www.sciencemag.org/cgi/content/full/315/5811/531/DC1

Supporting Online Material for

Damage to the Insula Disrupts Addiction to Cigarette Smoking

Nasir H. Naqvi, David Rudrauf, Hanna Damasio, Antoine Bechara*

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

Published 26 January 2007, *Science* **315**, 531 (2007)
DOI: 10.1126/science.1135926

This PDF file includes:

Materials and Methods
Figs. S1 and S2
Tables S1 to S5
References

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.

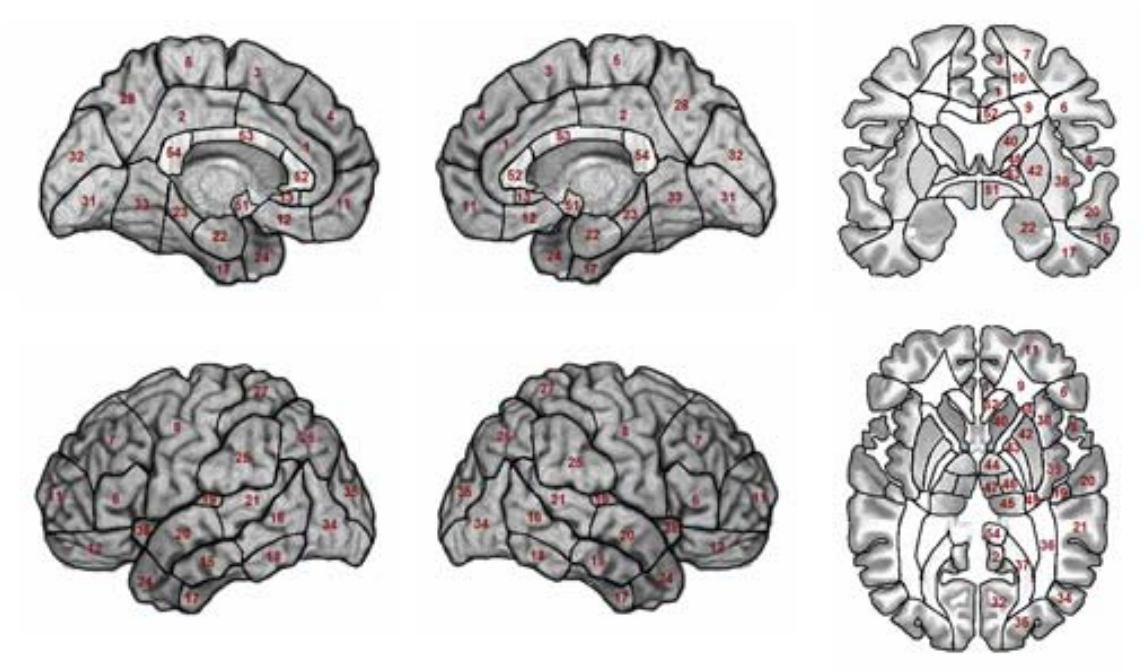
Penalized likelihood estimation contrasts the full model with a nested model that does not contain the independent variable of interest. This results in a penalized likelihood ratio that described the likelihood of having a particular behavioral outcome (e.g. quitting smoking) given the proportion of 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,

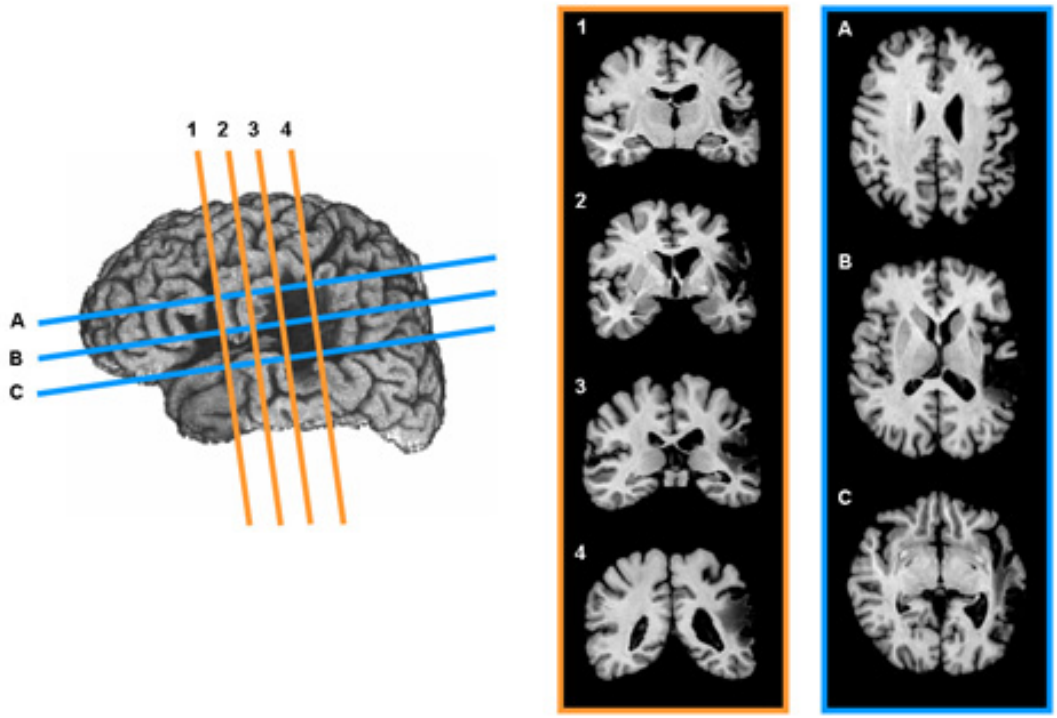
for each ROI, the minimum number of subjects necessary to reach significance in the case where the independent variable of interest perfectly predicted the dependent variable, controlling for the nuisance covariable. We used this number as a threshold in all of the statistical parametric maps, assigning 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.
2. D. Frith, *Biometrika* **80** (1993).
3. G. Heinze, M. Schemper, *Statistics in Medicne* **21**, 2409 (2002).
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).