                        | L          | -41                   | -68 | 36  | 3.77    | 37        |

ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; PFC = prefrontal cortex; L = left; R = right.

**Figure 2** Forty-three regions extracted from the whole-brain ANOVA corrected for multiple comparisons projected on the lateral (top) and medial (bottom) inflated surfaces of the left (on the left) and right (on the right) hemispheres. Green = regions from the Group  $\times$  Timepoint interaction effect; Yellow = regions from the Condition  $\times$  Timepoint interaction effect that have positive time-courses; Red = regions from the Condition  $\times$  Timepoint interaction effect that have negative time-courses



Time-courses for bilateral caudate regions are shown in Fig. 3. The larger BOLD activation in response to winning money replicates previous findings (Delgado et al. 2000, 2003). Time-courses for all other regions



**Figure 3** Time-courses in left (top) and right (bottom) caudate regions (circled). The yellow color of the regions overlaid on the brain surface denotes regions with positive time-courses in response to winning or losing money as shown in Fig. 2. See Fig. 1 legend for details

**Table 3** Regions identified from the Group  $\times$  Timepoint interaction.

| Group $\times$ Timepoint regions | Hemisphere | Talairach coordinates |     |    | Z value | No voxels |
|----------------------------------|------------|-----------------------|-----|----|---------|-----------|
|                                  |            | x                     | y   | z  |         |           |
| Medial frontal gyrus             | L          | -3                    | 1   | 60 | 4.24    | 42        |
| Superior frontal gyrus           | R          | 20                    | 13  | 49 | 3.61    | 37        |
| Middle frontal gyrus             | R          | 34                    | 3   | 53 | 4.09    | 47        |
| Middle frontal gyrus             | L          | -33                   | -4  | 48 | 3.62    | 26        |
| Inferior frontal gyrus           | R          | 43                    | 7   | 34 | 4.75    | 63        |
| Inferior frontal gyrus           | L          | -51                   | 0   | 32 | 4.13    | 34        |
| aPFC                             | R          | 22                    | 53  | 19 | 4.67    | 54        |
| Middle PFC                       | R          | 36                    | 42  | 19 | 3.6     | 31        |
| Supramarginal gyrus              | R          | 51                    | -32 | 33 | 3.87    | 36        |
| Angular gyrus                    | R          | 51                    | -57 | 34 | 4.83    | 64        |
| Precuneus                        | L          | -21                   | -77 | 42 | 3.98    | 37        |
| Insula                           | R          | 34                    | -3  | 15 | 3.57    | 24        |
| Anterior insula                  | R          | 27                    | 22  | 11 | 3.78    | 32        |

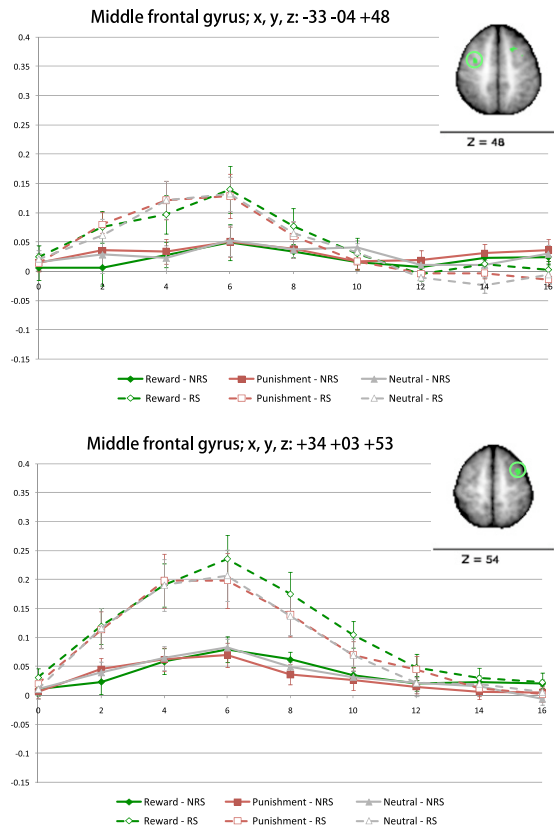
aPFC = anterior prefrontal cortex; PFC = prefrontal cortex; L = left; R = right.

with positive BOLD activations are shown in Supporting Information Fig. S2. Activation patterns for some regions were the same as that for the caudate with larger relative activation to winning money (medial frontal regions, inferior parietal lobule, putamen/globus pallidus, thalamus, occipital cortex and cerebellum). Other regions had a relatively greater activation to losing money (insula, superior frontal gyrus; more posterior medial frontal regions). All regions with negative time-courses had relatively smaller BOLD deactivation to monetary reward (Supporting Information Fig. S3).

Within-pair differences in the time-course of the BOLD response (significant Group  $\times$  Timepoint interaction) was seen in regions located in the frontal, parietal and insular cortex, mostly in the right hemisphere (Table 3, Fig. 2 green spheres). There was significantly greater BOLD activation in the RSs compared with the NRSs in all regions (Fig 4 & Supporting Information Fig. S4). We examined whether the larger activation in RSs was due to greater cognitive demands and found no significant group differences (Supplemental Results). In addition, *post hoc* regression models showed that group differences remained significant after adjustment for BDI (square root transformed for normality), CO (log transformed), movement (log transformed) and lifetime marijuana use, suggesting that group differences in brain activation were not explained by group differences in depressive symptoms, movement, smoking recency or lifetime marijuana use.

No regions were identified from the Condition  $\times$  Group  $\times$  Timepoint interaction, suggesting lack of a differential effect of processing of reward or punishment between RSs and NRSs.

Because of heterogeneity in smoking behavior among RSs, we examined whether within-pair activation



**Figure 4** Representative time-courses from left (top) and right (bottom) middle frontal gyrus (circled) with an effect of smoking exposure. The green color of the regions overlaid on the brain surface denotes regions with an effect of smoking exposure as shown in Fig. 2. See Fig. 1 legend for further details. There was no significant effect of winning or losing money in these regions as evidenced by the overlap of the time-courses in response to reward, punishment and neutral conditions in both groups

differences in the Group  $\times$  Timepoint regions were driven by current smokers or smokers with history of tobacco dependence. We compared average peak percent BOLD change (4 and 6 seconds after stimulus onset) between current ( $n = 9$ ) and former smokers ( $n = 6$ ) and between smokers with tobacco dependence history ( $n = 7$ ) and without such history ( $n = 8$ ) using one-way ANOVA (Supporting Information Table S5). Activation was overall very similar across subgroups of smokers, with the exception of significantly larger activation in the right angular gyrus of smokers with history of tobacco dependence compared with smokers without history of dependence.

## DISCUSSION

The aim of the present study was to investigate the effect of lifetime regular smoking on task-evoked brain activation using a reward and punishment guessing task and a study design that controls for genetic and family background factors on brain activation.

The modified number-guessing task evoked a pattern of reward-related brain activation consistent with the results of previous studies (Delgado *et al.* 2000, 2003; Knutson *et al.* 2001) lending validity to the implemented modifications. The time-course of activation in the caudate, with a later peak and more sustained response to reward than to punishment, is almost identical to the previously reported activation in this region (Delgado *et al.* 2000, 2003). Caudate activation in response to reward delivery is most frequently seen when subjects believe that their behavior affects reward delivery (Tricomi, Delgado & Fiez 2004), suggesting that the caudate may be involved in the learning of stimulus–outcome associations and thus in guiding future reward-related behavior and habit.

Previous studies have consistently shown blunted reward-related activation in the ventral striatum in smokers compared with controls using a priori hypothesis-driven ROI analysis or qualitative group comparisons (Martin-Solch *et al.* 2001, 2003; Buhler *et al.* 2010; van Hell *et al.* 2010; Luo *et al.* 2011; Peters *et al.* 2011). Consistent with the literature, our ROI analysis in ventral striatum showed attenuated response to reward and punishment in the RSs compared with the NRSs and this effect was significant in the right hemisphere. Peak response to reward in both left and right ventral striatum of RSs was the same as response to the neutral feedback, suggesting no effect of reward *per se*. However, while we do see an effect of regular smoking on reward processing in the ventral striatum consistent with the literature, it was only detected in the a priori ROI analysis and not the whole-brain analysis. Blunted activation to reward and punishment in the RSs is consistent with a direct effect of smoking on ventral striatum activation, rather than an effect of preexisting genetic factors, which are controlled for in the MZ twin pair design used in the current study. It could be that the ventral striatum is a region that is particularly sensitive to cigarette exposure, which could help explain group differences in adolescent smokers with very low lifetime exposure to cigarettes (Peters *et al.* 2011). Further, it could be that the effect of cigarette exposure on ventral striatum activity varies as a function of predisposing risk for smoking and we cannot make such a distinction with the available data.

Whole-brain analysis did not identify regions with an effect of smoking exposure on reward processing. It could be that heavy levels of smoking are necessary to robustly disrupt reward processing in the brain and the RSs in our sample were overall light smokers, though 46.7% ( $n = 7$  of 15) had a history of DSM-IV tobacco dependence. Some studies that show an effect of smoking on reward processing have included heavy smokers (Martin-Solch *et al.* 2001, 2003; Luo *et al.*



2011), but others have included a heterogeneous group of light to heavy smokers (van Hell *et al.* 2010). Thus, heavier levels of smoking do not seem necessary for blunted reward activation to be detected. However, these previous studies did not control for genetic influences on reward processing.

The group differences found in the frontal/parietal cortex were unexpected. Greater activation in these task control regions (Dosenbach *et al.* 2006, 2007, 2008) in the smokers could mean that smokers relied on greater cognitive effort than did non-smokers to perform the number guessing task. However, reaction time and button switching response data from the present study do not support any group difference in effort or demand. Others have also failed to demonstrate differences in cognitive effort in tasks designed to elucidate attentional bias to smoking cues (Luijten *et al.* 2011). Another interpretation is that group differences in frontal/parietal activation were driven by smokers with more extensive smoking history. Overall, current smokers or smokers with history of tobacco dependence had very similar peak BOLD response in these regions compared with former smokers or smokers with no history of tobacco dependence, respectively. Overall, these results support the epidemiological evidence that individuals who have smoked 100 or more cigarettes in their life, though with overall varying smoking histories, are different from those who have smoked less than 100 cigarettes. Nonetheless, these conclusions are tentative and made in the context of the small sample size in the subgroup analyses.

The findings of an effect of regular smoking in frontal/parietal regions are consistent with the interpretation that cigarette exposure is associated with greater reliance on frontal/parietal cortex to perform the task. The frontal/parietal regions are part of task control networks (Dosenbach *et al.* 2006, 2007, 2008) and a preponderance of regional activation in the right hemisphere has been associated with target detection at an attended location (Shulman *et al.* 2010). It could be that mechanisms of target detection reflect changes in attentional processing in smokers, which could be related to the general attention-enhancing properties of nicotine (Heishman, Kleykamp & Singleton 2010). Results in the frontal/parietal control regions could provide insights into methods for smoking cessation treatment, such as cognitive behavioral therapy which can alter processing in frontal/parietal and insular cortex regions (Huyser *et al.* 2010). A clinical trial of nicotine replacement therapy showed that smokers who did not maintain abstinence, compared with those who did, had relatively greater activation to smoking cues in many frontal/parietal regions prior to cessation (Janes *et al.* 2010), implicating control mechanisms in the maintenance of smoking behavior.

Further, greater activation of anterior and middle right insulae in regular compared with NRSs supports previous findings of the importance of the right insula in craving and tobacco addiction (Gray & Critchley 2007; Naqvi *et al.* 2007).

This study has several limitations. The RSs are a heterogeneous group of smokers comprising current and former smokers, as well as dependent and non-dependent smokers. It is possible that differences in exposure to cigarettes as a function of longer duration of smoking or as a function of heavier levels of smoking could have a different effect on brain reward processing than that shown in this paper. It is notable, though, that in frontal/parietal regions that showed group differences, peak BOLD activation was in large part similar between subgroups of smokers. There are individual differences in the past 12-month use of prescription medication. On the one hand, prescription medication could be a confounder in the study, but on the other hand, it provides a better representation of the general population. Larger samples are necessary to evaluate the impact of differences in smoking history and medication use on brain function. Finally, the number-guessing task does not distinguish anticipatory versus receipt phases of monetary reward. Reward-processing regions activated by the number-guessing task overlap with regions that activate both in response to anticipation and receipt of monetary reward (Knutson *et al.* 2001). It is possible that we may see more robust effects of smoking on reward processing using tasks that explicitly distinguish between the anticipation and receipt phases of reward processing. To the extent that there are dissociable mechanisms of anticipation versus receipt of monetary reward, both mechanisms are likely involved in the number-guessing task.

In conclusion, using a reward and punishment guessing task and a co-twin control study of smoking behavior that maximally controls for between-group differences on many potential confounding factors, we identified an effect of regular smoking on reward processing in a priori ROIs in the ventral striatum. In addition, we identified a set of frontal and parietal regions that showed larger activation in the RSs and no effect of reward or punishment. Considering that regular smoking is significantly heritable (Agrawal *et al.* 2005), RSs from MZ twin pairs discordant for smoking represent an unusual group of smokers, as they are at overall low genetic risk for smoking by virtue of having a MZ co-twin who is not an RS, yet they escalate in their smoking behavior to a point of dependence in some cases. The uniqueness of this group of smokers, and their apparent differential activation in attention and control regions of the cortex, could provide important insights into brain mechanisms involved in the development of tobacco addiction.

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## Authors Contribution

CNLS, PAFM and ACH were responsible for the study concept and design. PAFM and ACH developed the smoking assessments in the parent twin study. ACH is the principal investigator of the parent twin study. RLL, BLS and SEP contributed to the acquisition of neuro-imaging data. CNLS and RLL performed all analyses. SDK, BLS, KAB, SEP and DMB assisted with data analysis and interpretation of findings. CNLS wrote the manuscript. RLL, SDK, BLS, KAB, SEP, PAFM, ACH and DMB provided critical revision of the manuscript for important intellectual content. All authors approved the final version for publication. All authors have critically reviewed content and approved final version submitted for publication.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Number-guessing task

**Figure S2** Time-courses from regions extracted from the Condition  $\times$  Timepoint interaction effect of the whole-brain ANOVA.

**Figure S3** Time-courses from regions extracted from the Condition  $\times$  Timepoint interaction effect of the whole-brain ANOVA.

**Figure S4** Time-courses from regions extracted from the Group  $\times$  Timepoint interaction effect of the whole-brain ANOVA.

**Table S1** Comparison of diagnostic interview measures between never-regular and regular smokers in the entire twin cohort from which MZ twins for neuro-imaging were recruited

**Table S2** Smoking behavior from questionnaire data in 15 MZ twin pairs discordant for regular smoking

**Table S3** Diagnostic interview measures in the 15 MZ twin pairs discordant for regular smoking

**Table S4** Statistical significance and effect size estimations for pairwise comparisons of ventral striatum activation

**Table S5** Comparison of average peak percent BOLD change response (4 and 6 seconds post-stimulus) between current and former smokers and between lifetime dependent and non-dependent smokers