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Under pressure: adolescent substance users show exaggerated neural processing of aversive interoceptive stimuli

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ABSTRACT

Aims Adolescents with substance use disorders (SUD) exhibit hyposensitivity to pleasant internally generated (interoceptive) stimuli and hypersensitivity to external rewarding stimuli. It is unclear whether similar patterns exist for aversive interoceptive stimuli. We compared activation in the insular cortex and other brain regions during the anticipation and experience of aversive stimuli between adolescents with SUD and those without. **Design** Cross-sectional experimental study with two groups. **Participants** Adolescents (ages 15–17 years) with an alcohol or marijuana SUD ($n = 18$) and healthy comparison subjects (CON, $n = 15$). Participants were recruited by distributing flyers at local high schools. **Setting** Keck Imaging Center, University of California San Diego, CA, USA. **Measurements** Behavioral and neural responses to a continuous performance task with inspiratory breathing load recorded during an fMRI session. Questionnaires assessed life-time drug use, anxiety, sensation-seeking, impulsivity, affect and bodily awareness. Visual analog scales assessed drug craving and breathing load responses. **Findings** Across subjects, experience of breathing load elicited greater bilateral anterior and posterior insula (AI and PI, respectively) activation than anticipation ($F_{(1,31)} = 4.16$, $P < 0.05$). SUD exhibited greater left AI and bilateral PI activation during breathing load than anticipation, compared with CON ($F_{(1,31)} = 4.16$, $P < 0.05$). In contrast, CON showed greater activation during anticipation than breathing load in left PI, compared with SUD ($F_{(1,31)} = 4.16$, $P < 0.05$). **Conclusions** Adolescents with alcohol and marijuana substance use disorders may be hypersensitive to aversive interoceptive stimuli.

Keywords Adolescence, alcohol, breathing load, cannabis, fMRI, interoception.

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INTRODUCTION

Adolescence is an important time for exploring and risk-taking, which can involve experimentation with drugs. Among US 12th-graders, 68% have consumed alcohol and 46% have tried marijuana [1]. Given that substance use during adolescence increases risk for adulthood addiction [2–5], investigating potential risk markers for addiction may help to develop treatment targets and early interventions aimed at changing processing of visceral bodily sensations; for example, by mindfulness meditation [6].

Interoception [7,8], the processing of internal bodily sensations, is thought to moderate impaired decision processes that may drive addiction [9–13]. Adolescents may use substances to either amplify or dampen the influence of bodily sensations on decision-making. For example, an adolescent feeling nervous about attending a party may drink alcohol to reduce bodily sensations of anxiety. One characteristic of addiction is an impaired interoceptive system, causing inaccurate registration or correction of bodily sensations. Interoception is important for homeostasis [7,8], driving approach or avoidance of stimuli and allocating resources to regain perceived equilibrium [14]. One's predicted versus actual internal state (i.e. body prediction error [11]) may motivate the individual to take substances to feel better or to avoid withdrawal [9].

Insular cortex is a critical neural substrate for interoception and addiction [13,15–17]. Individuals with nicotine, marijuana and cocaine use disorders show increased insular reactivity to drug-related cues [18–20]. Research demonstrates that: (a) alcohol-dependent teenagers exhibit insular hyperactivity to alcohol cues [21]; and (b) insular alcohol cue response predicts increased drinking and alcohol-related problems in college students [22]. In contrast, studies involving non-drug cues reveal that lower insula activation is linked to adolescent alcohol/nicotine substance use disorders (SUD) within the context of decision-making tasks and/or non-drug rewards [23–25]. Taken together, individuals with SUD appear to exhibit insular hypersensitivity to substance-related stimuli, but hyposensitivity in other contexts.

Interoception research indicates that hedonic aspects of drinking moderate left insula white matter volume and frequency of binge drinking in adolescents [26]. Further, cigarette-smoking teenagers show an exposure-dependent decrease in right insular cortical thickness [27]. Adults with SUD exhibit anterior insula (AI) attenuation during anticipation and experience of a soft brush stroke to the palm/forearm as a pleasant interoceptive manipulation [28]. In contrast, adolescents with SUD exhibit AI hyperactivity during soft touch, suggesting that neural patterns of interoceptive responsiveness might differ in early versus chronic stages of SUD [29]. Although hedonic aspects of interoception have been investigated with cued reward paradigms, less is known about links between aversive interoception and SUD in adolescents. Investigating aversive stimuli is crucial, considering discomfort experienced by addicted users during withdrawal, which might trigger future use.

An inspiratory breathing load paradigm [30–32] was used to investigate aversive interoception in adults with SUD and showed lower insular and/or anterior cingulate cortex (ACC) activation during breathing load than controls [30,31], suggesting that the aversive interoceptive system is hyporesponsive. Given that adolescents exhibit exaggerated brain activation for pleasant stimuli (decreased insula activation for adults [28] versus increased activation for adolescents [29]), adolescents with SUD may show a hyper-reactive interoceptive response to aversive stimuli. In support of this assertion, adolescents at high risk for alcohol problems are more sensitive to negative stimuli and more motivated to drink alcohol in negative emotion-arousing situations than low-risk adolescents [33].

Another aspect of aversive stimuli is anticipation of negative events, where predictability can lessen psychological impact. Inability to anticipate an aversive stimulus could reflect an inhibitory control deficit linked to SUD development [34]. Studies show that adolescents who: (a) fail to anticipate aversive events are more likely to be alcohol- and nicotine-dependent [34] and (b) do not take advantage of the predictability of aversive stimuli (considered poor modulators) have more SUDs than good modulators [35]. Lack of cognitive control in adolescent SUD can be explained by a less mature cognitive control system and a hyperactive reward system [36–38]. This dual-process model [39] suggests that top–down cognitive control limitations result in reduced ability to regulate bottom-up urges.

To examine whether SUD adolescents possess a dysfunctional aversive interoceptive system, the present investigation used functional magnetic resonance imaging (fMRI) to examine insula activation in adolescents with current alcohol or marijuana SUD. It was hypothesized that: (a) SUD adolescents would show greater insular activation during the experience of breathing load than healthy controls and (b) SUD would exhibit greater ACC and prefrontal cortex activation than controls, as insula integrates activity from ACC and dorsolateral pre-frontal cortex (DLPFC [7,8]), both of which play a role in aversive interoceptive processing in healthy individuals [40] and adults with SUD [30].

METHODS

Participants

The University of California San Diego Human Research Protections Program approved this study. Written informed consent was provided by one parent or legal guardian of the adolescent participant, who provided assent. Adolescents (aged 15–17 years) were recruited by distributing flyers at local high schools. Adolescents were screened by telephone to rule out: life-time Diagnostic and Statistical Manual of Mental Disorders [41] Axis I psychiatric disorder independent of SUD; current use of psychoactive medications; history of major neurological or medical disorder, head trauma with loss of consciousness > 5 minutes, learning disability, serious physical health problems; complicated or premature birth; fMRI contraindications (e.g. irremovable metal); left handedness, non-correctable vision or hearing problems and prenatal alcohol/drug exposure. Participants were compensated $180 for their participation.

Fifteen SUD (nine male, six female) and 18 healthy controls (CON; eleven male, seven female) participated in this study. SUD were defined by: (a) current endorsement of $\geq$2 DSM-5 [41] SUD criteria for either alcohol or marijuana and (b) alcohol or marijuana use within the last 3 months. The primary diagnosis for 27% of SUD was alcohol use disorder; the other 73% endorsed marijuana use disorder. The number of SUD criteria endorsed ranged from 2–8 [mean = 3.4; standard deviation (SD) = 2.0]. CON had very limited life-time alcohol/marijuana uses and no other illicit drug use (see Table 1). Although most research has focused

Table 1 Subject characteristics by group.

	CON n = 18		*SUD n = 15*				
Characteristics	*Mean*	*SD*	*Mean*	*SD*	*df*	t/χ^2	*P*
Age	16.50	0.62	16.60	0.63	31	−0.92	0.65
Education	10.67	0.59	10.67	0.90	31	0.00	1.00
BSMSS	48.5[a]	12.12	51.18[b]	11.14	29	−0.64	0.53
Estimated verbal IQ	111.22	13.98	110.40	16.58	31	0.16	0.88
YSR internalizing *t*-score	45.00	10.36	53.27	10.90	31	−2.23	**0.03**
YSR externalizing *t*-score	44.94	10.22	58.33	9.20	31	−3.92	**0.00**
Demographics	*n*	%	*n*	%			
Female	7	38.90	5	33.3	1	0.11	0.74
Caucasian	15	83.33	12	80	1	0.06	0.81
Hispanic	3	16.70	3	20	1	0.06	0.81
Questionnaires	*Mean*	*SD*	*Mean*	*SD*			
Barratt Impulsivity Scale	58.00[c]	12.02	60.64[b]	9.14	28	−0.67	0.51
Sensation Seeking Scale	14.72	3.79	25.14[b]	6.05	30	−5.97	**0.00**
Body Awareness Questionnaire	81.17	20.22	79.13	14.19	31	0.33	0.75
STAI-T Trait	33.44	9.82	39.27	11.44	31	−1.57	0.13
PANAS positive	27.67	7.97	27.67	6.04	31	0.00	1.00
PANAS-negative	12.50	3.99	13.73	4.74	31	−0.81	0.42
% *Drug use*	*n*	%	*n*	%			
% Used alcohol in life-time	6	33.3	15	100	1	15.71	**0.00**
% Used alcohol in past week	1	5.6	10	66.7	1	13.75	**0.00**
% Used marijuana in life-time	2	11.1	15	100	1	25.88	**0.00**
% Used marijuana in past week	0	0	10	66.7	1	17.22	**0.00**
% Used other drugs in life-time	0	0	11	73.3	1	19.8	**0.00**
% Used other drugs in past week	0	0	0	0.0	[d]	[d]	[d]
Life-time drug use	Mean	SD	Mean	SD	*U*	Z	*P*
Life-time alcohol use	2.44	5.26	108.33	105.37	0.00	−0.52	**0.00**
Life-time marijuana use	0.28	0.96	351.87	284.49	4.50	−4.84	**0.00**
Life-time other drug use	0	0.00	45.20	75.02	36.00	−4.27	**0.00**

[a]*n* = 17. [b]*n* = 14. [c]*n* = 16. [d] No statistics because 'other drugs used in past week' is a constant. CON = control group; SUD = Substance Use Disorder group; BSMSS = Barratt Simplified Measure of Social Status; YSR = Youth Self-Report; STAI-T = State–Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule; SD = standard deviation; *U* = Mann–Whitney *U*-test result.

on the effects of stimulants on the insula, alcohol and marijuana users are included in this study, as methamphetamine use is not as prevalent among 12th-graders [1]. Our sample was considered sufficient to detect a medium effect size, using a similar study with an experimental task in SUD and CON adolescents as an estimate [29]. Participants completed a clinical assessment session, which included self-report questionnaires, and abstained from substance use at least 72 hours prior to their fMRI session. Abstinence was confirmed by self-report, urine toxicology and breathalyzer screens.

Clinical interview session

The Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA [42]) was used to assess life-time DSM-5 substance use history and SUD diagnoses, which were confirmed by a psychiatrist (M.P.P.) and psychologist (S.F.T.). The time-line follow-back (TLFB [43]), a retrospective calendar-based measure, was used to assess substance use for the past 30 days.

Questionnaires were administered to assess characteristics known to correlate with SUD, including the Sensation Seeking Scale (SSS-V [44]), Barratt Impulsiveness Scale (BIS-11 [45]) and Youth Self Report (YSR [46]). Barratt Simplified Measure of Social Status (BSMSS [47]) was completed using parental responses to measure socio-economic status (SES). The Positive and Negative Affect Schedule (PANAS [48]) was used to measure affect, and trait anxiety with the State–Trait Anxiety Inventory (STAI-T [49]). Verbal IQ was measured with the Wide Range Achievement Test 4 (WRAT4 [50]). The Body Awareness Questionnaire (BAQ [51]) measured attentiveness to non-emotive bodily processes. Substance craving was measured with a 10-

cm visual analog scale (VAS) ranging from 'no craving' to 'most craving ever experienced'.

fMRI session: aversive inspiratory breathing load paradigm

This continuous performance task (CPT) requires participants to anticipate and experience an aversive interoceptive stimulus (see Fig. 1). Subjects wore a nose clip and breathed through a mouthpiece with a non-re-breathing valve (2600 series; Hans Rudolph, Inc., Shawnee, KS, USA). Breathing equipment was attached to the scanner head coil eliminating the need for subjects to contract mouth muscles. A hose connected the mouthpiece to the inspiratory resistance load of 40 $cmH_2O/l/sec$, which consisted of a sintered bronze disk in a Plexiglas tube. This disk partially limits the airflow through the breathing tube, producing the resistance load. Padding between the coil and headphones was used to keep the subjects' heads snug in the coil to minimize movement.

Prior to scanning, participants were given task instructions, experienced 60-sec segments of various breathing loads (no load, 10, 20 and 40 $cmH_2O/l/sec$), and then completed VAS ratings of breathing load on a 10 cm scale, from 'not at all' to 'extremely' on 16 dimensions (see Table 2 [32,52]). These loads were used prior to the scan to investigate whether increased load altered VAS and familiarize participants with equipment. After the scan, participants were asked to complete VAS ratings for breathing load experienced during the CPT.

Participants performed the CPT with intermittent inspiratory breathing load during fMRI recording. Subjects were asked to respond quickly and accurately to the orientation (left or right) of the arrow with the button box. Briefly, background color served as a breathing load cue. Blue indicated no breathing load, and a yellow background indicated a one in four (25%) chance of experiencing the load (see Fig. 1). Subjects experienced three conditions: (1) baseline: task performed with a blue background; (2) anticipation: a yellow background indicated a 25% chance of a breathing load; and (3) breathing load: participant experiences 40 sec of resistive loaded breathing. Both response accuracy and reaction time (RT) were obtained. CO_2 levels of respiration were recorded via nasal cannula at a rate of 40 Hz. Event-related fMRI data collection parameters are explained in detail elsewhere [31].

Table 2 Visual Analogue Scale (VAS) scores for breathing load.

	CON n = 18		*SUD n = 15*				
VAS item	*Mean*	*SD*	*Mean*	*SD*	*d.f.*	*t*	*P*
Pleasant	2.16	1.72	1.16	1.13	30	2.01	**0.05**
Unpleasant	5.54	2.97	5.86	3.01	30	−0.29	0.77
Intense	3.62	3.11	4.36	2.62	30	−0.71	0.48
Tingling sensations	1.93	2.83	1.44	1.98	30	0.56	0.58
Fear of losing control	1.21	1.93	1.51	2.18	30	−0.42	0.68
Faintness	1.53	2.32	0.99	1.71	30	0.73	0.47
Fear of dying	0.37	1.10	0.54	1.83	30	−0.33	0.75
Unreality	0.94	1.43	0.67	1.88	30	0.47	0.64
Hot/cold flushes	0.66	1.35	0.24	0.46	22	1.21	0.24
Trembling	0.42	1.12	0.91	1.82	30	−0.93	0.36
Choking	0.46	1.13	0.89	2.08	30	−0.75	0.46
Fear of going crazy	0.41	1.12	0.49	1.56	30	−0.18	0.86
Abdominal distress	0.75	1.82	0.71	1.68	30	0.07	0.95
Chest pain	0.61	1.35	0.81	1.88	30	−0.36	0.73
Palpitations	0.48	1.22	0.71	1.78	30	0.42	0.68
Sweating	0.36	1.16	0.11	0.23	30	0.80	0.43
Dizziness	1.16	1.89	1.19	1.83	30	−0.05	0.96

VAS = Visual Analogue Scale; CON = control group; SUD = substance use disorder group; SD = standard deviation; the variable hot/cold flushes had unequal variances.

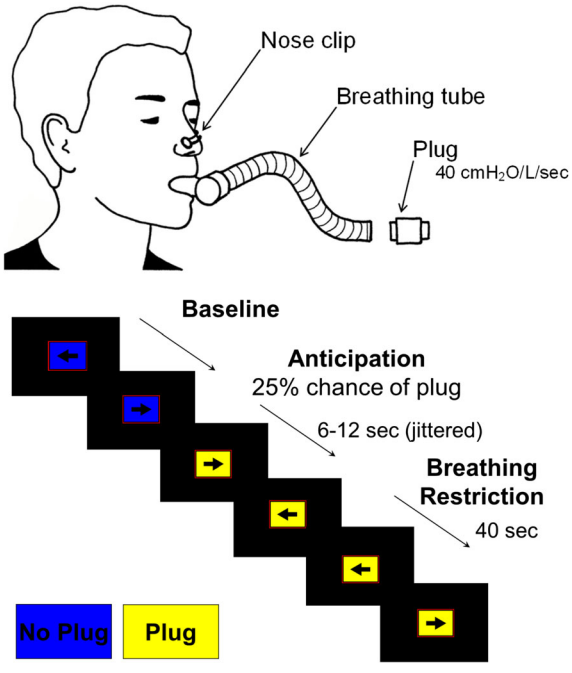

Figure 1 Inspiratory breathing load

fMRI data analysis

Single-subject image analysis pathway

fMRI data were pre-processed with the Analysis of Functional NeuroImages (AFNI) software package (Cox, 1996). GE slices were reconstructed into AFNI BRIK format. A temporal region containing the largest span with fewest voxel-wise outliers was used as a three-dimensional (3D) registration baseline. Six motion parameters (dx, dy, dz, roll, pitch, yaw) were obtained across the time–series.

Data were checked visually to ensure repetitions with movement were removed. Motion parameters were used as regressors to adjust EPI intensity changes due to motion artifacts. The functional EPI underwent automatic co-registration to the high-resolution anatomical image and was inspected visually to confirm successful alignment. New outliers were generated based on whether a given time-point greatly exceeded the mean number of voxel outliers for the time–series. Deconvolution was performed to determine breathing load task decision phase activations. Six movement regressors, a baseline/linear drift regressor, and two decision-making regressors (anticipation, breathing load) were convolved with a modified hemodynamic response function [53–55]. The baseline task condition served as the baseline for this analysis. A Gaussian spatial filter [4 mm full width at half maximum (FWHM)] was used to blur data to account for anatomical differences. Automated Talairach transformations were applied to anatomical images and EPIs were transformed subsequently into Talairach space. Percentage signal change (PSC) was determined by dividing the signal for each regressor of interest (anticipation, breathing load) by the baseline regressor and multiplying by 100. Five participants were excluded because of excessive movement during fMRI ($n = 2$), anatomical abnormality ($n = 1$), or fMRI acquisition errors ($n = 2$), leaving a total of 33 participants.

Group-level analyses

For each voxel, a linear mixed effects (LME) model was computed in R [56], using maximum likelihood estimation (MLE) to examine group differences in brain activation. Group (CON, SUD) and condition (anticipation, breathing load) were modeled as fixed factors, and subject was a random factor. The dependent measure was PSC. A threshold adjustment method based on Monte-Carlo simulations was used to guard against identifying false positive areas of activation. Based on AFNI AlphaSim, a voxel-wise $P < 0.01$ was associated with a 768 µl volume threshold (whole-brain mask; 12 contiguous voxels) for a clusterwise probability of $P < 0.05$ (two-sided). To examine a priori predictions, masks for bilateral insula and ACC, defined by the Talairach atlas [57], were employed with a minimum cluster volume of 384 µl (six contiguous voxels) for $P < 0.05$ (two-sided) corrected for multiple comparisons. Anticipation and breathing load activation were both initially compared to baseline activity. The group main effect is the difference between CON and SUD across all conditions. The condition main effect examines anticipation versus breathing load. The group × condition interaction examines between-group differences in anticipation and breathing load, and within-group differences between anticipation and breathing load.

Brain–behavior relationships

PSC (for brain regions with a significant group × condition interaction) were correlated within-group with life-time drug use, drug craving, STAI-T, BAQ, PANAS, BIS, SSS-V, YSR and VAS scores. These nine variables were correlated with six brain regions using SPSS version 22.0 (IBM Corp., Armonk, NY, USA), resulting in 51 comparisons. No Bonferroni corrections were applied, as correlations were considered exploratory.

Behavioral data analysis

SPSS version 22.0 (IBM Corp.) was used to analyze reaction time (RT) and accuracy using a repeated-measures analysis of variance (ANOVA) to examine within-subject differences as a function of condition (with baseline subtracted) and between-subject group differences. Pre- and post-VAS breathing ratings were examined using independent-samples *t*-tests between groups. CO_2 levels during anticipation and breathing load (with baseline subtracted) and between-subject group differences were examined using a repeated-measures ANOVA.

RESULTS

Subject characteristics

Demographics

Groups did not differ significantly in age, gender, education, verbal IQ or ethnicity. SUD endorsed higher sensation-seeking, internalizing and externalizing scores than CON, but groups did not differ on impulsivity (see Table 1). SUD reported greater life-time substance use than CON (see Table 1). Pre-scan VAS ratings showed that, across participants, responses were affected significantly by breathing load for pleasantness ($F_{(2,64)} = 5.8$, $P < 0.01$), unpleasantness ($F_{(1.62,\ 51.84)} = 5.01$, $P < 0.02$) and intensity ($F_{(2,64)} = 4.36$, $P < 0.02$). SUD had more life-time cigarette use [mean = 164.43, standard error (SE) = 73.76] than CON (mean = 0.17, SE = 0.17; $t_{(13)}$, $P = 0.04$). Multiple regressions predicting brain activation during breathing load within SUD using alcohol, marijuana and cigarette use as predictors found no significant effect of cigarette use on blood–oxygen-level dependent (BOLD) response. For post-scan VAS ratings, SUD rated breathing load as less pleasant (mean = 1.16, SD = 1.13) than CON (mean = 2.16, SD = 1.72; $t_{(30)}$, $P = 0.05$; see Table 2).

Behavioral responses

RT was similar for anticipation (mean = 645.98, SE = 21.84) and breathing load (mean = 630.63, SE = 29.00) for all participants ($F_{(1,24)} = 0.81$, $P = 0.38$) and did not differ

between groups for both anticipation ($mean_{CON} = 645.62$, $SE_{CON} = 32.92$; $mean_{SUD} = 646.47$, $SE_{SUD} = 27.35$) and breathing load ($mean_{CON} = 627.06$, $SE_{CON} = 44.58$; $mean_{SUD} = 634.02$, $SE_{SUD} = 31.29$), $F_{(1,24)} = 1.84$, $P = 0.19$. No significant interaction between group and condition emerged ($F_{(1,24)} = 0.05$, $P = 0.82$). Groups were equally accurate (> 97% correct on average) ($F_{(1,25)} = 0.003$, $P = 0.97$). There was a trend for both groups to be less accurate during anticipation (98.9%) than breathing load (99.4%; $F_{(1,25)} = 4.24$, $P = 0.05$). There was no group × condition interaction effect ($F_{(1,25)} = 0.20$, $P = 0.67$).

CO_2 levels

CO_2 output did not differ between groups ($F_{(1,24)} = 0.29$, $P = 0.64$); however, CO_2 was lower during breathing load than anticipation across participants ($F_{(1,24)} = 19.29$, $P < 0.01$). No group by condition interaction effect emerged ($F_{(1,24)} = 0.23$, $P = 0.64$), replicating prior work using this breathing paradigm [30,31,58]. CO_2 was not correlated significantly with breathing load PSC in any brain region from group LME analyses.

BOLD response contrast

Condition and group main effects

Across subjects, the region of interest (ROI) analysis showed that loaded breathing elicited greater bilateral AI and PI activation than anticipation ($F_{(1,31)} = 4.16$, $P < .05$; see Fig. 2). Table 3 indicates results from both the ROI and whole brain analyses that loaded breathing induced a large change in BOLD response affecting several areas of the brain ($F_{(1,31)} = 4.16$, $P < 0.05$). Furthermore, the whole brain analysis showed that CON exhibited greater pre-central gyrus activation than SUD across conditions ($F_{(1,31)} = 4.16$, $P < 0.05$; see Table 3). There was no group main effect for the insula or ACC.

Group × condition interaction

The whole brain analysis showed that groups differed by condition in several regions. Generally, CON showed greater activation during anticipation than SUD, whereas during breathing load SUD showed greater activation than CON (see Table 4). During anticipation, CON exhibited greater right PI activation than SUD. During breathing load, SUD showed greater activation than CON in right PI, left parahippocampal gyrus and left superior temporal gyrus. Moreover, the ROI analysis showed that CON exhibited greater left PI activation during anticipation than SUD.

Within-group differences indicate that SUD showed greater activation during breathing load than anticipation in bilateral PI, left AI, left middle frontal gyrus and right inferior frontal gyrus (see Figs 3 and 4). CON showed the opposite pattern, with greater activation during anticipation than breathing load in left PI (see Fig. 3).

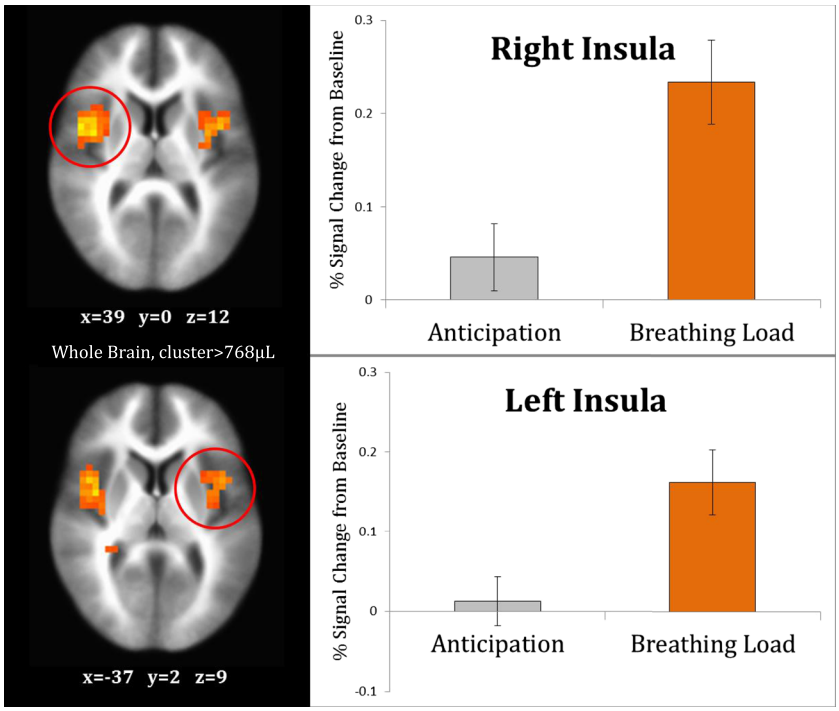

Figure 2 The main task effect showed greater bilateral insula activation during breathing load than anticipation across participants ($F_{(1,31)} = 4.16$, $P < 0.05$). Error bars reflect ± 1 standard error

Table 3 Task effect: functional magnetic resonance imaging (fMRI) results for main effect of condition and main effect of group.

		Main effect of condition							
Mask	*Voxels*	*Volume (μl)*	*X*	*Y*	*Z*	*L / R*	*BA*	*Center of mass*	*Result*
Whole brain	602	38 528	−47	−7	26	L		Precentral gyrus	Plug>Ant
Whole brain	585	37 440	49	−8	26	R		Precentral gyrus	Plug>Ant
Whole brain	417	26 688	−10	−70	−17	L		Declive	Plug>Ant
Whole brain	363	23 232	1	−17	58	R		Medial frontal gyrus	Plug>Ant
Whole brain	312	19 968	29	−42	13	R		Caudate	Ant>Plug
rROI	118	7552	39	0	12	R		Anterior and posterior insula	Plug>Ant
rROI	89	5696	−37	2	9	L		Anterior and posterior insula	Plug>Ant
Whole brain	85	5440	−1	−14	−26	L		Brain	Ant>Plug
Whole brain	76	4864	−41	−25	−9	L		Parahippocampal gyrus	Ant>Plug
Whole brain	49	3136	−26	−46	9	L		Parahippocampal gyrus	Plug>Ant
Whole brain	32	2048	17	5	−2	R		Lentiform nucleus	Plug>Ant
Whole brain	27	1728	−5	−50	58	L		Precuneus	Plug>Ant
Whole brain	19	1216	−34	37	36	L		Middle frontal gyrus	Plug>Ant
Whole brain	19	1216	−9	−12	43	L		Paracentral lobule	Plug>Ant
Whole brain	17	1088	−7	61	−2	L		Superior frontal gyrus	Plug>Ant
Whole brain	16	1024	48	−48	21	R		Superior temporal gyrus	Plug>Ant
Whole brain	14	896	−21	0	38	L		Cingulate gyrus	Ant>Plug
Whole brain	13	832	−56	−45	−12	L		Middle temporal gyrus	Plug>Ant
Whole brain	12	768	−31	52	−3	L		Middle frontal gyrus	Plug>Ant
Whole brain	12	768	−2	−95	2	L		Cuneus	Ant>Plug
Whole brain	12	768	−13	−17	9	L		Thalamus	Plug>Ant
		Main effect of group							
Whole brain	12	768	−27	−5	33	L		Precentral gyrus	CON>SUD

Plug = breathing load; Ant = anticipation; CON = control group; SUD = substance use disorder group; L = left hemisphere; R = right hemisphere; rROI = restricted region of interest (ROI) based on hypothesis; Talairach coordinates reflect center of mass. Regions reflect significant clusters of at least 12 contiguous voxels (whole brain; 768 μl) or six contiguous voxels (rROI; 384 μl) meeting the $F_{(1,31)} = 4.16$, $P < 0.05$ threshold corrected for multiple comparisons via AlphaSim.

Table 4 Functional magnetic resonance imaging (fMRI) results for interaction effect of group by condition.

Mask	*Voxels*	*Volume (μl)*	*X*	*Y*	*Z*	*L / R*	*Center of mass*	*BA*	*Ant*	*Plug*
Whole brain	36	2304	45	−7	17	R	Posterior insula	BA 13	CON>SUD	SUD>CON
Whole brain	26	1664	−33	43	25	L	Middle frontal gyrus	BA 10	CON = SUD	SUD>CON
Whole brain	22	1408	−24	−21	−22	L	Parahippocampal gyrus	BA 35	CON>SUD	CON = SUD
Whole brain	20	1280	−30	5	−27	L	Uncus	BA 38	CON = SUD	SUD>CON
Whole brain	15	960	−59	−36	−11	L	Middle temporal gyrus	BA 21	CON = SUD	SUD>CON
Whole brain	14	896	−31	24	10	L	Anterior insula	BA 13	CON = SUD	SUD>CON
Whole brain	13	832	−45	4	−7	L	Superior temporal gyrus	BA 38	CON>SUD	CON = SUD
Whole brain	13	832	41	25	10	R	Inferior frontal gyrus	BA 13	CON = SUD	SUD>CON
rROI	8	512	−45	4	−6	L	Posterior insula	BA 38	CON>SUD	CON = SUD

L = left hemisphere; R = right hemisphere; Ant = anticipation; Plug = breathing load; CON = control group; SUD = substance use disorder group; rROI = restricted region of interest (ROI) based on hypothesis; Talairach coordinates reflect center of mass; BA = Brodmann Area. Regions reflect significant clusters of at least 12 contiguous voxels (whole brain; 768 μl) or six contiguous voxels (rROI; 384 μl) meeting the $F_{(1,31)} = 4.16$, $P < 0.05$ threshold corrected for multiple comparisons via AlphaSim.

Brain–behavior relationships

To address greater brain activation during breathing load in SUD compared to (a) anticipation in SUD and (b) breathing load in CON, self-report and drug use measures were correlated within brain activation within SUD during breathing load. SUD with lower scores on the BAQ showed greater left middle frontal gyrus activation ($r = -0.62$, $P = 0.02$; see Fig. 5a) and right PI activation ($r = -0.58$, $P = 0.02$; see Fig. 5b) during breathing load.

DISCUSSION

This investigation examined aversive interoceptive processing in adolescents with SUD and yielded three main results. First, consistent with our prediction,

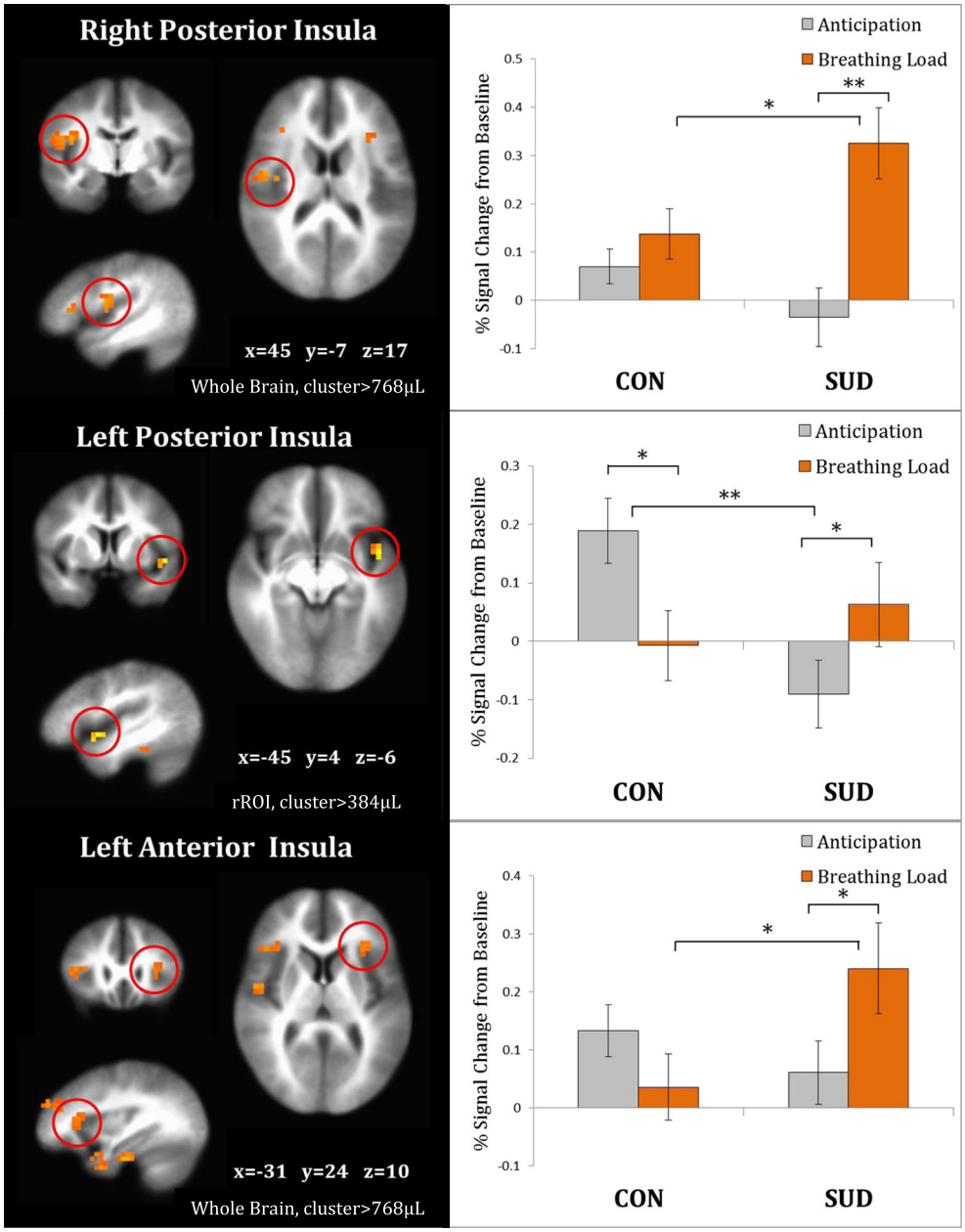

Figure 3 The group by condition interaction showed: (1) between-group differences, wherein adolescents with substance use disorder (SUD) exhibited greater right posterior insula and left anterior insula activation than healthy controls (CON) during breathing load; and (2) within-group differences, wherein SUD exhibited consistent modulation in bilateral posterior insula and left anterior insula as a function of anticipation and breathing load conditions, but CON did not. Error bars reflect ± 1 standard error: $^{*}P < 0.05$; $^{**}P < 0.01$

SUD showed greater insula activation during breathing load than CON. Secondly, SUD showed greater breathing-load activation than CON in left middle frontal gyrus and right inferior frontal gyrus. SUD, but not CON, exhibited consistent modulation in these frontal regions as a function of condition. Thirdly, SUD rated the breathing load as less pleasant than CON. Taken together, findings suggest that the insula of SUD adolescents is hypersensitive to aversive stimuli.

Our results are consistent with heightened sensitivity exhibited by adolescent SUD during pleasant stimuli processing [29]. Not only do adolescents generally show greater striatal sensitivity to valenced stimuli than adults [59], but adolescents with SUD demonstrate greater responses than CON to aversive stimuli in the present study. Thus, SUD adolescents may not be able to predict physiological bodily changes accurately. Differences seen between anticipation and breathing load in SUD might reflect a bodily prediction error. This is illustrated in the PI difference between anticipation and breathing load for SUD (see Fig. 3). SUD show a diminished anticipatory response in conjunction with an exaggerated response during

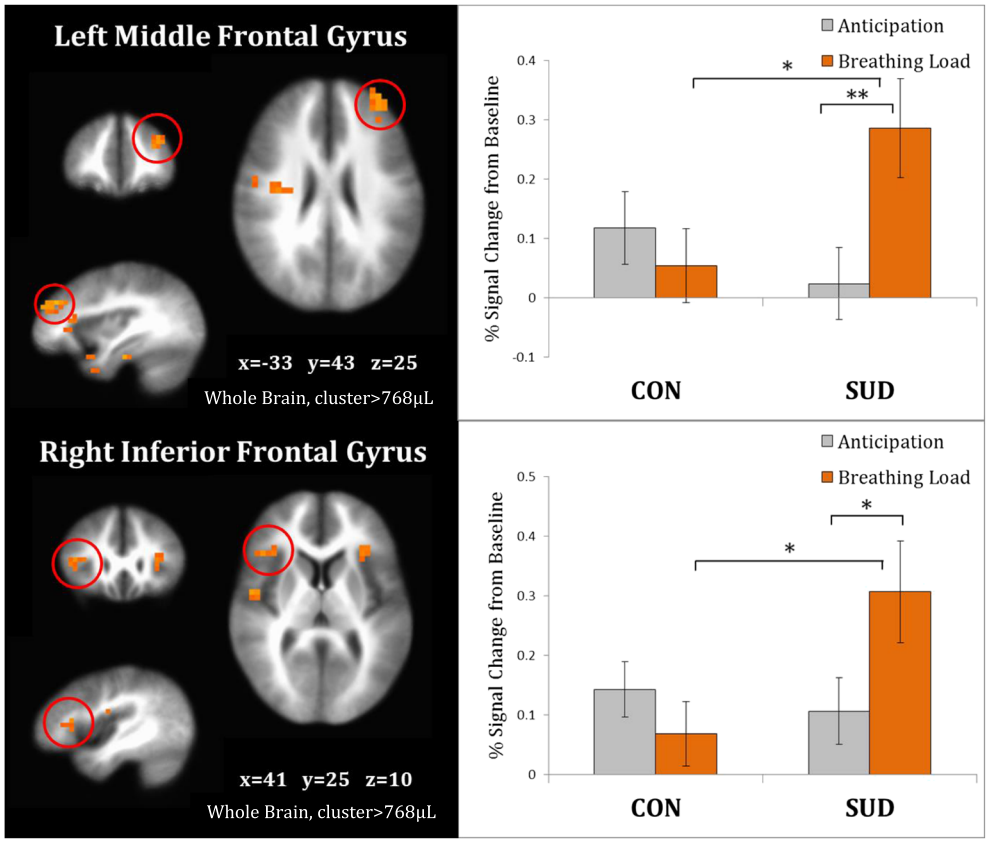

Figure 4 The group by condition interaction showed: (1) between-group differences, wherein adolescents with substance use disorder (SUD) exhibited greater left middle frontal gyrus and right inferior frontal gyrus than healthy controls (CON) during breathing load; and (2) within-group differences, wherein SUD exhibited consistent modulation in these regions as a function of anticipation and breathing load conditions, but CON did not. Error bars reflect ± 1 standard error. *$P < 0.05$; **$P < 0.01$

breathing load. Perhaps this discrepancy illustrates an inability to make use of the predictability of the aversive event, evident in prior research [34,35]. Addiction may represent a chronic imbalance of a homeostatic condition of the body, leading to maladaptive regulation of the internal state through substances [11]. Negative reinforcement mechanisms of drug seeking to avoid aversive consequences of withdrawal has been thought to be crucial in addiction development [60]. However, we did not find a relationship between drug craving and insula activation in SUD. Previous research shows that SUD exhibit increased insular cue reactivity to drug-related stimuli, thought to be linked to craving [18–20]. Craving in cigarette-smoking adolescents is correlated negatively with cortical thickness of right ventral AI [27]. Perhaps craving measurements should be completed during the CPT, while the participant is still in the scanner. Insula integrates information from ACC and DLPFC to facilitate goal representation and motivation [17]. The present study suggests that this integration may be disrupted in SUD, potentially resulting in craving, thereby over-ruling non-drug taking goals.

Insular cortex is a crucial component of a neural salience network involved in switching between the default mode network (involved with craving and withdrawal symptoms) and the executive control network (involved in decision-making) [61–63]. Increased insula activation in SUD might be related to the inability to disengage from the default mode network, and consequently the inability to switch to the executive control network for top–down control.

Contrary to our prediction, ACC did not show differential activation between groups. ACC activation has been found using the same aversive breathing task in SUD and CON adults, and is thought to monitor and inhibit reactions to aversive stimuli [30,31,40]. Perhaps ACC plays a different role in behavioral control for adolescents than for adults. For example, when making risky choices, adults exhibited greater ACC activation than adolescents [64]. Moreover, adults showed greater ACC activation than both adolescents and young adults when anticipating pleasant interoceptive stimuli [65]. However, youth at high risk for SUD show greater ACC, pre-frontal and AI activation than CON in response to risky decisions involving high potential for negative outcomes [66]. Thus, it is unclear what role ACC plays in adolescents.

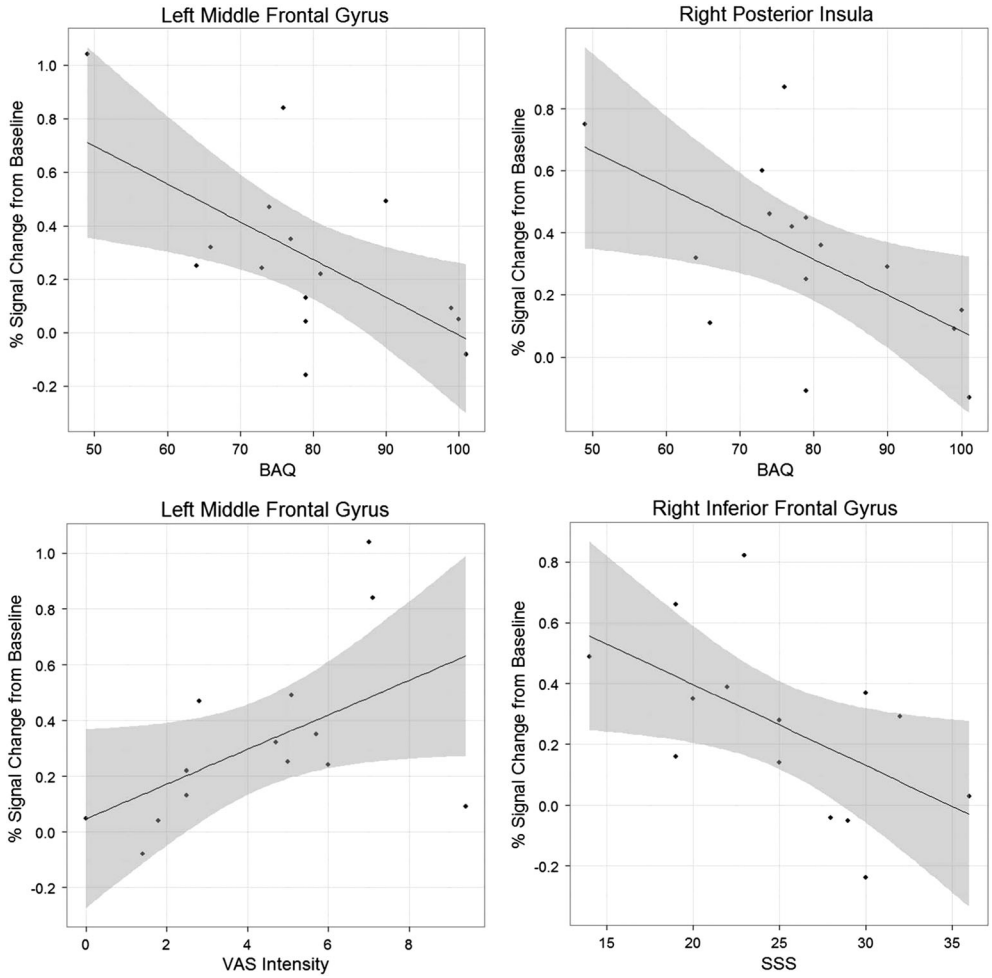

Figure 5 Brain activation during breathing load in adolescents with substance use disorder (SUD). SUD exhibited a negative relations between (a) left middle frontal gyrus ($r = -0.62$, $P = 0.02$) and (b) right posterior insula ($r = -0.58$, $P = 0.02$) and Body Awareness Questionnaire (BAQ) scores. Brain activation during breathing load for SUD shows a positive relationship between (c) left middle frontal gyrus and visual analogue scale (VAS) intensity of the breathing load ($r = 0.52$, $P = 0.05$). SUD exhibited a negative relationship between (d) right inferior frontal gyrus and sensation-seeking scale (SSS; $r = -0.56$, $P = 0.04$)

This study has several limitations. First, our sample size was modest and limited to adolescents who endorsed alcohol or marijuana SUD. Future research should recruit larger samples to investigate potential neural differences between substances. A larger sample could also investigate differences between gender, as functional and structural imaging studies have shown differential patterns for heavy drinking adolescents, with females generally showing more abnormalities than males [24,67–69]. Secondly, this cross-sectional study cannot determine whether the dysfunctional interoceptive system reflects a pre-existing condition or a consequence of neurotoxic effects of substance use. Future studies could investigate this directionality similarly to research showing that children with mothers who smoked displayed more impulsivity than children of non-smokers on a delayed discounting task [70]. If adolescents at risk of developing SUD show altered interoceptive function compared to those without family history of SUD, this would support that it is a pre-existing condition.

Despite these limitations, this investigation shows altered interoceptive functioning in adolescents with alcohol/marijuana SUD indicated by: (a) increased insular activation during breathing load and (b) modulation in insular and frontal regions as a function of anticipation and breathing load compared to CON. Our findings suggest that adolescents with SUD are hypersensitive to aversive stimuli, which may lead to drug-seeking behavior.

Declaration of interests

None.

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References

1. Johnston L. D., O'Malley P. M., Bachman J. G., Schulenberg J. E., Miech R. A. Monitoring the Future National Survey Results on Drug Use, 1975–2013. Ann Arbor: Institute for Social Research, University of Michigan; 2014; pp. 1–659.
2. Anthony J. C., Petronis K. R. Early-onset drug use and risk of later drug problems. *Drug Alcohol Depend* 1995; **40**: 9–15.
3. Grant B. F., Stinson F. S., Harford T. C. Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: a 12-year follow-up. *J Subst Abuse* 2001; **13**: 493–504.
4. Bonomo Y. A., Bowes G., Coffey C., Carlin J. B., Patton G. C. Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. *Addiction* 2004; **99**: 1520–8.
5. Grant J. D., Scherrer J. F., Lynskey M. T., Lyons M. J., Eisen S. A., Tsuang M. T. *et al.* Adolescent alcohol use is a risk factor for adult alcohol and drug dependence: evidence from a twin design. *Psychol Med* 2006; **36**: 109–18.
6. Farb N. A. S., Segal Z. V., Anderson A. K. Mindfulness meditation training alters cortical representations of interoceptive attention. *Soc Cogn Affect Neurosci* 2013; **8**: 15–26.
7. Craig A. D. How do you feel? Interoception: the sense of the physiological condition of the body. *Nat Rev Neurosci* 2002; **3**: 655–66.
8. Craig A. D. B. How do you feel—now? The anterior insula and human awareness. *Nat Rev Neurosci* 2009; **10**: 59–70.
9. Naqvi N. H., Bechara A. The insula and drug addiction: an interoceptive view of pleasure, urges, and decision-making. *Brain Struct Funct* 2010; **214**: 435–50.
10. Paulus M. P. Neural basis of reward and craving—a homeostatic point of view. *Dialogues Clin Neurosci* 2007; **9**: 379–87.
11. Paulus M. P., Tapert S. F., Schulteis G. The role of interoception and alliesthesia in addiction. *Pharmacol Biochem Behav* 2009; **94**: 1–7.
12. Verdejo-Garcia A., Clark L., Dunn B. D. The role of interoception in addiction: a critical review. *Neurosci Biobehav Rev* 2012; **36**: 1857–69.
13. Paulus M. P., Stewart J. L. Interoception and drug addiction. *Neuropharmacology* 2014; **76**: 342–50.
14. Craig A. D. Interoception: the sense of the physiological condition of the body. *Curr Opin Neurobiol* 2003; **13**: 500–5.
15. Naqvi N. H., Rudrauf D., Damasio H., Bechara A. Damage to the insula disrupts addiction to cigarette smoking. *Science* 2007; **315**: 531–4.
16. Naqvi N. H., Bechara A. The hidden island of addiction: the insula. *Trends Neurosci* 2009; **32**: 56–67.
17. Naqvi N. H., Gaznick N., Tranel D., Bechara A. The insula: a critical neural substrate for craving and drug seeking under conflict and risk. *Ann NY Acad Sci* 2014; **1316**: 53–70.
18. Goudriaan A. E., de Ruiter M. B., van den Brink W., Oosterlaan J., Veltman D. J. Brain activation patterns associated with cue reactivity and craving in abstinent problem gamblers, heavy smokers and healthy controls: an fMRI study. *Addict Biol* 2010; **15**: 491–503.
19. Filbey F. M., Schacht J. P., Myers U. S., Chavez R. S., Hutchison K. E. Marijuana craving in the brain. *Proc Natl Acad Sci USA* 2009; **106**: 13016–21.
20. Bonson K. R., Grant S. J., Contoreggi C. S., Links J. M., Metcalfe J., Weyl H. L. *et al.* Neural systems and cue-induced cocaine craving. *Neuropsychopharmacology* 2002; **26**: 376–86.
21. Tapert S. F., Brown G. G., Baratta M. V., Brown S. A. fMRI BOLD response to alcohol stimuli in alcohol dependent young women. *Addict Behav* 2004; **29**: 33–50.
22. Dager A. D., Anderson B. M., Rosen R., Khadka S., Sawyer B., Jiantonio-Kelly R. E. *et al.* Functional magnetic resonance imaging (fMRI) response to alcohol pictures predicts subsequent transition to heavy drinking in college students. *Addiction* 2014; **109**: 585–95.
23. Crowley T. J., Dalwani M. S., Mikulich-Gilbertson S. K., Du Y. P., Lejuez C. W., Raymond K. M. *et al.* Risky decisions and their consequences: neural processing by boys with antisocial substance disorder. *PLOS ONE* 2010; **5**: e12835.
24. Caldwell L. C., Schweinsburg A. D., Nagel B. J., Barlett V. C., Brown S. A., Tapert S. F. Gender and adolescent alcohol use disorders on BOLD (blood oxygen level dependent) response to spatial working memory. *Alcohol Alcohol* 2005; **40**: 194–200.
25. Rubinstein M. L., Luks T. L., Dryden W. Y., Rait M. A., Simpson G. V. Adolescent smokers show decreased brain responses to pleasurable food images compared with nonsmokers. *Nicotine Tob Res* 2011; **13**: 751–5.
26. Chung T., Clark D. B. Insula white matter volume linked to binge drinking frequency through enhancement motives in treated adolescents. *Alcohol Clin Exp Res* 2014; **38**: 1932–40.
27. Morales A. M., Ghahremani D., Kohno M., Hellemann G. S., London E. D. Cigarette exposure, dependence, and craving are related to insula thickness in young adult smokers. *Neuropsychopharmacology* 2014; **39**: 1816–22.
28. May A. C., Stewart J. L., Migliorini R., Tapert S. F., Paulus M. P. Methamphetamine dependent individuals show attenuated brain response to pleasant interoceptive stimuli. *Drug Alcohol Depend* 2013; **131**: 238–46.
29. Migliorini R., Stewart J. L., May A. C., Tapert S. F., Paulus M. P. What do you feel? Adolescent drug and alcohol users show altered brain response to pleasant interoceptive stimuli. *Drug Alcohol Depend* 2013; **133**: 661–8.
30. Stewart J. L., May A. C., Poppa T., Davenport P. W., Tapert S. F., Paulus M. P. You are the danger: attenuated insula response in methamphetamine users during aversive interoceptive decision-making. *Drug Alcohol Depend* 2014; **142**: 110–9.
31. Stewart J. L., Juavinett A. L., May A. C., Davenport P. W., Paulus M. P. Do you feel alright? Attenuated neural processing of aversive interoceptive stimuli in current stimulant users. *Psychophysiology* 2014; **52**: 249–62.
32. Davenport P. W., Vovk A. Cortical and subcortical central neural pathways in respiratory sensations. *Respir Physiol Neurobiol* 2009; **167**: 72–86.
33. Randall D. M., Cox W. M. Experimental mood inductions in persons at high and low risk for alcohol problems. *Am J Drug Alcohol Abuse* 2001; **27**: 183–7.
34. Taylor J., Carlson S. R., Iacono W. G., Lykken D. T., McGue M. Individual differences in electrodermal responsivity to predictable aversive stimuli and substance dependence. *Psychophysiology* 1999; **36**: 193–8.
35. Taylor J. Electrodermal reactivity and its association to substance use disorders. *Psychophysiology* 2004; **41**: 982–9.
36. Volkow N. D., Fowler J. S., Wang G.-J., Swanson J. M. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry* 2004; **9**: 557–69.
37. Wiers R. W., Bartholow B. D., van den Wildenberg E., Thush C., Engels R. C. M. E., Sher K. J. *et al.* Automatic and controlled processes and the development of addictive behaviors in adolescents: a review and a model. *Pharmacol Biochem Behav* 2007; **86**: 263–83.
38. Casey B. J., Jones R. M. Neurobiology of the adolescent brain and behavior: implications for substance use disorders. *J Am Acad Child Adolesc Psychiatry* 2010; **49**: 1189–201; quiz 1285.

39. Somerville L. H., Jones R. M., Casey B. J. A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. *Brain Cogn* 2010; **72**: 124–33.
40. Paulus M. P., Flagan T., Simmons A. N., Gillis K., Kotturi S., Thom N. *et al.* Subjecting elite athletes to inspiratory breathing load reveals behavioral and neural signatures of optimal performers in extreme environments. *PLOS ONE* 2012; **7**: e29394.
41. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. Washington, DC: American Psychiatric Association; 2013.
42. Pierucci-Lagha A., Gelernter J., Feinn R., Cubells J. F., Pearson D., Pollastri A. *et al.* Diagnostic reliability of the Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA). *Drug Alcohol Depend* 2005; **80**: 303–12.
43. Sobell L. C., Maisto S. A., Sobell M. B., Cooper A. M. Reliability of alcohol abusers' self-reports of drinking behavior. *Behav Res Ther* 1979; **17**: 157–60.
44. Zuckerman M. Item revisions in the Sensation Seeking Scale Form V (SSS-V). *Pers Individ Dif* 1996; **20**: 515.
45. Patton J. H., Stanford M. S., Barratt E. S. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol* 1995; **51**: 768–74.
46. Achenbach T. M., Rescorla L. A. Manual for the ASEBA School-Age Forms and Profiles. Burlington, VT: University of Vermont. Research Center for Children; 2001.
47. Barratt W. The Barratt simplified measure of social status (BSMSS). Terre Haute, IN: Indiana State University; 2006.
48. Watson D., Clark L. A., Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol* 1988; **54**: 1063–70.
49. Spielberger C. D., Gorsuch R. L., Lushene R., Vagg P. R. Manual for the State–Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists' Press; 1983.
50. Wilkinson G. S., Robertson G. J. Wide Range Achievement Test (WRAT4). *Professional Manual Psychological Assessment Resources*, Lutz, FL; 2006.
51. Shields S. A., Mallory M. E., Simon A. The Body Awareness Questionnaire: reliability and validity. *J Pers Assess* 1989; **53**: 802–15. DOI: 101207/s15327752jpa5304_16.
52. Chan P.-Y. S., Davenport P. W. Respiratory-related evoked potential measures of respiratory sensory gating. *J Appl Physiol* 2008; **105**: 1106–13.
53. Boynton G. M., Engel S. A., Glover G. H., Heeger D. J. Linear systems analysis of functional magnetic resonance imaging in human V1. *J Neurosci* 1996; **16**: 4207–21.
54. Cohen M. Parametric analysis of fMRI data using linear systems methods. *Neuroimage* 1997; **6**: 93–103.
55. Friston K. J., Frith C. D., Turner R., Frackowiak R. S. J. Characterizing evoked hemodynamics with fMRI. *Neuroimage* 1995; **2**: 157–65.
56. Pinheiro J., Bates D., Saikat D., Sarkar D., the R Development Core Team. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-111. Vienna, Austria: R Development Core Team; 2013.
57. Lancaster J. L., Woldorff M. G., Parsons L. M., Liotti M., Freitas C. S., Rainey L. *et al.* Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* 2000; **10**: 120–31.
58. Stewart J. L., Parnass J. M., May A. C., Davenport P. W., Paulus M. P. Altered frontocingulate activation during aversive interoceptive processing in young adults transitioning to problem stimulant use. *Front Syst Neurosci* 2013; **7**: 1–15.
59. Galván A., McGlennen K. M. Enhanced striatal sensitivity to aversive reinforcement in adolescents versus adults. *J Cogn Neurosci* 2013; **25**: 284–96.
60. Koob G. F., Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science* 1997; **278**: 52–8.
61. Lerman C., Gu H., Loughead J., Ruparel K., Yang Y., Stein E. A. Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. *JAMA Psychiatry* 2014; **71**: 523–30.
62. Sutherland M. T., McHugh M. J., Pariyadath V., Stein E. A. Resting state functional connectivity in addiction: lessons learned and a road ahead. *Neuroimage* 2012; **62**: 2281–95.
63. Weiland B. J., Sabbineni A., Calhoun V. D., Welsh R. C., Hutchison K. E. Reduced executive and default network functional connectivity in cigarette smokers. *Hum Brain Mapp* 2015; **36**: 872–82.
64. Eshel N., Nelson E. E., Blair R. J., Pine D. S., Ernst M. Neural substrates of choice selection in adults and adolescents: development of the ventrolateral prefrontal and anterior cingulate cortices. *Neuropsychologia* 2007; **45**: 1270–9.
65. May A. C., Stewart J. L., Tapert S. F., Paulus M. P. The effect of age on neural processing of pleasant soft touch stimuli. *Front Behav Neurosci* 2014; **8**: 52.
66. Hulvershorn L. A., Hummer T. A., Fukunaga R., Leibenluft E., Finn P., Cyders M. A. *et al.* Neural activation during risky decision-making in youth at high risk for substance use disorders. *Psychiatry Res* 2015; **233**: 102–111.
67. Medina K. L., McQueeny T., Nagel B. J., Hanson K. L., Yang T. T., Tapert S. F. Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. *Addict Biol* 2009; **14**: 457–68.
68. Schweinsburg B. C., Alhassoon O. M., Taylor M. J., Gonzalez R., Videen J. S., Brown G. G. *et al.* Effects of alcoholism and gender on brain metabolism. *Am J Psychiatry* 2003; **160**: 1180–3.
69. Squeglia L. M., Sorg S. F., Schweinsburg A. D., Wetherill R. R., Pulido C., Tapert S. F. Binge drinking differentially affects adolescent male and female brain morphometry. *Psychopharmacology (Berl)* 2012; **220**: 529–39.
70. Reynolds B., Leraas K., Collins C., Melanko S. Delay discounting by the children of smokers and nonsmokers. *Drug Alcohol Depend* 2009; **99**: 350–3.