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NICOTINE ABSTINENCE INFLUENCES THE CALCULATION OF SALIENCE IN DISCRETE INSULAR CIRCUITS

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Abstract

Background—Insular subdivisions show distinct patterns of resting state functional connectivity (rsFC) with specific brain regions, each with different functional significance. Seeds in these subdivisions are employed to characterize the effects of acute nicotine abstinence on rsFC between insula subdivisions and brain networks implicated in addiction and attentional control.

Methods—In a within subjects design, resting-state BOLD data were collected from treatment-seeking smokers (n=20) following smoking satiety and again following 48 hours of nicotine abstinence. Three right hemisphere insular regions of interest (dorsal, ventral, and posterior) served as seeds for analyses. Indices of both static and dynamic rsFC were obtained and correlated with indices of subjective withdrawal and behavioral performance.

Results—Abstinence-induced physiological, subjective, and cognitive differences were observed. Overall dynamic rsFC was reduced during abstinence, and circuits containing each insular seed showed changes in rsFC as a function of nicotine abstinence. Specifically, dorsal and posterior insular connections to the default mode and salience networks were enhanced, while a previously undescribed ventral insular connection to the executive control network was reduced. Further, static rsFC was significantly correlated with subjective ratings of aversive affect and withdrawal in the modified ventral and posterior insular-seeded circuits.

Conclusions—As predicted, divergent connections between insula subdivisions and anticorrelated resting brain networks were observed during abstinence. These changes reflect an attentional bias *toward* aversive affective processing and not directly *away from* exogenous cognitive processing, suggesting a coordinated modulation of circuits associated with interoceptive and affective processing that instantiates an aversive state during nicotine abstinence.

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DISCLOSURES

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

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Keywords

Abstinence; Insula; functional connectivity; affect; nicotine; cessation

INTRODUCTION

The cynical adage says quitting smoking is easy, it's staying quit that is difficult. Smoking cessation attempts regularly fail within days of attempting full nicotine abstinence (1, 2), and many smokers relapse before even 24 hours of continuous abstinence (3). These observations suggest abstinence during the initial stages of a quit attempt is a key early barrier to long-term smoking cessation (4). Thus, understanding the neurobiological mechanisms of acute nicotine abstinence is a foundational step toward improving clinical outcomes.

The disruptions associated with acute abstinence include both cognitive (5, 6) and affective processes (7–12). Critically, the insula has a well-described role as a node of integration between cognitive and affective processing (13–15), and plays a central role both in adaptive cognitive control (16, 17) and the integration of visceral, autonomic, and homeostatic stimuli that influence the calculation of salience (18). Using resting state functional connectivity (rsFC), the insula has been shown to interact with constituent nodes of its own and other large-scale brain networks to influence endogenous and exogenous attention orientation via a hypothesized tripartite network model (19). Briefly, this model describes a Default Mode Network (DMN)—including rostral and posterior cingulate cortices, parahippocampal gyrus and bilateral inferior parietal cortex (20)—and an anticorrelated Executive Control Network (ECN)—including bilateral dorsolateral prefrontal and parietal cortices (21). During interoceptive processing, the DMN is relatively more active while the ECN is relatively deactivated (22, 23). During exteroceptive processing the reverse is true. The toggling between these two networks is thought to be mediated by the Salience Network (SN)—comprised of bilateral insula and dorsal anterior cingulate cortex (24–26).

Using nicotine as an exemplar, the tripartite model has been extended to address abstinence-related dysfunction. During abstinence, the insula is more strongly correlated with DMN and reciprocally more weakly correlated with ECN, facilitating a shift from exteroceptive to interoceptive attentional focus (27, 28). In support of this hypothesis, enhanced insula-DMN connectivity has been observed in acutely abstinent smokers (12, 29–31). However, these confirmatory studies have generally not shown a downregulated insula-ECN connection. This is surprising given the described anticorrelation between DMN and ECN (22, 23) as well as the disruptions in sustained attention associated with the acute withdrawal state (5). This disparity may reflect a tendency to treat the insula as a unitary structure, though recent exceptions are noted (32, 33).

Compelling evidence exists for both hemispheric differences and anatomical subdivisions of insular structural and functional connectivity. Recent structure/function neuroimaging results have demonstrated multiple diverse insular subdivisions (34–38), including dorsal and ventral subunits of the anterior insula and one or more posterior subunits (34, 39–41). Right insula is implicated in sympathetic nervous system activation (42, 43) and attention

orientation and arousal (35, 44). Further, right insula plays a significant role in smoking behavior maintenance. A seminal lesion study (45) showed injury to right insula led to increased disruption in smoking behavior (see also (46–48)). Taken together, these data suggest interrogation of right insula subdivisions (e.g. (34)) may more fully characterize the central role this structure plays in the integrative calculation of salience and the potential disruptions in these calculations induced by acute nicotine abstinence.

Dysfunction in the ability of right insular subdivisions to efficiently toggle between interoceptive and exteroceptive attentional focus may provide, at least in part, a mechanism contributing to the allostatic state thought to maintain drug addiction (49). For example, during withdrawal, enhanced insula-DMN and/or reduced insula-ECN connectivity may lead to an overrepresentation of endogenous signals perceived as aversive. These overrepresented signals may then serve as negative reinforcers, biasing insular calculations of salience towards withdrawal-related dysphoria and leading to an increased motivation to smoke. By directly quantifying changes in insular connectivity in response to the stress of acute abstinence, we seek to provide a systems-level description of the effects of acute abstinence and potential hurdles to protracted abstinence.

In the present within-subjects study, we interrogate both static (s-rsFC) and dynamic (d-rsFC) rsFC measures between right insular subdivisions (34) and targets within large-scale networks comprising the tripartite network model relevant to attention and addiction (19, 27, 28). S-rsFC, assessed over many minutes, allows for direct interrogation of circuits based on prior insular parcellation, and thus links known insula function with changes in network connectivity. In contrast, d-rsFC characterizes time-varying fluctuations in connectivity on the order of seconds (50, 51), and provides a global index of network flexibility across all networks of interest (DMN, ECN, SN). This additional characterization may be critical, as recent results in healthy controls show insular connections are among the most dynamically flexible and spatially distributed within the human brain (52, 53). As a compliment to s-rsFC measures, d-rsFC may provide an index of systems-level regulatory function disrupted by acute abstinence.

As predicted by the tripartite abstinence model (27), we hypothesized insula-DMN s-rsFC during abstinence would be increased, particularly in insula subdivisions associated with interoceptive processing (i.e. posterior), and insula-ECN s-rsFC would be decreased, specifically in subdivisions associated with attentional processing (i.e. dorsal). Based on the prior model, we had no directional hypothesis for insula-SN s-rsFC during abstinence. Additionally, we hypothesized that d-rsFC would be reduced during abstinence, with less flexible network configurations and an increased probability of remaining in a given state over time.

Finally, to ascribe clinical relevance to the abstinence-sensitive circuits identified, we examined the relationship between both d-rsFC and s-rsFC measures and validated clinical indices of affective dysphoria (54) and cognitive processes including sustained attention (6, 55–57) disrupted by abstinence. Here, we hypothesized affective measures would correlate with insula-DMN connection increases, while cognitive measures would correlate with reductions in insula-ECN connectivity.

METHODS

Participants

Twenty smoking cessation treatment-seeking participants completed all experimental procedures. Participants were required to be right-handed, 18–60 years of age, free of active drug or alcohol abuse/dependence (other than nicotine), report no current psychiatric or neurological disorders, and have no contraindications for magnetic resonance imaging (MRI) (Table 1). Written informed consent was obtained in accordance with the NIDA-IRP Institutional Review Board.

Experimental Design

In this longitudinal, within-subjects design, each participant completed two MR scanning sessions, one during ad lib sated smoking, followed by an acute abstinence scan with last cigarette ~48 hours prior to scan. As participants were enrolled in a smoking cessation protocol, the order of the two scans was fixed, with the sated scan preceding the abstinent scan by an average of 67 days (median 28 days). (See supplement for general procedures)

Behavioral task measure—Following brief medical assessment, participants completed a 12-minute rapid visual information processing (RVIP) task (55) administered at a computer outside the MRI scanner to assess sustained attention processing (See supplement for task details).

Subjective withdrawal measure—Directly before each scanning session (sated and abstinent), subjective ratings of withdrawal-related symptoms were assessed via the Wisconsin Smoking Withdrawal Scale (WSWS), a 28 item self-report test comprised of 7 subscales (Anger, Anxiety, Concentration, Craving, Hunger, Sadness, Sleep), and TOTAL score (54). As pre-scan procedures generally took up to 120 min, participants were given a <10 min smoking break immediately prior to the withdrawal assessment and MRI scan during the sated scan session only.

MRI Scanning—Following WSWS administration, participants immediately entered the scanner and completed an 8-min, eyes open resting scan at onset of a longer scan session which also included several cognitive tasks (see (58)). (See supplement for MRI data acquisition and preprocessing parameters)

Abstinence-Specific Procedures—Upon completion of the sated scan session, participants began weekly meetings with a cessation counselor who, together with the participant, set a target quit date and time for completion of the 48-hour abstinence period and scan session.

DATA ANALYSIS

Behavioral data—RVIP task performance was assessed via sensitivity index, $d' = Z(\text{hit rate}) - Z(\text{false alarm rate})$. SESSION (abstinence (–) sated) effects were calculated via paired t-test of RVIP d' values.

Subjective Ratings—Withdrawal was assessed via WSWs scores for each scanning session. SESSION (abstinence (–) sated) effects were calculated via paired t-test, corrected for multiple comparisons based on the 7 subscales and total score assessed (p-corrected < 0.05/8 = 0.00625).

Resting-State Functional Connectivity

Single-subject analysis—For each subject, correlation coefficient (CC) images were computed by correlating each voxel's motion-censored time course with a reference time course from the 3 right insula seeds (Dorsal (DOR), Ventral (VEN), and Posterior (POS)) defined via probabilistic voxel maps created by Chang et al. (34). Any voxel with >85% inclusion probability in each insular subdivision was included in the mask for that subdivision, resulting in three non-overlapping insular subdivision seeds. (Fig 1, Table S1)

The resulting subject-level CC images were Fisher's Z-transformed ($z = \frac{1}{2} \log \left(\frac{1+r}{1-r} \right)$) to better approximate a normal distribution. In addition, as a *post hoc* test for potential hemispheric differences, right insula seed masks were reflected across the midline to left insula (Fig S2a) and similar subject-level CC images were computed.

rsFC target network identification—Seed based analyses were *a priori* constrained to voxels within each of the three functionally generated masks of large-scale brain networks consistently shown relevant to nicotine addiction (27, 28): DMN, ECN, and SN. These brain network masks were identified via group-level independent component analysis (gICA), (59) resulting in voxel masks for each network (Fig 1, See Supplemental materials for gICA method details). For each gICA-identified network, an individual mask of resultant voxels (Fig 1) was used in s-rsFC and d-rsFC analyses.

Static rsFC analysis—Paired t-tests of SESSION difference in s-rsFC strength between each of the 3 insular seeds and voxels within each of the 3 large-scale network masks were performed (i.e. seed-ECN voxels; seed-SN voxels; seed-DMN voxels) with correction for multiple comparisons (3 seeds * 3 target networks, 1 sided tests for DMN and ECN rsFC, 2 sided tests for SN rsFC) and cluster-correction applied (p-voxel < 0.0167, p-corrected < 0.05/9 = 0.0056 for each test). *Post hoc* tests for hemispheric differences employing left insula seeds were conducted with identical statistical corrections (p-voxel < 0.0167, p-corrected < 0.0056).

Dynamic rsFC analysis—The three right insular seeds (DOR, VEN, POS) and the three brain network masks (ECN, DMN, SN) were resampled to 4×4×4 mm voxel size to reduce computational load. Mean time series from insular seeds were extracted and used as seed time courses. D-rsFC between the 3 insula seeds and 3 brain network targets were estimated using a sliding window method (51, 60). By sliding a Hamming window with window width of 22 TRs (44 secs) and sliding steps of 1 TR, correlation matrices between each insula seed time course and the time series of each voxel in each of the 3 brain network masks within each time window were computed. These windowed correlation matrices were then transformed using Fisher's Z-transformation and concatenated to form a 3D matrix, sized 3×5510×209 for each subject and session.

To assess the structure of reoccurring connectivity patterns, k-means clustering algorithm was applied to windowed correlation matrices and repeated 500 times with random initialization of centroid positions to avoid converging into local minima. The resulting centroid of each cluster ($n=6$ based on the silhouette criterion (61); Fig S1) was recognized as a state. After clustering, each windowed correlation matrix was assigned to one of the 6 identified states. For each subject in each session, a state transition process was described using a Markov matrix. SESSION effects were assessed via a paired t-test ($P(\text{remain in current state})$)—defined as sum of the diagonal of the Markov matrix.

Correlation analyses—Measures of static and dynamic rsFC showing a significant SESSION effect were extracted for both the abstinent and sated conditions for each participant, with the magnitude of rsFC SESSION effect correlated with the magnitude of SESSION effects on both RVIP d' and WSWS subscale subjective ratings. These correlations were corrected for multiple comparisons based on the number of rsFC measures tested ($n=5$: 4 s-rsFC circuits identified, 1 d-rsFC measure) * state measures tested ($n=6$: RVIP d' (1), WSWS subscales (5)) ($p\text{-corrected} < 0.05/30 = 0.00167$).

Finally, *post hoc* correlations between rsFC measures and cognitive and subjective withdrawal measures were calculated at both abstinence and satiety individually. In each case, state-specific correlations were only evaluated for those insular-seeded rsFC circuits that showed a SESSION effect. As above, these follow-up tests were corrected for multiple comparisons based on the number of rsFC measures ($n=5$), subjective measures ($n=6$), and states ($n=2$) tested ($p\text{-corrected} < 0.05/60 = 0.00083$).

RESULTS

Abstinence Verification/SESSION effects

Physiological—Consistent with participant self-reports of abstinence, expired CO (ppm) showed a significant SESSION effect ($t(19) = -9.35$, $p < 0.0001$), with abstinence CO values (mean = 2.35 (SD = 1.09)) significantly lower than sated (23.8 (10.8)). All participants self-reported successful abstinence of ~48 hours (48.6 hours (1.5)) prior to the abstinence scan (Table 2).

Sustained Attention Behavior—RVIP task d' showed a significant SESSION effect ($t(19) = -2.28$, $p = 0.017$), with abstinence d' (1.25 (0.7)) significantly lower than sated d' (1.46 (0.9)).

Subjective Withdrawal—Of the 7 WSWS subscales and TOTAL score, five (Anger, Concentration Disruption, Craving, Sadness, and WSWS TOTAL score) showed a SESSION effect, with significantly increased ratings during abstinence, after correction for multiple comparisons. (Table 2)

Static rsFC—Following motion censoring, there was no significant difference in % of total TRs retained across sessions ($t(19) = 1.53$, $p = 0.14$), with 98.1% (7.71) of total TRs retained in sated resting scan and 96.4% (10.12) retained in the abstinent resting scan.

Four functional s-rsFC circuits between the 3 interrogated insular subdivisions (DOR, VEN, POS) and the 3 large-scale resting networks of interest (DMN, ECN, SN) showed significant SESSION effects (Fig 2). A circuit between VEN and the left dorsal lateral prefrontal cortex (dlPFC) within the larger ECN showed decreased connectivity strength during abstinence when compared with the sated state (Fig 2a). In contrast, circuits between POS and dorsal ACC (dACC) and POS and right inferior frontal cortex (IFC), both within the larger SN, showed increased functional connectivity during abstinence (Fig 2b). Similarly, an increase in connectivity strength between DOR and a cluster in right inferior parietal lobule (IPL) within the larger DMN was seen in the abstinence vs. sated session (Fig 2c).

Post hoc analyses employing left insula seeds identified a similar (left) POS-dACC circuit with increased connectivity as a function of abstinence. (Fig S2b) No other right insula seeded circuit results were replicated with left insula seeds.

Dynamic rsFC—The probability of staying in a given state ($P(\text{remain in state})$) showed a significant SESSION effect ($t(19) = 2.35$ $p = 0.030$), such that this measure of d-rsFC was increased in abstinence ($P(\text{remain in state}) = 0.912$ (0.016)) as compared to sated ($P(\text{remain in state}) = 0.900$ (0.017)) state. (Fig 3)

Correlations between rsFC and clinical measures

Sated correlation—Of the clinical measures obtained in the *sated* condition, s-rsFC in the POS-IFC (SN) circuit was positively correlated only with WSWs sadness subscale ($r = 0.74$, $p < 0.0005$). In addition, s-rsFC in the POS-dACC (SN) circuit showed a significant positive correlation only with the anger subscale of the WSWs ($r = 0.671$, $p < 0.0005$) (Fig 4a). No additional subjective measures or identified s-rsFC circuits showed a significant correlation during satiety (Table S2). Also of note, the correlation with WSWs anger was not observed in the analogous left POS-dACC circuit identified in *post hoc* analysis (Fig S2b). No significant correlations were observed between $P(\text{remain in state})$ and clinical measures during satiety.

Abstinent correlation—During the *abstinent* condition, s-rsFC in the VEN-dlPFC (ECN) circuit showed a significant negative correlation with the craving subscale of the WSWs ($r = -0.795$, $p < 0.0005$) (Fig 4b). No additional subjective measures or identified s-rsFC circuits showed a significant correlation during abstinence (Table S2). No significant correlations were observed between $P(\text{remain in state})$ and any clinical measures during abstinence.

SESSION effect correlation—None of the insula subdivision-seeded s-rsFC circuits identified in the SESSION analysis, nor $P(\text{remain in state})$ showed a significant correlation between the magnitude of SESSION effect and magnitude of SESSION effect for any clinical measure (RVIP d' , WSWs subscales) after correction for multiple comparisons (Table S2).

DISCUSSION

Our results demonstrate that acute, 48-hour abstinence precipitates an overall less flexible network configuration, whereby specific connections seeded in right insula subdivisions

show distinct, but coherent patterns of modulation. As predicted by the tripartite model of nicotine addiction (27), divergent connections between insula subdivisions and both DMN and ECN are observed during abstinence. However, contrary to the model, it is not that right insula directly modulates the exchange of an exogenous orientation during satiety for an endogenous orientation during abstinence. Instead, the current data show that only those insula-seeded connections related to endogenous processing are modulated as a function of abstinence. These modulated insular circuits appear to facilitate the communication of subjectively aversive interoceptive signals during abstinence. As such, the identified circuits likely play a primary role in instantiating the aversive affective state characteristic of withdrawal. The significant correlations between a subset of these plastic, insula-seeded circuits and subjective ratings of withdrawal—but importantly not cognitive performance—are consistent with this interpretation and suggest a role for the identified circuits in processes that promote continued substance abuse via negative reinforcement (62).

During abstinence, positive correlations seen in satiety were both selectively enhanced (i.e. POS-dACC and POS-IFC) and reduced (VEN-dIPFC), while negative correlations were only enhanced (DOR-IPL). Divergent directional circuit modulations argue against any non-specific effects of nicotine abstinence on the BOLD signal. Instead, these modulations emphasize the distinct, oppositional functional contributions of insular subdivisions and related circuitry to abstinence-related dysfunction.

In abstinence, the enhanced circuit connectivity between DOR and IPL within the larger DMN (63) is consistent with previous results showing similar increases in insula-DMN connectivity related to an enhanced salience of interoceptive signals (12, 29–31). The DOR insula has a previously-described role in cognitive processes associated with attentional orientation and salience calculation (36, 37, 64). Thus, the enhanced connectivity between this region and the endogenously-oriented DMN suggests that information processed in the DMN is preferentially communicated with DOR during nicotine abstinence. Indeed, such an interoceptive bias of attentional processing has been previously linked to both abstinence and the maintenance of addiction (27, 65, 66).

In a similar vein, the enhanced circuit strength between POS and dACC as well as between POS and IFC is consistent with the idea that interoceptive or somatosensory input is given priority in the calculation of salience during abstinence. To wit, POS has been shown to be preferentially active in response to general interoceptive signals (14, 42) as well as smoking cues (9, 67). In much the same manner as the increased DOR-IPL s-rsFC provides an avenue for information transfer between DMN and a node within the SN (e.g. DOR), the enhanced POS-dACC and POS-IFC circuits may provide a complimentary means for interoceptive signals processed by POS to be shared with additional nodes within the SN. The observed correlations between POS-dACC and POS-IFC connectivity and aversive affective ratings during satiety (described below) support this inference.

Finally, the previously-unreported reduction in connectivity between the VEN and dIPFC within the ECN (68) may signal a reduction in top-down control of affective processing. This reduction may subsequently increase the contribution of affective signals integrated in VEN (42, 69, 70) to overall salience calculations during abstinence. This finding suggests

the decoupling of insula-ECN during abstinence is localized to the insular subdivision associated with affective (VEN) as opposed to cognitive (DOR) processing. This disruption in communication between top-down control and affective processing may in turn importantly contribute to the nicotine withdrawal syndrome. Speaking to this point, VEN-dIPFC connectivity strength is correlated with subjective craving during abstinence (described below).

More broadly, d-rsFC as measured across each right insula subdivision and DMN, ECN, and SN is significantly reduced as a function of acute abstinence. From moment to moment, abstinent smokers tend to be confined within an identified network state rather than transitioning between states. These d-rsFC findings are notable, as prior evidence in healthy participants identified insular nodes among the most flexible and interconnected within the human brain (52, 53), with this flexibility related to executive function (52, 71). Thus, the reduced flexibility observed, while not related to cognitive performance in our sample, is nonetheless maladaptive. Decreased d-rsFC is directionally consistent with the observation that chronically taxed biological systems display a lack of dynamic response to acute stress (49, 72). The acute stress of nicotine abstinence precipitates both an overall reduction in d-rsFC as well as modulation of a diverse set of insular circuits, suggesting reduced d-rsFC may be an overarching signal of maladaptive circuit response to abstinence. This inflexibility in network configuration from moment to moment may in turn provide a more static environment to instantiate (or disrupt) the specific insular connections described above. Together, the observed changes in d-rsFC and s-rsFC provide systems-level insight into how the affective disruptions associated with smoking abstinence arise.

Clinical relevance for the modified circuit connections was identified via correlations with affective measures sensitive to nicotine abstinence. During satiety, positive correlations between POS-dACC and WSWs anger subscale and between POS-IFC and WSWs sadness are consistent with predictions of the allostatic model of addiction (62). This model posits that chronic exposure to nicotine induces neurobiological changes—for example upregulation of nicotinic receptor expression (73)—leading to an alteration of homeostatic set point which may promote ongoing smoking behavior via maintenance of a dysphoric state. Here, the magnitude of this subjective aversive disruption is correlated with POS seeded circuit connectivity strength, which suggests a role for these circuits in instantiating aversive affective sensations even when nicotine is present.

In contrast, the VEN-dIPFC circuit shows a negative correlation with subjective craving during abstinence. When perturbed by abstinence, the degree of decoupling between VEN and dIPFC significantly predicts subjective craving. Prior task-based results in abstinent smokers are in line with this observed decoupling such that dIPFC activation is reduced (74, 75) and both VEN-ins activation (70) and regional cerebral blood flow (76) are increased during acute abstinence. Thus, the decoupling of dIPFC and VEN may lead to a reduction in top-down regulation of insular-modulated affective processing and a subsequent increase in abstinence-induced craving.

While interpreting the relationship between insula connectivity and subjective withdrawal is straightforward, the absence of significant correlation between abstinence-modulated

circuits and RVIP (sustained attention) performance is somewhat surprising. This is especially true given prior evidence linking DOR insula activation with RVIP task performance (56, 77, 78) and attenuation during acute abstinence (56, 77). The absence of an insular rsFC-behavior relationship in the current data suggests the observed decline in RVIP performance may be modulated by nodes and circuits exclusive of the right insula. For example, abstinence-related modulations of dlPFC activation have been tied to decrements in working memory performance (79, 80). The present findings indicate that modulations of insular connectivity are more closely related to affective as opposed to cognitive disruptions during abstinence.

The results described should be viewed considering several limitations. Specifically, the sample size of the current study is small ($n=20$). Additional studies using treatment-seeking smokers are needed to replicate the observed modulations in insular connectivity. In addition, the interventional nature of the smoking cessation protocol and fixed order of scans (sated, abstinent) potentially introduces order effects. However, it should be noted that the current results do replicate prior results showing similar DOR-DMN circuit modulations as a function of *counterbalanced* abstinence vs. satiety in non-treatment seeking smokers. (12) Finally, the current design confounds changes in affect with change in nicotine state. The influence of mood on insular connectivity cannot be dissociated from the influence of abstinence. Future studies that independently modulate mood and/or abstinence are needed to adequately assess the effects of both modulations.

Taken together, the present results suggest a coordinated modulation of circuits associated with interoceptive and affective processing sets the stage for negative reinforcement by instantiating a potentially aversive state during nicotine abstinence. The insula's multifaceted role as a hub of affective integration is apparent when directly examining each of its three main subdivisions—Dorsal, Ventral, Posterior—and their coordinated response to acute abstinence. That the constellation of circuits identified are more closely associated with interoception and affective processing than with exogenous cognitive processing, and are modulated in a drug state coherent manner, suggests preferential processing via the insula of these endogenous signals during abstinence. Further, these findings suggest pursuing cessation treatment focused on mitigating affective as opposed to cognitive disruption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Piasecki TM. Relapse to smoking. Clin Psychol Rev. 2006; 26:196–215. [PubMed: 16352382]

2. Messer K, Trinidad DR, Al-Delaimy WK, Pierce JP. Smoking Cessation Rates in the United States: A Comparison of Young Adult and Older Smokers. *Am J Public Health*. 2008; 98:317–322. [PubMed: 18172143]
3. Westman EC, Behm FM, Simel DL, Rose JE. Smoking Behavior on the First Day of a Quit Attempt Predicts Long-term Abstinence. *Arch Intern Med*. 1997; 157:335. [PubMed: 9040302]
4. Ashare RL, Wileyto EP, Perkins KA, Schnoll RA. The first 7 days of a quit attempt predicts relapse: validation of a measure for screening medications for nicotine dependence. *J Addict Med*. 2013; 7:249–54. [PubMed: 23669629]
5. McClernon FJ, Addicott MA, Sweitzer MM. Smoking abstinence and neurocognition: implications for cessation and relapse. *Curr Top Behav Neurosci*. 2015; 23:193–227. [PubMed: 25655892]
6. Ashare RL, Falcone M, Lerman C. Cognitive function during nicotine withdrawal: Implications for nicotine dependence treatment. *Neuropharmacology*. 2014; 76:581–91. [PubMed: 23639437]
7. Wang Z, Faith M, Patterson F, Tang K, Kerrin K, Wileyto EP, et al. Neural substrates of abstinence-induced cigarette cravings in chronic smokers. *J Neurosci*. 2007; 27:14035–40. [PubMed: 18094242]
8. Froeliger B, Modlin L, Wang L, Kozink RV, McClernon FJ. Nicotine withdrawal modulates frontal brain function during an affective Stroop task. *Psychopharmacology (Berl)*. 2012; 220:707–718. [PubMed: 21989805]
9. Engelmann JM, Versace F, Robinson JD, Minnix JA, Lam CY, Cui Y, et al. Neural substrates of smoking cue reactivity: a meta-analysis of fMRI studies. *Neuroimage*. 2012; 60:252–62. [PubMed: 22206965]
10. Grillon C, Avenevoli S, Daurignac E, Merikangas KR. Fear-Potentiated Startle to Threat, and Prepulse Inhibition Among Young Adult Nonsmokers, Abstinent Smokers, and Nonabstinent Smokers. *Biol Psychiatry*. 2007; 62:1155–1161. [PubMed: 17543892]
11. Fedota JR, Sutherland MT, Salmeron BJ, Ross TJ, Hong LE, Stein EA. Reward Anticipation Is Differentially Modulated by Varenicline and Nicotine in Smokers. *Neuropsychopharmacology*. 2015; 40:2038–2046. [PubMed: 25742873]
12. Sutherland MT, Carroll AJ, Salmeron BJ, Ross TJ, Hong LE, Stein EA. Down-regulation of amygdala and insula functional circuits by varenicline and nicotine in abstinent cigarette smokers. *Biol Psychiatry*. 2013; 74:538–546. [PubMed: 23506999]
13. Zaki J, Davis JJ, Ochsner KN. Overlapping activity in anterior insula during interoception and emotional experience. *Neuroimage*. 2012; 62:493–499. [PubMed: 22587900]
14. Craig ADB. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci*. 2009; 10:59–70. [PubMed: 19096369]
15. Gu X, Hof PR, Friston KJ, Fan J. Anterior insular cortex and emotional awareness. *J Comp Neurol*. 2013; 521:3371–88. [PubMed: 23749500]
16. Cole MW, Reynolds JR, Power JD, Repovs G, Anticevic A, Braver TS. Multi-task connectivity reveals flexible hubs for adaptive task control. *Nat Neurosci*. 2013; 16:1348–55. [PubMed: 23892552]
17. Power JD, Schlaggar BL, Lessov-Schlaggar CN, Petersen SE. Evidence for Hubs in Human Functional Brain Networks. *Neuron*. 2013; 79:798–813. [PubMed: 23972601]
18. Critchley HD, Harrison NA. Visceral Influences on Brain and Behavior. *Neuron*. 2013; 77:624–638. [PubMed: 23439117]
19. Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct*. 2010; 214:655–67. [PubMed: 20512370]
20. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A*. 2001; 98:676–82. [PubMed: 11209064]
21. Honey CJ, Kötter R, Breakspear M, Sporns O. Network structure of cerebral cortex shapes functional connectivity on multiple time scales. *Proc Natl Acad Sci U S A*. 2007; 104:10240–5. [PubMed: 17548818]
22. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A*. 2005; 102:9673–8. [PubMed: 15976020]

23. Keller CJ, Bickel S, Honey CJ, Groppe DM, Entz L, Craddock RC, et al. Neurophysiological Investigation of Spontaneous Correlated and Anticorrelated Fluctuations of the BOLD Signal. *J Neurosci.* 2013; 33:6333–6342. [PubMed: 23575832]
24. Uddin LQ. Salience processing and insular cortical function and dysfunction. *Nat Rev Neurosci.* 2014; 16:55–61. [PubMed: 25406711]
25. Sridharan D, Levitin DJ, Menon V. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci U S A.* 2008; 105:12569–74. [PubMed: 18723676]
26. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci.* 2007; 27:2349–56. [PubMed: 17329432]
27. Sutherland MT, McHugh MJ, Pariyadath V, Stein EA. Resting state functional connectivity in addiction: Lessons learned and a road ahead. *Neuroimage.* 2012; 62:1–15. [PubMed: 22569063]
28. Fedota JR, Stein EA. Resting-state functional connectivity and nicotine addiction: prospects for biomarker development. *Ann N Y Acad Sci.* 2015; 1349:64–82. [PubMed: 26348486]
29. Lerman C, Gu H, Loughhead J, Ruparel K, Yang Y, Stein EA. Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. *JAMA psychiatry.* 2014; 71:523–30. [PubMed: 24622915]
30. Huang W, King JA, Ursprung WWS, Zheng S, Zhang N, Kennedy DN, et al. The development and expression of physical nicotine dependence corresponds to structural and functional alterations in the anterior cingulate-precuneus pathway. *Brain Behav.* 2014; 4:408–17. [PubMed: 24944870]
31. Wang K, Yang J, Zhang S, Wei D, Hao X, Tu S, Qiu J. The neural mechanisms underlying the acute effect of cigarette smoking on chronic smokers. *PLoS One.* 2014; 9:e102828. [PubMed: 25051341]
32. Addicott MA, Sweitzer MM, Froeliger B, Rose JE, McClernon FJ. Increased Functional Connectivity in an Insula-Based Network is Associated with Improved Smoking Cessation Outcomes. *Neuropsychopharmacology.* 2015; 40:2648–2656. [PubMed: 25895453]
33. Drouman V, Read SJ, Bechara A. Revisiting the role of the insula in addiction. *Trends Cogn Sci.* 2015; doi: 10.1016/j.tics.2015.05.005
34. Chang LJ, Yarkoni T, Khaw MW, Sanfey AG. Decoding the role of the insula in human cognition: functional parcellation and large-scale reverse inference. *Cereb Cortex.* 2013; 23:739–49. [PubMed: 22437053]
35. Touroutoglou A, Hollenbeck M, Dickerson BC, Feldman Barrett L. Dissociable large-scale networks anchored in the right anterior insula subserve affective experience and attention. *Neuroimage.* 2012; 60:1947–1958. [PubMed: 22361166]
36. Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, et al. Functional network organization of the human brain. *Neuron.* 2011; 72:665–78. [PubMed: 22099467]
37. Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct Funct.* 2010; 214:519–534. [PubMed: 20512376]
38. Simmons WK, Avery JA, Barcalow JC, Bodurka J, Drevets WC, Bellgowan P. Keeping the body in mind: insula functional organization and functional connectivity integrate interoceptive, exteroceptive, and emotional awareness. *Hum Brain Mapp.* 2013; 34:2944–58. [PubMed: 22696421]
39. Cauda F, D'Agata F, Sacco K, Duca S, Geminiani G, Vercelli A. Functional connectivity of the insula in the resting brain. *Neuroimage.* 2011; 55:8–23. [PubMed: 21111053]
40. Deen B, Pitskel NB, Pelphrey KA. Three systems of insular functional connectivity identified with cluster analysis. *Cereb Cortex.* 2011; 21:1498–506. [PubMed: 21097516]
41. Klein TA, Ullsperger M, Danielmeier C. Error awareness and the insula: links to neurological and psychiatric diseases. *Front Hum Neurosci.* 2013; 7:14. [PubMed: 23382714]
42. Hassanpour MS, Yan L, Wang DJJ, Lapidus RC, Arevian AC, Simmons WK, et al. How the heart speaks to the brain: neural activity during cardiorespiratory interoceptive stimulation. *Philos Trans R Soc London B Biol Sci.* 2016:371.

43. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. *Neurology*. 1992; 42:1727–32. [PubMed: 1513461]
44. Kann S, Zhang S, Manza P, Leung H-C, Li C-SR. Hemispheric Lateralization of Resting-State Functional Connectivity of the Anterior Insula: Association with Age, Gender, and a Novelty-Seeking Trait. *Brain Connect*. 2016; 6:724–734. [PubMed: 27604154]
45. Naqvi NH, Rudrauf D, Damasio H, Bechara A. Damage to the insula disrupts addiction to cigarette smoking. *Science*. 2007; 315:531–4. [PubMed: 17255515]
46. Abdolahi A, Williams GC, Benesch CG, Wang HZ, Spitzer EM, Scott BE, et al. Damage to the insula leads to decreased nicotine withdrawal during abstinence. *Addiction*. 2015; 110:1994–2003. [PubMed: 26347067]
47. Gaznick N, Tranel D, McNutt A, Bechara A. Basal ganglia plus insula damage yields stronger disruption of smoking addiction than basal ganglia damage alone. *Nicotine Tob Res*. 2014; 16:445–53. [PubMed: 24169814]
48. Suner-Soler R, Grau A, Gras ME, Font-Mayolas S, Silva Y, Davalos A, et al. Smoking Cessation 1 Year Poststroke and Damage to the Insular Cortex. *Stroke*. 2012; 43:131–136. [PubMed: 22052507]
49. Koob G, Le Moal M. Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*. 2001; 24:97–129. [PubMed: 11120394]
50. Preti MG, Bolton TA, Van De Ville D. The dynamic functional connectome: State-of-the-art and perspectives. *Neuroimage*. 2016; doi: 10.1016/j.neuroimage.2016.12.061
51. Calhoun VD, Miller R, Pearlson G, Adalı T. The Chronnectome: Time-Varying Connectivity Networks as the Next Frontier in fMRI Data Discovery. *Neuron*. 2014; 84:262–274. [PubMed: 25374354]
52. Chen, T., Cai, W., Ryali, S., Supekar, K., Menon, V., Bressler, S., et al. Distinct Global Brain Dynamics and Spatiotemporal Organization of the Salience Network. In: Sporns, O., editor. *PLOS Biol*. Vol. 14. 2016. p. e1002469
53. Nomi JS, Farrant K, Damaraju E, Rachakonda S, Calhoun VD, Uddin LQ. Dynamic functional network connectivity reveals unique and overlapping profiles of insula subdivisions. *Hum Brain Mapp*. 2016; 37:1770–1787. [PubMed: 26880689]
54. Welsch SK, Smith SS, Wetter DW, Jorenby DE, Fiore MC, Baker TB. Development and validation of the Wisconsin Smoking Withdrawal Scale. *Exp Clin Psychopharmacol*. 1999; 7:354–61. [PubMed: 10609970]
55. Wesnes K, Warburton DM. Effects of smoking on rapid information processing performance. *Neuropsychobiology*. 1983; 9:223–9. [PubMed: 6646394]
56. Beaver JD, Long CJ, Cole DM, Durcan MJ, Bannon LC, Mishra RG, Matthews PM. The Effects of Nicotine Replacement on Cognitive Brain Activity During Smoking Withdrawal Studied with Simultaneous fMRI/EEG. *Neuropsychopharmacology*. 2011; 36:1792–1800. [PubMed: 21544072]
57. McClernon FJ, Kozink RV, Rose JE. Individual differences in nicotine dependence, withdrawal symptoms, and sex predict transient fMRI-BOLD responses to smoking cues. *Neuropsychopharmacology*. 2008; 33:2148–57. [PubMed: 17987060]
58. Fedota JR, Matous AL, Salmeron BJ, Gu H, Ross TJ, Stein EA. Insula Demonstrates a Non-Linear Response to Varying Demand for Cognitive Control and Weaker Resting Connectivity with the Executive Control Network in Smokers. *Neuropsychopharmacology*. 2016; 41:doi: 10.1038/npp.2016.62
59. Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci*. 2005; 360:1001–13. [PubMed: 16087444]
60. Hutchison RM, Womelsdorf T, Allen EA, Bandettini PA, Calhoun VD, Corbetta M, et al. Dynamic functional connectivity: promise, issues, and interpretations. *Neuroimage*. 2013; 80:360–78. [PubMed: 23707587]
61. Rousseeuw PJ. Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *J Comput Appl Math*. 1987; 20:53–65.
62. Koob GF. Negative reinforcement in drug addiction: the darkness within. *Curr Opin Neurobiol*. 2013; 23:559–563. [PubMed: 23628232]

63. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A*. 2003; 100:253–8. [PubMed: 12506194]
64. Menon V. Large-scale brain networks and psychopathology: a unifying triple network model. *Trends Cogn Sci*. 2011; 15:483–506. [PubMed: 21908230]
65. Naqvi NH, Gaznick N, Tranel D, Bechara A. The insula: a critical neural substrate for craving and drug seeking under conflict and risk. *Ann N Y Acad Sci*. 2014; 1316:53–70. [PubMed: 24690001]
66. Paulus MP, Stewart JL. Interoception and drug addiction. *Neuropharmacology*. 2014; 76(Pt B): 342–50. [PubMed: 23855999]
67. Addicott MA, Froeliger B, Kozink RV, Van Wert DM, Westman EC, Rose JE, McClernon FJ. Nicotine and non-nicotine smoking factors differentially modulate craving, withdrawal and cerebral blood flow as measured with arterial spin labeling. *Neuropsychopharmacology*. 2014; 39:2750–9. [PubMed: 24820539]
68. Dosenbach NUF, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RAT, et al. Distinct brain networks for adaptive and stable task control in humans. *Proc Natl Acad Sci U S A*. 2007; 104:11073–8. [PubMed: 17576922]
69. Woo C-W, Koban L, Kross E, Lindquist MA, Banich MT, Ruzic L, et al. Separate neural representations for physical pain and social rejection. *Nat Commun*. 2014; 5:5380. [PubMed: 25400102]
70. Zelle SL, Gates KM, Fiez JA, Sayette MA, Wilson SJ. The first day is always the hardest: Functional connectivity during cue exposure and the ability to resist smoking in the initial hours of a quit attempt. *Neuroimage*. 2016; doi: 10.1016/j.neuroimage.2016.03.015
71. Braun U, Schäfer A, Walter H, Erk S, Romanczuk-Seiferth N, Haddad L, et al. Dynamic reconfiguration of frontal brain networks during executive cognition in humans. *Proc Natl Acad Sci U S A*. 2015; 112:11678–83. [PubMed: 26324898]
72. McEwen B. Allostasis and Allostatic Load Implications for Neuropsychopharmacology. *Neuropsychopharmacology*. 2000; 22:108–124. [PubMed: 10649824]
73. De Biasi, M., Dani, JA. Reward, Addiction, Withdrawal to Nicotine. 2011. <http://dx.doi.org/10.1146/annurev-neuro-061010-113734>
74. Azizian A, Nestor LJ, Payer D, Monterosso JR, Brody AL, London ED. Smoking reduces conflict-related anterior cingulate activity in abstinent cigarette smokers performing a Stroop task. *Neuropsychopharmacology*. 2010; 35:775–82. [PubMed: 19907418]
75. Loughhead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, Lerman C. Working Memory-Related Neural Activity Predicts Future Smoking Relapse. *Neuropsychopharmacology*. 2014; doi: 10.1038/npp.2014.318
76. Wang Z, Ray R, Faith M, Tang K, Wileyto EP, Detre JA, Lerman C. Nicotine abstinence-induced cerebral blood flow changes by genotype. *Neurosci Lett*. 2008; 438:275–280. [PubMed: 18499348]
77. Kozink RV, Lutz AM, Rose JE, Froeliger B, McClernon FJ. Smoking withdrawal shifts the spatiotemporal dynamics of neurocognition. *Addict Biol*. 2010; 15:480–90. [PubMed: 21040240]
78. Lawrence NS, Ross TJ, Stein EA. Cognitive Mechanisms of Nicotine on Visual Attention. *Neuron*. 2002; 36:539–548. [PubMed: 12408855]
79. Falcone M, Wileyto EP, Ruparel K, Gerraty RT, Laprate L, Detre JA, et al. Age-related differences in working memory deficits during nicotine withdrawal. *Addict Biol*. 2013; doi: 10.1111/adb.12051
80. Loughhead J, Ray R, Wileyto EP, Ruparel K, Sanborn P, Siegel S, et al. Effects of the alpha4beta2 partial agonist varenicline on brain activity and working memory in abstinent smokers. *Biol Psychiatry*. 2010; 67:715–21. [PubMed: 20207347]

Figure 1. Seeds and Targets

Clusters in Dorsal, Ventral and Posterior insula served as seeds for static rsFC (see Table S2 for coordinates) with targets comprised of group ICA-derived voxel maps of each of three large-scale networks relevant to attention and addiction: Executive Control Network (ECN), Salience Network (SN), and Default Mode Network (DMN). All rsFC comparisons were corrected for multiple comparisons.

Figure 2. s-rsFC results

Maps of circuits showing a significant SESSION effect (left) and extracted s-rsFC values from identified clusters during satiety (green) and abstinence (red) in A) VEN (purple), B) POS (orange), and C) DOR (yellow) insula. Bar plot of the observed effect is included to aid interpretation.

Figure 3. Dynamic rsFC results

Markov matrices describing transitions between identified states during satiety (top) and abstinence (bottom). P(remain in state) calculated as sum of diagonal within each matrix. A significant effect of SESSION on P(remain in state) is observed.

Figure 4. rsfc Correlations

Significant correlations between s-rsFC and WSWS measures are observed between A) POS-IFC and WSWS sadness (1) and POS-dACC circuit and WSWS anger (2) during satiety, and between B) VEN-dlPFC circuit and WSWS Craving during abstinence

Table 1

Participant Demographics

	n=20
Gender (M/F)	11/9
Race (AA/C)	10/10
Age at Scan 1	39.7 (11.3)
WASI IQ	98.0 (12.7)
Years Edu	13.5 (2.4)
FTND ^a	4.8 (1.6)
CPD ^b	13.8 (5.1)
Years Smoking	20.3 (10.2)

^aFagerstrom Test of Nicotine Dependence

^bCigarettes per day

Table 2

Abstinence Verification

	Sated (scan 1)	Abstinent (scan 2)	t-stat	p
Physiological				
expired CO (ppm)	23.4 (10.8)	2.35 (1.1)	-9.35	<0.0001
Behavioral: RVIP				
d'	1.46 (0.88)	1.25 (0.68)	-2.28	0.017
Subjective: WSWs				
Anger	3.25 (2.3)	5.75 (2.5)	3.60	0.0009*
Anxiety	6.10 (3.1)	7.70 (2.0)	2.16	0.0215
Concentration	2.84 (1.6)	4.85 (2.9)	3.09	0.0030*
Craving	9.26 (3.3)	11.47 (3.4)	2.83	0.0053*
Hunger	10.75 (3.0)	12.45 (4.2)	2.18	0.0218
Sadness	3.21 (2.5)	4.89 (2.3)	3.14	0.0027*
Sleep	6.05 (2.8)	8.70 (4.8)	2.56	0.0097
TOTAL	41.7 (13.4)	56.4 (13.5)	4.25	0.0002*

*
p < 0.0063