because of additional mechanisms suppressing stable B-T interactions in GCs.

During the selection process in GCs, many GC B cells die and are visible by histology as "tingible bodies" inside macrophages (*1*). The cell bodies of dying GFP$^+$ GC B cells were observed to undergo fragmentation (Fig. 4G), but surprisingly, this occurred outside of macrophages (movie S11). Blebs of dead GFP$^+$ GC B cells appeared to be taken up by multiple macrophages (movie S11), although some blebs moved rapidly away from the original location of cell death, as if carried by motile cells (movie S2). Indeed, some GFP$^+$ B cell blebs were attached to and carried by rapidly migrating CFP$^+$ T cells (Fig. 4, H and I, and movie S12). All T cells that carried GFP$^+$ B cell blebs had a median velocity greater than 10 μm/min (Fig. 4E), suggesting that they were not undergoing stable interactions with living B cells. The GFP$^+$ GC B cells represent only about 1 to 2% of GC B cells, and we observed about 0.5% of T cells carrying GFP$^+$ blebs; by extrapolation, at least one quarter of the GC T cells may be associated with one or more blebs from dead GC B cells. A higher frequency of bleb–T cell interactions were stable compared with live B cell–T cell interactions (Fig. 4, C and J), suggesting that these dead B cell fragments may affect the availability of T cell help in GCs.

Our findings reveal that GC B cells are highly motile and exhibit a probing behavior as they travel over the antigen-bearing FDC network. The lack of GC B cell pausing suggests that the selection mechanism does not involve competition for adhesion to FDCs, whereas the rapid movement of B cells in close proximity to each other raises the possibility that high-affinity cells remove surface-bound antigen from lower-affinity cells. The observed migration of GC B cells from light to dark zones is consistent with GC B cells undergoing repeated rounds of mutation and selection within a given GC (*17*). Our estimate that GC B cells spend only several hours in the light zone suggests a limited amount of time to access helper T cells. Given that stable interactions of GC B cells with GC T cells were infrequent, it seems possible that T cell help is a limiting factor driving selection of higher-affinity B cell clones. In vitro studies have shown that T cells responding to antigen-presenting B cells can be sensitive to variations in the affinity of the B cell receptor across several orders of magnitude (*18*). We propose a selection model in which newly arising mutated GC B cells with higher affinity for antigen obtain and process greater amounts of antigen in a given period of time and then outcompete the surrounding B cells and B cell blebs for the attention of GC T cells.

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Supporting Online Material

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Materials and Methods
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Damage to the Insula Disrupts Addiction to Cigarette Smoking

Nasir H. Naqvi,[1] David Rudrauf,[1,2] Hanna Damasio,[3,4] Antoine Bechara[1,3,4]*

A number of brain systems have been implicated in addictive behavior, but none have yet been shown to be necessary for maintaining the addiction to cigarette smoking. We found that smokers with brain damage involving the insula, a region implicated in conscious urges, were more likely than smokers with brain damage not involving the insula to undergo a disruption of smoking addiction, characterized by the ability to quit smoking easily, immediately, without relapse, and without persistence of the urge to smoke. This result suggests that the insula is a critical neural substrate in the addiction to smoking.

Cigarette smoking, the most common preventable cause of morbidity and mortality in the developed world (*1*), is an addictive behavior. Despite being aware of negative consequences, many smokers have difficulty quitting, and even those who quit experience urges to smoke and tend to relapse (*2*, *3*). These phenomena appear to arise from long-term adaptations within specific neural systems. Subcortical regions, such as the amygdala, the nucleus accumbens, and the mesotelencephalic dopamine system, have been shown in animal models to promote the self-administration of drugs of abuse (*4*, *5*). Functional imaging studies have shown that exposure to drug-associated cues activates cortical regions such as the anterior cingulate cortex, the orbitofrontal cortex, and the insula (*6*–*13*). Among these regions, the insula is of particular interest because of its potential role in conscious urges. The insula has been proposed to function in conscious emotional feelings through its role in the representation of bodily (interoceptive) states (*14*–*16*). Activity within the insula on both sides of the brain has been shown to correlate with subjective cue-induced drug urges (*7*, *8*, *11*). It has also been shown that a high amount of activity in the right insula during a simple decision-making task is associated with relapse to drug use (*17*). Given its potential role in cognitive and emotional processes that promote drug use, the question arises as to whether the insula is necessary for maintaining addiction to smoking. We hypothesized that the insula is a critical neural substrate in the addiction to smoking. We predicted, therefore, that damage to the insula would disrupt addiction to smoking.

We identified 19 cigarette smokers who had acquired brain damage that included the insula (*18*). Six of these patients had right insula damage, and 13 had left insula damage. We also

[1]Division of Cognitive Neuroscience, Department of Neurology, University of Iowa Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA. [2]Laboratory of Computational Neuroimaging, Department of Neurology, University of Iowa Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA. [3]Dornsife Cognitive Neuroscience Imaging Center, SGM 501, University of Southern California, Los Angeles, CA 90089, USA. [4]Brain and Creativity Institute, HNB B26, University of Southern California, Los Angeles, CA 90089, USA.

*To whom correspondence should be addressed. E-mail: bechara@usc.edu

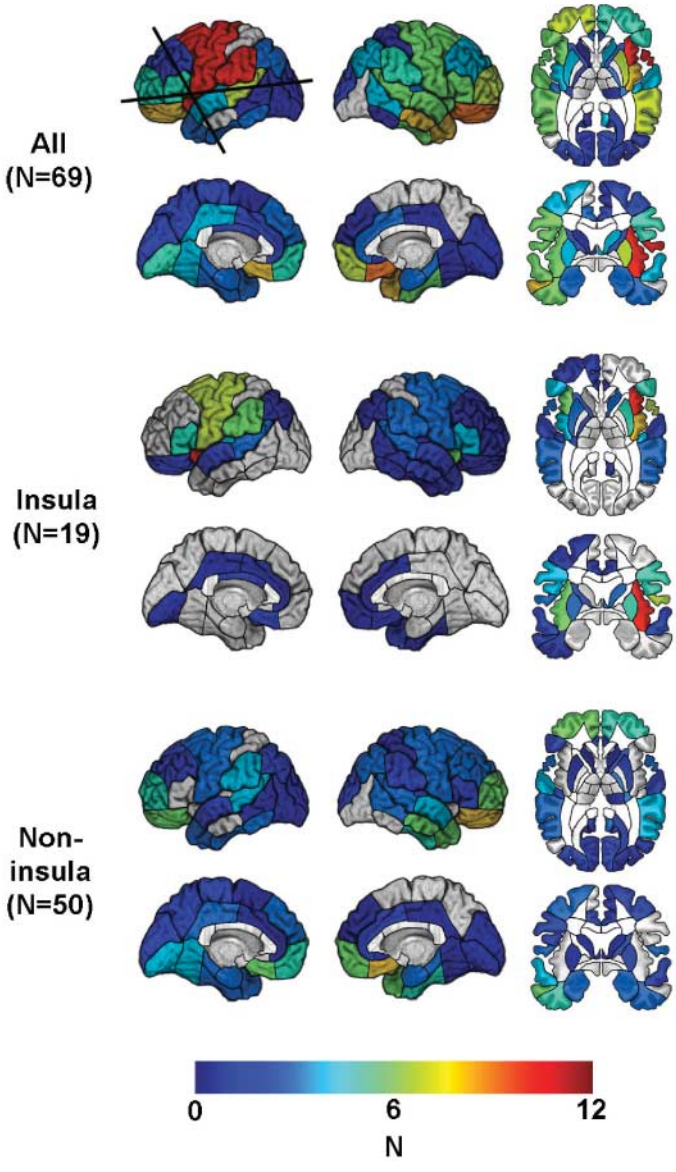

Fig. 1. Number (*N*) of patients with lesion in each of the regions identified in this study, mapped onto a reference brain. Boundaries of anatomically defined regions are drawn on the brain surface. Regions names are provided in the Materials and Methods. Regions not assigned a color contained no lesions. (**Top**) All patients. The horizontal line marks the transverse section of the brain shown in the top row. The vertical line marks the coronal section shown in the bottom row. (**Middle**) Patients with lesions that involved the insula. (**Bottom**) Patients with lesions that did not involve the insula.

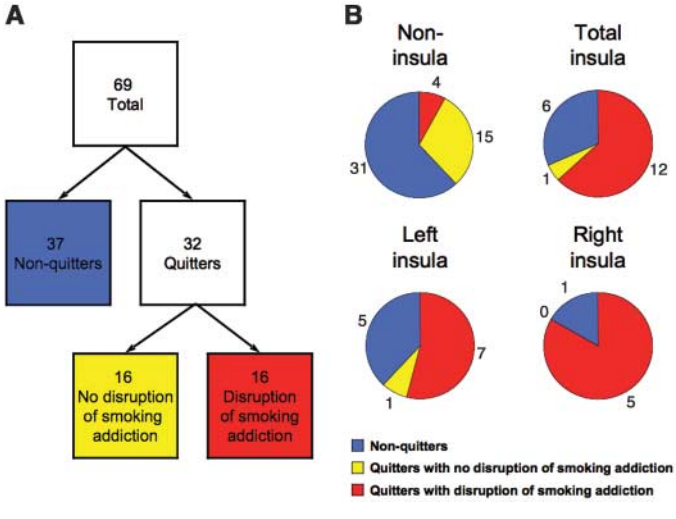

Fig. 2. Patients who quit smoking after lesion onset and patients who underwent a disruption of smoking addiction after lesion onset. (**A**) Tree diagram showing the behavioral classification of patients. (**B**) Pie charts illustrating the proportion of patients in each anatomical group who fell into each of the behavioral categories. The colors correspond to the behavioral group depicted in (A). These actual proportions are shown in the Materials and Methods. The proportion of patients with a disruption of smoking addiction was higher among both left insula–lesioned patients and right insula–lesioned patients compared with among noninsula-lesioned patients.

identified a group of 50 cigarette smokers who had acquired damage that did not include the insula. All of these patients had been smoking more than five cigarettes per day for more than 2 years at the time of lesion onset. The groups were matched with respect to several characteristics, including the number of cigarettes they were smoking at lesion onset, the total number of years they had been smoking at lesion onset, and the etiology of their brain damage (Fig. 1 and table S1).

First, we performed a logistic regression analysis in which the dependent variable was whether or not patients quit smoking some time after lesion onset (i.e., whether or not they were smoking at the time of the study). The independent variable of interest was the extent of damage in the insula on a given side. An estimate of the total extent of the lesion was entered as a nuisance covariable (Materials and Methods). We found that the likelihood of quitting smoking after a lesion in either the right or the left insula was not significantly higher than the likelihood of quitting after a noninsula lesion (odds ratio = 2.94, χ^2 = 2.74, and P = 0.10). When we examined the right and left insulae separately, we found that the likelihood of quitting smoking was not significantly higher after a right insula lesion than after a noninsula lesion (odds ratio = 2.53, χ^2 = 2.98, and P = 0.08), nor was it significantly higher after a left insula lesion compared with after a noninsula lesion (odds ratio = 1.44, χ^2 = 1.12, and P = 0.29) (Fig. 2 and table S3). One explanation of this null finding is that, whereas the insula-lesioned patients may have quit smoking due to a disruption of smoking addiction, the noninsula-lesioned patients may have quit smoking at a similar rate because they were concerned about the negative consequences of smoking. Simply determining whether the patients were smoking at the time of the study did not address this distinction.

To more specifically assess a disruption of smoking addiction, we asked all of the patients who quit smoking after lesion onset a set of questions aimed at their recollection of the experience of quitting. Patients were classified as having had a disruption of smoking addiction if they fulfilled all four of the following criteria: (i) reporting quitting smoking less than 1 day after lesion onset, (ii) reporting that they did not start smoking again after they quit, (iii) rating the difficulty of quitting as less than three on a scale of one to seven, and (iv) reporting feeling no urges to smoke since quitting. According to these criteria, 16 of the patients who quit smoking after lesion onset were classified as having a disruption of smoking addiction. The 16 quitters who failed to meet all four of these criteria, along with all 37 nonquitters, were considered to have no disruption of smoking addiction (Fig. 2).

We performed a logistic regression in which the dependent variable was whether or not patients underwent a disruption of smoking addiction after lesion onset as defined by the above

criteria. As before, the independent variable of interest was the extent of damage to the insula on a given side, whereas the estimate of the total extent of the lesion was entered as a nuisance covariable. We found that the likelihood of having a disruption of smoking addiction after a lesion in either the right or the left insula was significantly higher than the likelihood of having a disruption of smoking addiction after a noninsula lesion (odds ratio = 22.05, χ^2 = 16.64, and P = 0.0005). When we examined the right and left insulae separately, we found that the likelihood of having a disruption of smoking addiction was significantly higher after a right insula lesion than after a noninsula lesion (odds ratio = 10.87, χ^2 = 12.90, and P = 0.0003) and was also significantly higher after a left insula lesion compared with after a noninsula lesion (odds ratio = 3.61, χ^2 = 10.33, and P = 0.001) (Fig. 2 and table S3). Although it appears that effects may be somewhat larger with right insula lesions compared with left insula lesions, the sample sizes were too small to confirm this statistically.

We then conducted a similar logistic regression that included only the patients in our sample who quit smoking after lesion onset (thus, we were not required to assume that patients who continued to smoke after lesion onset had an intact smoking addiction). We found that five of five of the patients who quit smoking after a right insula lesion and seven of eight of the patients who quit smoking after a left insula lesion met the criteria for having a disruption of smoking addiction, compared to 4 of 19 of the patients who quit smoking after a noninsula lesion (right insula–lesioned patients versus noninsula-lesioned patients: odds ratio = 6.55, χ^2 = 7.76, and P = 0.005; left insula–lesioned patients versus noninsula-lesioned patients: odds ratio = 7.19, χ^2 = 10.06, and P = 0.002). Putting the right and left sides together, 12 of 13 patients who quit smoking after a lesion in the insula did so with a disruption of smoking addiction. Relative to noninsula-lesioned patients, this translates into an odds ratio of 136.49 as estimated by the logistic regression (χ^2 = 15.48 and P = 0.00008) (Fig. 2 and table S3).

In our sample, the patients with insula lesions tended also to have damage in adjacent areas (Fig. 1). This raises the question of whether the observed effects were necessarily due to insula damage or whether they required damage in one or more areas adjacent to the insula. To address this issue, we performed a region-by-region logistic regression analysis that estimated, for each region of the brain that we sampled, the likelihood of having a disruption of smoking addiction after a lesion that included the region compared to a lesion that did not include the region. This analysis included all of the patients in the sample. We found that the only regions in which lesions were significantly associated with an increased likelihood of having a disruption of smoking addiction were the right and left insulae (Fig. 3). On the left side, there were near-significant effects in regions adjacent to the insula, such as the putamen. We cannot rule out the possibility that some of these regions independently or cumulatively play a role in smoking addiction. For example, evidence from animal studies suggests that the dorsal striatum, which includes the putamen, is involved in learning and expression of drug-use habits (*4*). However, for most of these regions the patients with lesions who had a disruption of smoking addiction also had damage in the insula (table S4), suggesting that apparent effects of lesions in these regions were due to a bystander effect. We did find four patients who had a disruption of smoking addiction after suffering from brain damage that did not involve the insula. When we examined their lesions, we found that each of them had damage in a unique set of regions (table S5). This raises the possibility that certain patients may undergo a disruption of smoking addiction as a general effect of suffering from a brain injury.

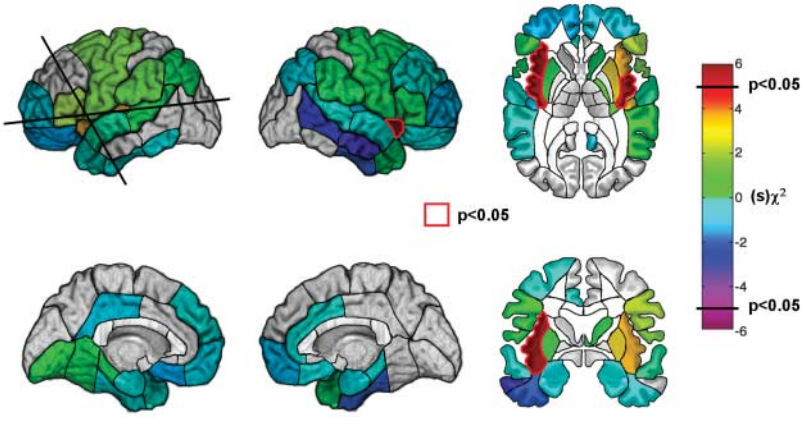

Fig. 3. Whole-brain region-by-region logistic regression analysis. The color of each region corresponds to a χ^2 statistic given the sign of regression coefficient obtained from the logistic regression analysis. The only regions that were assigned a color were those for which the number of patients was sufficient to detect a statistically significant effect (Materials and Methods). Regions for which there was a statistically significant association between a lesion and a disruption of smoking addiction (P < 0.05, uncorrected) are highlighted in red. The insula is the only region on either side of the brain where a lesion was significantly associated with a disruption of smoking addiction. There were nonsignificant effects in regions on the left side that are adjacent to the insula; however, patients with damage in these regions also tended to have damage in the insula (Materials and Methods). The likelihood of having a disruption of smoking addiction was not increased after damage in the orbitofrontal cortex.

The results indicate that smokers who acquire insula damage are very likely to quit smoking easily and immediately and to remain abstinent. In addition, smokers with insula damage are very likely to no longer experience conscious urges to smoke after quitting. These findings are consistent with previous functional imaging evidence showing that activity in the insula is correlated with subjective drug urges (*7*, *8*, *11*). Additionally, the results provide evidence that subjective urges are an important factor in maintaining smoking addiction. However, urges may not be the only factor that promotes smoking. Recent theories of addiction propose that usual drug use in addicted individuals is driven primarily by automatic or implicit motivational processes, such as habits (*4*) and incentive salience wanting (*19*). Conscious urges come into play when there is an impediment to drug use, such as an attempt to quit or to resist relapse (*20*). The present results are consistent with this view. However, it remains to be seen whether insula damage spares the automatic tendency to smoke. It also remains to be seen whether patients with insula damage still obtain pleasure from smoking, because pleasure and urge may be dissociable facets of smoking reward (*19*).

Our sample included a number of patients with damage to the orbitofrontal cortex (Fig. 1), a region that, like the insula, has been implicated by functional imaging studies to play a role in conscious drug urges (*6*, *8*, *9*, *11*–*13*). We found no association between lesions in the orbitofrontal cortex and a disruption of smoking addiction (Fig. 3 and table S4). One explanation of this result is that smokers who acquire orbitofrontal damage experience a reduction in conscious urges but continue to smoke because their automatic tendency to smoke is still intact. At the same time, these patients may have a low likelihood of attempting to quit smoking after suffering from a brain injury, because the orbitofrontal region is critical for decisions that override the automatic tendency to obtain immediate rewards in order to avoid future negative consequences (*21*, *22*). Insula-lesioned patients, in contrast, may not have such severe decision-making deficits and thus may be likely to attempt to quit smoking after suffering from a brain injury.

The results of this study suggest that the insula is a critical neural substrate for the urge to

smoke, although they do not in themselves indicate why the insula, a region known to play a role in the representation of the bodily states (*16*), would play such an important role in urge. A clue may be provided by the account of one patient in our sample who quit smoking immediately after he suffered a stroke that damaged his left insula. He stated that he quit because his "body forgot the urge to smoke" (*23*). His experience suggests that the insula plays a role in the feeling that smoking is a bodily need. Indeed, much of the pleasure and satiety that is obtained from smoking is derived from its bodily effects, in particular its impact on the airway (*24*, *25*). In addition, nicotine withdrawal is associated with changes in autonomic and endocrine function (*26*, *27*), which may contribute to its unpleasantness. Current evidence suggests that the insula plays a role in conscious feelings by anticipating the bodily effects of emotional events (*14*, *15*). The insula may therefore function in the conscious urge to smoke by anticipating pleasure from the airway effects of smoking and/or relief from the aversive autonomic effects of nicotine withdrawal. Thus, damage to the insula could lead a smoker to feel that his or her body has "forgotten" the urge to smoke.

An important question pertains to whether insula lesions cause a disruption of motivated behaviors other than smoking. In a follow-up survey, we found that none of the patients with insula damage who had a disruption of smoking addiction admitted to any reductions in their pleasure from eating, their desire to eat, or their intake of food. This does not preclude the possibility that these patients had some impairment of taste perception (*28*, *29*) or had deficits in other motivated behaviors that we did not assess. One possibility is that motivated behaviors that are fundamental to survival, such as eating, are supported by redundant neural mechanisms that are difficult to disrupt with a lesion in a single brain region. A related possibility is that the insula is critical for behaviors whose bodily effects become pleasurable through learning; although the bodily effects of eating are inherently pleasurable, the bodily effects of smoking are initially aversive and become pleasurable as addiction develops (*25*). It would be interesting to see how insula damage affects other learned pleasures.

Our findings suggest that therapies that modulate the function of the insula will be useful in helping smokers quit. For example, sensory replacements for smoking, such as denicotinized cigarettes and irritant inhalers, are highly effective in reducing urges and promoting abstinence (*30*, *31*). Such therapies may work by engaging sensory representations of the airway within the insula, thereby satisfying the "bodily need" to smoke. Future pharmacologic therapies may target neurotransmitter receptors that are expressed within the insula. In addition, the efficacy of various smoking cessation therapies may be monitored by measuring activity within the insula with functional brain imaging. Lastly, the findings of this study demonstrate that conscious feelings, such as urges, are an important component of addiction.

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18. All of the patients in this study were selected from the patient registry of the Division of Behavioral Neurology and Cognitive Neuroscience, Department of Neurology, University of Iowa. Selection criteria are detailed in the Materials and Methods. The insula was defined here as the region bounded anteriorly by the limen insulae and posteriorly, inferiorly, and superiorly by the circular insular sulcus. This included both anterior and posterior insular cortices and underlying white matter.
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23. Patient N. is a right-handed male who was 38 years old when we interviewed him. N. started smoking at the age of 14. At the time of his stroke, he was smoking more than 40 unfiltered cigarettes per day and was enjoying smoking very much. By his own admission, he was heavily addicted to smoking. He recalled that he used to experience frequent urges to smoke, especially upon waking, after eating, when he drank coffee or alcohol, and when he was around other people who were smoking. He often found it difficult to refrain from smoking in situations where it was inappropriate, e.g., at work or when he was sick and bedridden. He was aware of the health risks of smoking before his stroke but was not particularly concerned about those risks. Before his stroke, he had never tried to stop smoking, and he had had no intention of doing so. N. smoked his last cigarette on the evening before his stroke. When asked about his reason for quitting smoking, he stated simply, "I forgot that I was a smoker." When asked to elaborate, he said that he did not forget the fact that he was a smoker but rather that "my body forgot the urge to smoke." He felt no urge to smoke during his hospital stay, even though he had the opportunity to go outside to smoke. His wife was surprised by the fact that he did not want to smoke in the hospital, given the degree of his prior addiction. N. recalled how his roommate in the hospital would frequently go outside to smoke and that he was so disgusted by the smell upon his roommate's return that he asked to change rooms. He volunteered that smoking in his dreams, which used to be pleasurable before his stroke, was now disgusting. N. stated that, although he ultimately came to believe that his stroke was caused in some way by smoking, suffering a stroke was not the reason why he quit. In fact, he did not recall ever making any effort to stop smoking. Instead, it seemed to him that he had spontaneously lost all interest in smoking. When asked whether his stroke might have destroyed some part of his brain (fig. S2) that made him want to smoke, he agreed that this was likely to have been the case.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5811/531/DC1
Materials and Methods
Figs. S1 and S2
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10.1126/science.1135926

SUPPORTING MATERIALS

Detailed Methods

Subjects. All of the patients included in this study were drawn from the Patient Registry of the Division of Behavioral Neurology and Cognitive Neuroscience, Department of Neurology, University of Iowa. We reviewed the patients in the Registry to determine if they met the following inclusion criteria: they did not suffer from amnesia; they were not severely aphasic; their lesions were stable (i.e. non-progressive) and chronic (>6 months old); their lesions could be visualized using T1-weighted MRI or CT; and they were not addicted to other drugs of abuse at the time of lesion onset per their medical records. A total of 307 patients who met these inclusion criteria were contacted for this study to determine their smoking history. One hundred and seventy-nine of these patients reported never smoking. Fifty-nine reported smoking at some time, but quitting a number of years before lesion onset. Sixty-nine reported that they were smoking more than 5 cigarettes per day for more than 2 years at the time of lesion onset. These patients were the subjects of this study.

We recorded the following information for each subject: sex, current age, age at lesion onset, years since lesion onset, number of cigarettes smoked per day at lesion onset, current number of cigarettes smoked per day (current smokers only), number of years smoking at lesion onset, length of hospital stay, and psychotropic drugs administered during the hospital stay, including antidepressants, antipsychotics, anxiolytics and antiseizure medications. Medication records were obtained from the medical chart. For

patients with strokes, the time of lesion onset was defined as the day on which the stroke occurred. For patients with surgical resection of meningiomas and epileptic foci, the time of lesion onset was defined as the day of the surgery. Insula lesioned patients and non-insula lesioned patients were compared with respect to each of these parameters, using unpaired t-tests to compare means and $\chi 2$ tests to compare proportions (Supporting Table 1).

Behavioral Classification. The patients who were smoking at lesion onset were administered a brief interview in order to determine their smoking patterns before lesion onset and how these changed in relation to lesion onset. Information was obtained from collaterals when necessary. This interview was conducted by someone who did not know the anatomy of the lesion. All of the patients were asked whether or not they had smoked in the past month. Patients who reported not smoking in the past month were classified as "quitters." Patients who reported smoking during the past month were classified as "non-quitters."

All of the quitters were asked a series of retrospective questions aimed at their experience of quitting smoking in relation to the onset of their lesions. These were: 1) "How soon after your brain injury did you quit smoking?" 2) "How difficult was it to quit smoking after your brain injury, on a scale of 1-7, with 1 being very easy and 7 being very difficult?" 3) "How many times have you started smoking again since your brain injury?" and 4) "Have you experienced any urge to smoke since you (most recently) quit smoking?" Patients who reported that they quit smoking less than 1 day after their brain injury, who rated the difficulty of quitting as less than 3 on a scale of 1-7, who reported

not starting smoking again since their brain injury, and who reported that they felt no urge to smoke since quitting were classified as having a "disruption of smoking addiction."

Anatomy. Most of the patients underwent T1-weighted MR imaging in order to visualize their lesions. Several patients underwent CT imaging instead of MR imaging due to the presence of ferromagnetic elements in their bodies. Lesions were examined by an expert (H.D.) who determined the proportion of damage to each of 54 different regions of interest (ROIs) (Supporting Figure 1, Supporting Table 2). These ROIs correspond to the historical research interests of our laboratory. The parcellation of ROIs is based upon sulci, gyri and other gross anatomical landmarks, as previously described (*1*). All cortical regions included both gray matter and sub-adjacent white matter.

The proportion of damage to each ROI was specified as follows: 0 = no lesion at all within the ROI, 1 = 0-25% of the ROI damaged by the lesion, 2 = 25-75% of the ROI damaged by the lesion and 3 = 75-100% of the ROI damaged by the lesion. For each patient, 3 different parameters were calculated to describe the extent of damage to the insula. First the proportion of damage to the insula on a given side was estimated by averaging the numbers representing the proportions of damage to the anterior and posterior insulae, respectively, on that side. Next, the proportion of damage to the total insula (left or right) was estimated by averaging the numbers representing the proportions of insula damage to the anterior and posterior insula on the right and left sides. This calculation treated the right and left insulae as a single region.

For each subject, we estimated an index of the total extent of the lesion by adding the numbers representing the proportion of damage in a region across all of the regions damaged in that subject. The index of total lesion extent was found to be significantly larger for subjects with insula lesions (mean = 15.1, S.D. = 10.9) than for subjects with non-insula lesions (mean = 7.7, S.D. = 5.7) [$t(68) = 3.28$, $p = 0.002$]. This raised the possibility that effects seemingly due to insula lesions were instead due to a greater number of anatomically distinct regions affected. For this reason, the index of total lesion extent was entered as a nuisance covariable in all of the logistic regression analyses (see below).

To illustrate how the various lesion-related parameters were calculated, we will describe the lesion of patient N., who reported that his "body forgot the urge to smoke." (Supporting Figure 2). The proportion of damage in the different ROIs affected by the lesion was as follows: 2 in the left transverse temporal gyrus, 3 in the left posterior superior temporal gyrus, 2 in the left supramarginal gyrus, 1 in the left anterior insula, 3 in the left posterior insula, and 1 in the left putamen. The estimated proportion of damage to the left insula was 2 [$(1+3)/2 = 2$], corresponding to 25-75% of damage. The estimated proportion of damage to the right insula was 0, since the lesion did not include any damage on the right side. The estimated proportion of total insula damage was 1 [$(1+3+0+0)/4 = 1$], corresponding to 0-25% of total insula damage. The estimated total lesion extent was 12 ($2+3+2+1+3+1 = 12$).

Statistical Analysis and Data Processing. Three different sets of logistic regression analyses were performed that were focused on different behavioral effects of insula

lesions. In the first set of analyses, the binary dependent variable was whether a patient was classified as being a quitter ("1") or a non-quitter ("0") after lesion onset. In the second set of analyses, the binary dependent variable was whether a patient met all of the criteria for having a disruption of smoking addiction after lesion onset ("1") or did not meet all of these criteria ("0"). This set of analyses included all 69 patients, including the 37 patients who did not quit smoking after lesion onset. By definition, patients who did not quit smoking after lesion onset did not meet the criteria for having a disruption of smoking addiction (i.e. they were assigned a "0"). In the third set of analyses, the binary dependent variable was again whether a patient met all of the criteria for having a disruption of smoking addiction ("1") or did not meet all of these criteria ("0"). However, this third set of analyses was limited to the 32 subjects who quit smoking after lesion onset. Because this analysis excluded non-quitters, it did not require us to assume that non-quitters had an intact smoking addiction.

The first analysis in each set compared the effects of insula lesions on either side of the brain to the effects of non-insular lesions. For this analysis, the independent variable of interest was the estimated proportion of the total insula lesioned, as calculated above. The second analysis in each set compared the effects of left insula lesions to the effects of non-insular lesions. For this analysis, the independent variable of interest was the estimated proportion of damage to the left insula, as calculated above. This analysis excluded subjects with right insula lesions. The third analysis compared the effects of right insula lesions to the effects of non-insular lesions. For this analysis, the independent variable of interest was the estimated proportion of damage to the right insula, as calculated above. This analysis excluded subjects with left insula lesions. For

each analysis, the index of the total lesion extent was entered as a nuisance covariable. The thresholds for statistical significance were Bonferroni corrected, to adjust for multiple comparisons (uncorrected $\alpha = 0.05$).

Next, a whole-brain analysis was performed to address the possibility that apparent effects of insula lesions on smoking addiction were actually due to lesions in regions adjacent to the insula. This analysis included all of the patients in the sample. Each region of the brain was treated as a separate analysis. For each region, the independent variable of interest was the proportion of damage to that region, as estimated above. The binary dependent variable was whether the patient met all of the criteria for having a disruption of smoking addiction after lesion onset ("1") or did not meet all of these criteria ("0"). The index of the total lesion extent was entered as a nuisance covariable. The thresholds for statistical significance were uncorrected, so that significant effects in regions near the insula were less likely to be excluded due to Type-II error.

Note that for the analyses of the effects of insula lesions vs. non-insula lesions (Table 1), patients with lesions in the insula on a given side were compared to patients without insula lesions (i.e. patients with lesions in the contralateral insula were excluded). In contrast, in the whole-brain region-by-region analysis, patients with insula lesions on a given side were compared to patients with lesions in all other regions, *including the contralateral insula.* This could in part explain differences in results between these two analyses. Further differences may be explained by the fact that whereas the whole brain analysis considered the anterior and posterior insula as separate regions, the comparison of insula lesions to non-insula lesions did not.

All of the logistic regression analyses used Frith's penalized likelihood estimation (*2*), adapted for logistic regression (*3*). This approach is preferable to the more commonly used Wald test since it reduces bias in maximum likelihood estimates and provides a solution to the problem of separation, or monotonous likelihood. This occurs when one of the independent variables perfectly predicts the dependent variable, which is more likely to occur in small samples. For example, only 4 subjects in our sample had lesions in the right posterior insula and all of them met the criteria for having a disruption of smoking addiction.

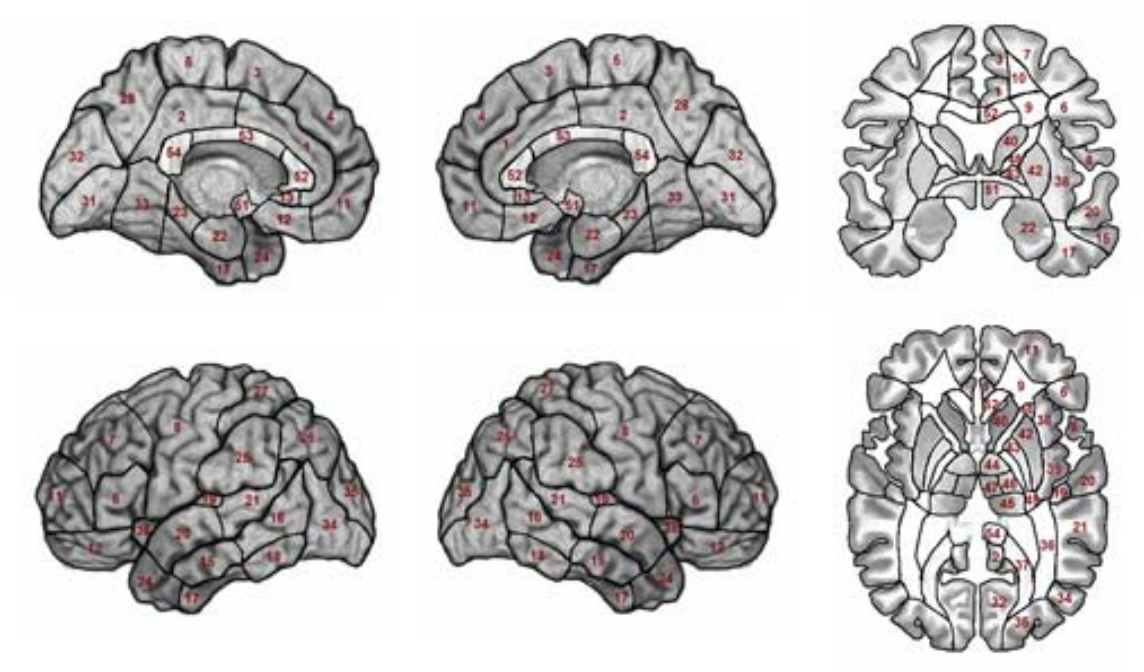
Penalized likelihood estimation contrasts the full model with a nested model that does not contain the independent variable of interest. This results in a penalized likelihood ratio that described the likelihood of having a particular behavioral outcome (e.g. quitting smoking) given the proportion of damage within a specific region (the independent variable), controlling for the estimated total extent of the lesion (the nuisance covariable). The log of this penalized likelihood ratio is multiplied by a coefficient to obtain a parameter that is equivalent to a $\chi 2$ statistic. Statistical significance is then tested using a standard $\chi 2$ distribution, with the degrees of freedom equal to the number of covariates in the full model minus the number of covariates in the nested model (there was 1 degree of freedom for all of the analyses that we performed).

For certain ROIs in the whole-brain region-by-region analysis, only a very small number of subjects had a lesion in the region, leading to problems of statistical power. We therefore attempted to differentiate between ROIs in which significant results could not be observed because of a low sample size and ROIs in which significant results could not be observed because of the absence of an actual effect. We did this by calculating,

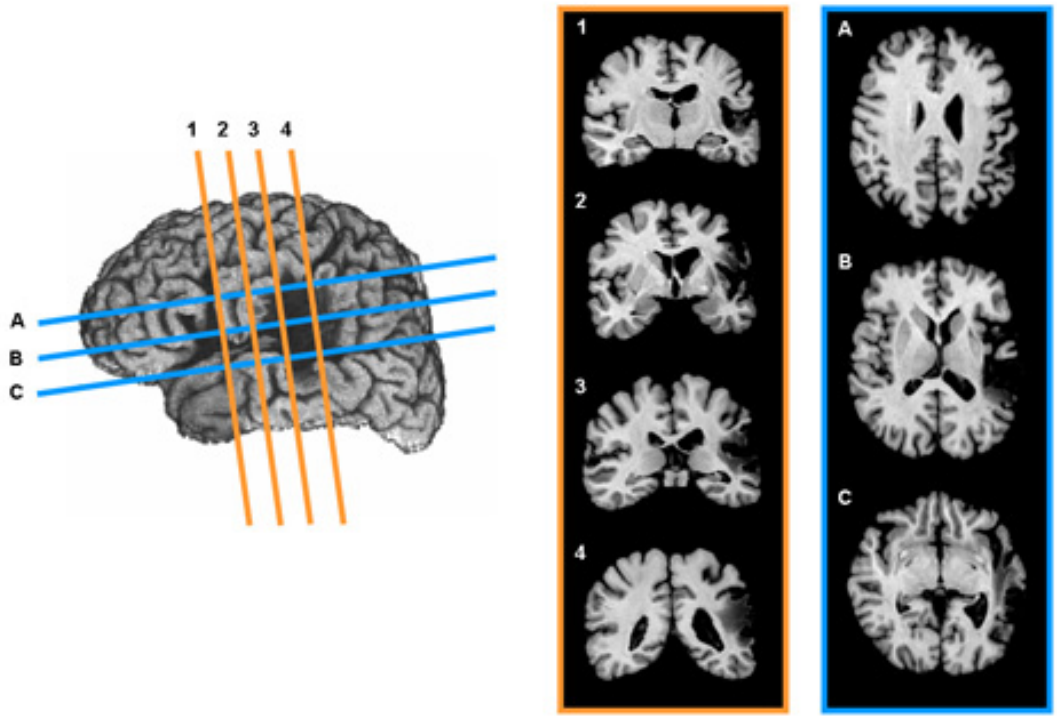
for each ROI, the minimum number of subjects necessary to reach significance in the case where the independent variable of interest perfectly predicted the dependent variable, controlling for the nuisance covariable. We used this number as a threshold in all of the statistical parametric maps, assigning values/colors only to ROIs that passed this threshold. Note that this threshold depended upon the total number of subjects with lesions in the ROI, which is the same for all the analyses, as well as upon the total number of subjects who had the behavioral outcome of interest, which differed depending upon the specific behavioral outcome being examined.

The analyses were performed in Matlab (MathWorks, Inc., Natick, MA), which invoked an R package (www.r-project.org) that performed the logistic regression (*4*). The data describing the number of subjects with lesions in each ROI and the $\chi 2$ values resulting from the logistic regression for each ROI were mapped, using Matlab, onto lateral, mesial, coronal and horizontal views of the same reference brain used in all of the figures. The ROIs were traced onto the reference brain using the aforementioned parcellation scheme. In order to facilitate the interpretation of the results, we mapped the $\chi 2$ statistic using the sign of the regression coefficient describing the slope of the relationship between the dependent variable and the independent variable of interest. This allowed us to indicate both the strength and direction of the effect using a single color scale. As stated above, only ROIs in which there were a sufficient number of subjects to detect statistically significant effects if they existed were assigned a color. Regions in which the $\chi 2$ value surpassed the threshold for statistical significance ($p<0.05$, 2-tailed, uncorrected) were highlighted in red.

Supporting Figure 1



Supporting Figure 2



Supporting Table 1

	Insula (N=19)	Non-insula (N=50)	t(67)/χ2(1)
N females	6	19	0.24
Age	57.2 (9.6)	53.7 (11.4)	1.20
Age at lesion onset	48.4 (14.1)	45.4 (12.0)	0.88
Years since lesion onset	8.8 (8.3)	8.2 (7.5)	0.26
Cigarettes/day at lesion onset	27.0 (13.9)	27.1 (14.6)	0.03
Years smoking at lesion onset	27.8 (12.8)	26.74 (12.4)	0.31
Days in hospital	12.1 (11.7)	11.4 (13.5)	0.18
N antidepressant in hospital	2	3	0.41
N anti-anxiety in hospital	2	6	0.01
N anti-seizure in hospital	4	5	1.48
N antipsychotic in hospital	1	1	0.43

Means were compared using t-tests (standard deviations are in parentheses). Proportions were compared using χ^2 tests. There were no significant differences between the two groups with respect to any of these parameters ($p<0.05$, uncorrected).

Supporting Table 2

Number	Region Name
1	anterior cingulate gyrus
2	posterior cingulate gyrus
3	supplementary motor area
4	medial prefrontal region
5	medial somatomotor region
6	frontal operculum
7	prefrontal region
8	lateral somatomotor region
9	frontal paraventricular white matter
10	frontal supraventricular area
11	frontal pole
12	orbitofrontal cortex
13	basal forebrain
14	subventricular region
15	anterior middle temporal gyrus
16	posterior middle temporal gyrus
17	anterior inferior temporal gyrus
18	posterior inferior temporal gyrus
19	transverse temporal gyrus
20	anterior superior temporal gyrus
21	posterior superior temporal gyrus
22	anterior parahippocampal gyrus
23	posterior parahippocampal gyrus
24	temporal pole
25	supramarginal gyrus
26	angular gyrus
27	lateral superior parietal lobule

Number	Region Name
28	medial superior parietal lobule
29	parietal paraventricular region
30	parietal supraventricular region
31	infracalcarine region
32	supracalcarine region
33	temporo-occipital junction
34	lateral inferior occipital region
35	medial superior occipital region
36	occipital paraventricular area
37	forceps major
38	anterior insula
39	posterior insula
40	head caudate nucleus
41	body caudate nucleus
42	putamen
43	globus pallidus
44	anterior thalamus
45	posterior thalamus
46	lateral thalamus
47	mesial thalamus
48	anterior limb internal capsule
49	posterior limb internal capsule
50	genu internal capsule
51	hypothalamus
52	genu corpus callosum
53	body corpus callosum
54	splenium corpus callosum

The numbers identify the brain regions in Supplementary Figure 1

Supporting Table 3

	Left insula	Right insula	Total insula	Non-insula
% Quitting	61.5	83.3	68.4	38.0
% DSA - all patients	53.8*	83.3**	63.2**	8.0
% DSA - quitters only	87.5*	100*	92.3**	21.1

DSA: disruption of smoking addiction. Symbols next to the percentages reflect p-values for the comparisons between patients in a particular insula lesioned group and patients with non-insula lesions, calculated using logistic regression (*p< 0.05; **p<0.005, Bonferroni corrected).

Supporting Table 4

Side	Region	Total N	N DSA - total	N DSA - insula also lesioned	β_0	β_1	β_2	Pseudo-R^2	Odds ratio	χ^2	p
R	**Anterior insula**	6	5	5	-1.49	1.19	0.52	10.37	3.27	6.41	0.01
R	**Posterior insula**	4	4	4	-1.42	1.47	0.48	8.81	4.35	5.47	0.02
R	**Frontal operculum**	7	4	3	-1.31	0.27	0.57	0.73	1.31	0.45	0.50
R	**Somatomotor region**	6	3	3	-1.26	0.16	0.59	0.13	1.17	0.08	0.77
R	**Supramarginal gyrus**	6	3	3	-1.26	0.10	0.61	0.07	1.10	0.04	0.84
R	**Putamen**	4	2	2	-1.32	0.48	0.59	1.41	1.61	0.88	0.35
R	**Orbitofrontal cortex**	9	1	0	-1.15	-0.32	0.75	0.74	0.74	0.45	0.50
L	**Anterior insula**	12	7	7	-1.52	0.55	0.51	5.98	1.73	3.64	0.06
L	**Posterior insula**	9	6	6	-1.55	0.74	0.52	9.06	2.09	5.54	0.02
L	**Frontal operculum**	6	4	3	-1.39	0.49	0.51	3.54	1.64	2.17	0.14
L	**Somatomotor region**	10	5	4	-1.43	0.47	0.56	3.01	1.60	1.85	0.17
L	**Supramarginal gyrus**	11	5	5	-1.42	0.40	0.60	2.66	1.50	1.63	0.20
L	**Putamen**	8	5	5	-1.45	0.56	0.55	5.17	1.75	3.17	0.08
L	**Orbitofrontal cortex**	8	1	1	-1.11	-0.50	0.70	2.48	0.61	1.52	0.22

Total N: the total number of patients with damage involving the region. N DSA - total: the number of patients with damage in the region who had a disruption of smoking addiction. N DSA - insula also lesioned: the number of patients with damage in the region who had a disruption of smoking addiction and who also had damage in the insula. The β_0, β_1, β_2, pseudo-R^2, odds ratio and χ^2 are all parameters calculated by the logistic regression analyses.

Supporting Table 5

675	2662	2991	3165
L - frontal operculum	R - orbitofrontal cortex	L - parahippocampal gyrus	R - supplementary motor area
L - somatomotor cortex	R - temporal pole	L - infracalcarine cortex	R - medial somatomotor area
		L - temporoccital junction	
		L - posterior thalamus	
		R - temporoccital junction	

Patients with brain damage that did not include the insula who underwent a disruption of smoking addiction. The patient ID is listed in the top row. Each column contains the regions damaged in that patient. Each patient has damage in a unique set of brain regions, i.e, there is no overlap of brain damage.

SUPPORTING FIGURE LEGENDS

Supporting Figure 1. Regions of interest (ROIs) included in this study. A few ROIs that are not displayed in this figure were included, but these contained very few subjects. The numbers correspond to the regions listed in Supporting Table 2. Radiological convention (left on the figure = patient's right side) is used in all brain maps included in this study.

Supporting Figure 2. T1-weighted MR images of N.'s brain, showing brain damage caused by a stroke. The lines drawn on the lateral view indicate the planes of coronal (orange) and horizontal (blue) section. The main area of damage is in the left hemisphere, in the posterior half of the superior temporal gyrus, the lower portion of the supra-marginal gyrus immediately above, and in the posterior two thirds of the insula (the insula includes the cortex, along with the underlying white matter). There is also some damage in the most posterior aspect of the frontal operculum. There is minimal damage to the left putamen.

SUPPORTING NOTES

1. H. Damasio, A. Damasio, *Lesion Analysis in Neuropsychology* (Oxford University Press, New York, 1989), pp.
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4. G. Heinze, M. Ploner, "A SAS macro, S-Plus library and R package to perform logistic regression without convergence problems" *Tech. Report No. 2/2004* (Medical University of Vienna, 2004).