Inactivation of the Interoceptive Insula Disrupts Drug Craving and Malaise Induced by Lithium

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Inactivation of the Interoceptive Insula Disrupts Drug Craving and Malaise Induced by Lithium

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Addiction profoundly alters motivational circuits so that drugs become powerful reinforcers of behavior. The interoceptive system continuously updates homeostatic and emotional information that are important elements in motivational decisions. We tested the idea that interoceptive information is essential in drug craving and in the behavioral signs of malaise. We inactivated the primary interoceptive cortex in amphetamine-experienced rats, which prevented the urge to seek amphetamine in a place preference task. Interoceptive insula inactivation also blunted the signs of malaise induced by acute lithium administration. Drug-seeking and malaise both induced Fos expression, a marker of neuronal activation, in the insula. We conclude that the insular cortex is a key structure in the perception of bodily needs that provides direction to motivated behaviors.

An important factor that contributes to drug-seeking in addicted individuals is the negative affective state that results from abstinence, described as increased anxiety, irritability, and sadness (*1*). Also important, at least at initial stages of addiction, are the bodily changes underlying the reinforcing properties of drugs. We hypothesized that affective states are monitored by the interoceptive system and particularly by the insular cortex, known to process homeostatic and emotional information (*2*, *3*). To test this idea, we reversibly inactivated the primary insular cortex in amphetamine-experienced rats and tested them with the place preference paradigm. We also injected naïve rats with malaise-inducing LiCl injections, monitored behavioral measures of malaise, and reversed those signs by inactivating the insula. A preliminary account has been presented (*4*).

Place preference tests (PPTs) used a two-compartment biased apparatus (*5*, *6*), with one white compartment paired with amphetamine (or saline) administration, connected by a brown alley to a black compartment paired with saline. Rats were placed in the connecting alley at the beginning of each 10-min session. The procedure took 30 days (Fig. 1A). Drug-naïve rats (all rats at PPT1, Fig. 1B) and rats treated with saline in the white compartment (fig. S1) spent more time in the black compartment, following their innate preference for darker places. Amphetamine-treated rats considerably increased the time spent in the white compartment on PPT2. One hour later, they received bilateral injections of a Na^+ channel blocker (2% lidocaine, 1 μl per side) that is effective to reversibly block cortical structures for about 20 min (*7*). Insular cortex inactivation changed the place preference back to the black compartment (Fig. 1B, PPT3) while producing no effects on general ambulation (fig. S2). The effect of insular cortex inactivation was reversible, because amphetamine-treated rats chose the white compartment on PPT4. The specificity of insular cortex inactivation was demonstrated by bilateral injections of lidocaine into the adjacent primary somatosensory cortex or by saline injection into the insular cortex of amphetamine-experienced rats. These rats significantly preferred the white compartment paired with amphetamine (Fig. 1B, PPT3). Amphetamine-experienced rats showed behavioral sensitization, a neural consequence of repeated psychostimulant exposure (fig. S3).

Place conditioning to amphetamine was paralleled by increased Fos immunoreactivity (Fos-ir), a marker of neuronal activation, in the insular cortex (Fig. 2, A and B). In contrast, the primary somatosensory cortex showed no significant activation in amphetamine-experienced compared with saline-treated rats (fig. S4). In addition, amphetamine-experienced rats showed increased Fos-ir in lateral hypothalamic area (LHA) orexin neurons 1 hour after PPT2, as previously demonstrated for cocaine and morphine (*8*) (Fig. 2, C and D).

If the inactivation of the insula disrupted the interoceptive feelings associated with drug clues, then its inactivation should also blunt the behavioral effects of a well-known malaise-inducing agent like LiCl (*9*). To test this idea, we bilaterally injected lidocaine into the insular cortex 5 min before administration of LiCl (0.15 M; 5 ml/kg intraperitoneally). Behavioral assessment started immediately and lasted for 30 min.

Rats injected with saline into the insular cortex or with lidocaine into the primary somatosensory cortex (Fig. 3) before the LiCl injection showed evident signs of malaise (*10*). They quickly laid on their bellies (Fig. 3, A and B), a postural index of malaise, dramatically decreased ambulation, and failed to respond with attentive movements when their cage was tapped. In contrast, the inactivation of the insular cortex blunted the behavioral consequences of LiCl administration (Fig. 3, A and B) for 15 min, a temporal course compatible with the inactivating effect of lidocaine at the dose we used (*7*).

To further evaluate the participation of the insular cortex in the responses to LiCl, we studied Fos-ir in a different group of rats killed 1 hour after LiCl or saline intraperitoneal injections. We found that LiCl administration induced a significant increase in Fos-ir in the anterior (bregma 0.95 to –0.51) insular cortex (Fig. 3C). In humans, the anterior insula is activated by the feeling of disgust induced by certain odors and during the observation of disgusted faces in others (*11*, *12*). Also, electrical stimulation of the insula frequently elicited upsetting gastrointestinal feelings, often associated with nausea (*13*).

The guide cannulae were aimed at the primary interoceptive cortex, located in the posterior granular insular cortex (*7*, *14*–*16*) (Fig. 4A). To confirm that we inactivated this cortical area, in addition to a cytoarchitectonic analysis of the cannulae tracks, we microinjected a different group of rats with the anterograde axonal tracer biotinylated dextran amine (BDA) or the retrograde tracer cholera toxin (CtB) into the same coordinates as the cannula placement (Fig. 4B). These tracer injections were restricted to the posterior granular insular cortex (*14*) and labeled axon terminals (BDA) or cell bodies (CtB) in the visceral thalamic nucleus, VPLpc, indicating that we were inactivating the primary interoceptive cortex (*15*). This interoceptive information is then distributed through more rostral and ventral insular cortices to prefrontal cortices, as well as to limbic structures (*17*, *18*).

Withdrawal symptoms vary a great deal across different drugs, but negative affect symptoms like anxiety, irritability, and sadness are common to all drugs (*1*). A recent study reported that patients with damage to the insular cortex could easily quit smoking because they lost the

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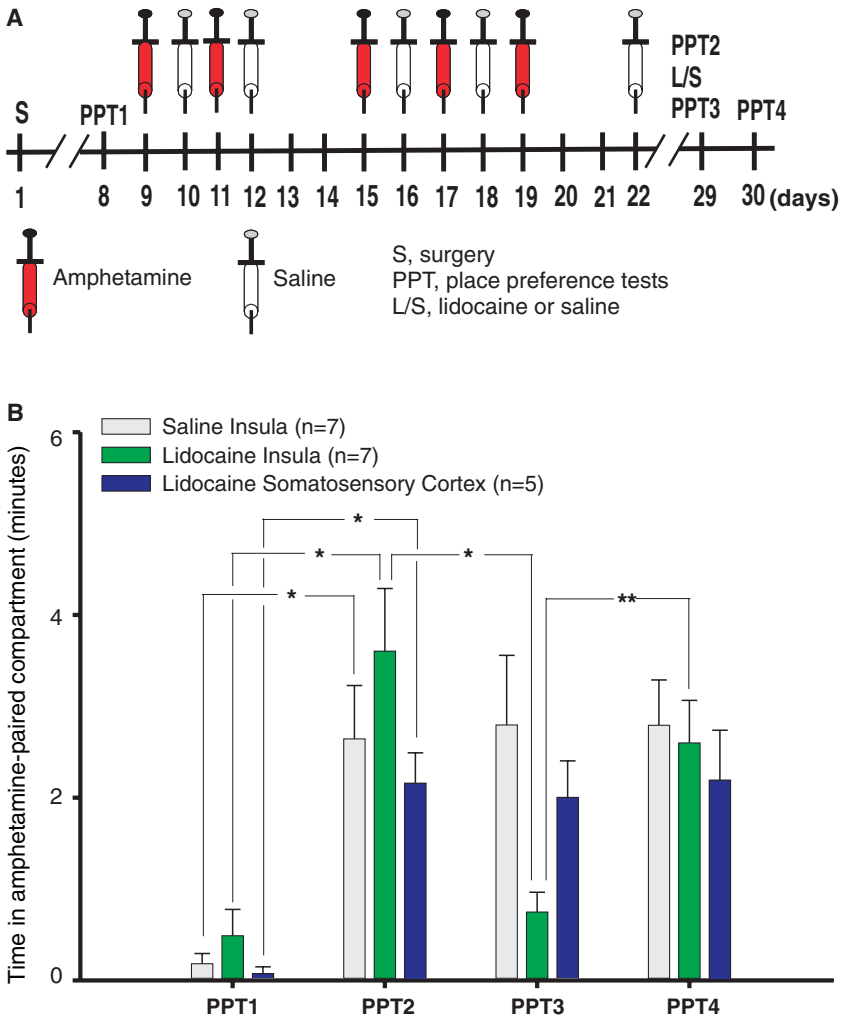

Fig. 1. Amphetamine-experienced rats changed their preference for the drug-paired (white) compartment in a place preference test (PPT) when the insular cortex was reversibly inactivated. (**A**) Time line of amphetamine administration and PPT. (**B**) Time spent in the drug-paired compartment during 10-min PPT sessions. Drug naïve rats strongly chose the black compartment (PPT1). After repeated amphetamine injections, all three groups were conditioned to prefer the white compartment (PPT2). One hour after PPT2, each rat was microinjected into either the primary somatosensory cortex or the insular cortex with lidocaine or saline in the insula 5 min before PPT3 began. Only rats microinjected with lidocaine in the insula reverted to preferring the default black compartment. The next day (PPT4), all groups chose the amphetamine-paired compartment. Single asterisks, $P < 0.001$; double asterisks, $P = 0.004$. Error bars indicate SEM; *n* is the number of rats.

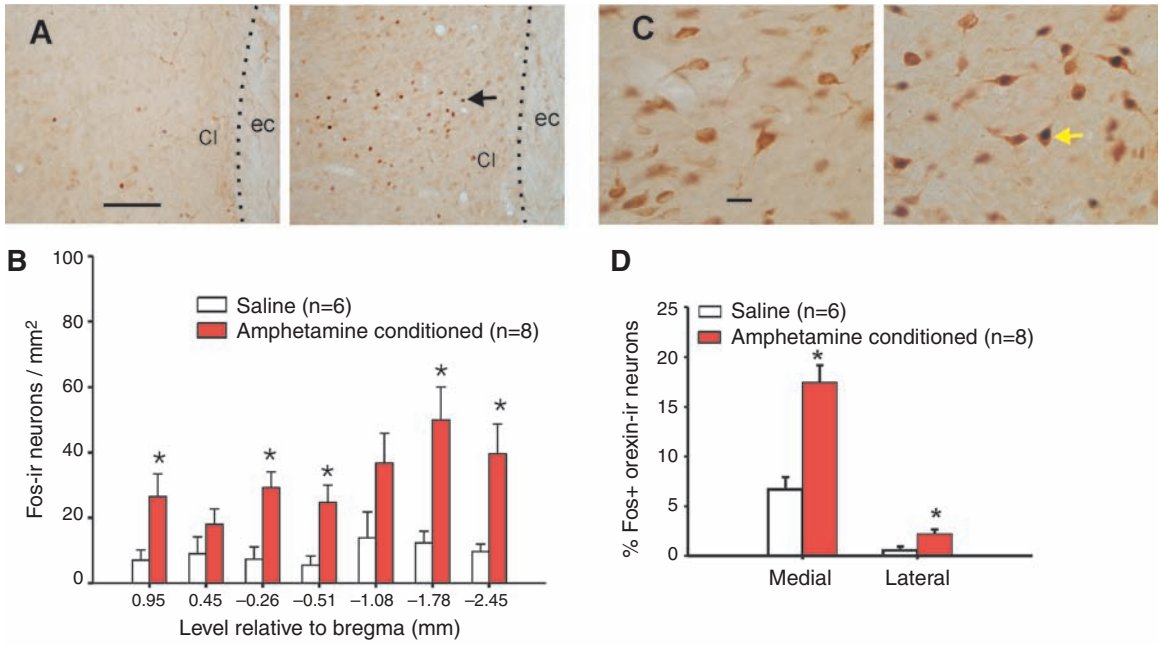

Fig. 2. Amphetamine-experienced rats had significant increases in Fos-ir in the insular cortex and in LHA orexin neurons after the PPT2. (**A**) Deep layers of the granular insular cortex showed near-absence of Fos-ir in a saline-treated rat (left) and significant increase in Fos-ir (arrow) in an amphetamine-experienced rat (right). (**B**) Quantification of Fos-ir in the granular insular cortex at different anteroposterior levels. Asterisks, $P < 0.042$. (**C**) Increased Fos-ir in LHA orexin neurons (arrow) in amphetamine-experienced rats (right) but not in saline controls (left). (**D**) Quantification of Fos-ir in orexin neurons. Note the stronger response of medial LHA neurons. Asterisks, $P = 0.001$ for the medial and $P = 0.011$ for the lateral LHA orexin neurons. Cl, claustrum; ec, external capsule; LHA, lateral hypothalamic area. Scale bars indicate 100 μm for (A) and 20 μm for (C).

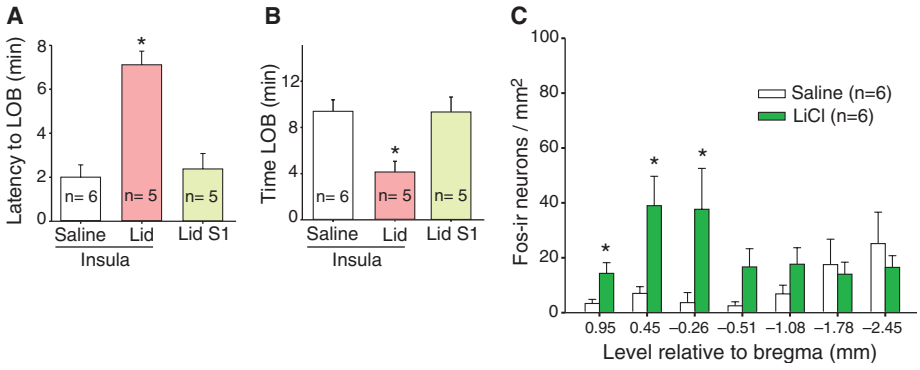

Fig. 3. Inactivation of the insular cortex attenuated malaise induced by LiCl. (**A**) Significant increase ($P = 0.002$) in the latency to lie on belly (LOB) and (**B**) in the time spent LOB ($P = 0.006$) of rats whose insular cortex was inactivated with lidocaine (Lid) compared with rats who received saline injection in the insula or lidocaine into the primary somatosensory cortex (S1). (**C**) LiCl intraperitoneal administration, but not saline, increased Fos-ir in rostral granular insular cortex. Asterisks, $P < 0.025$. Error bars indicate SEM.

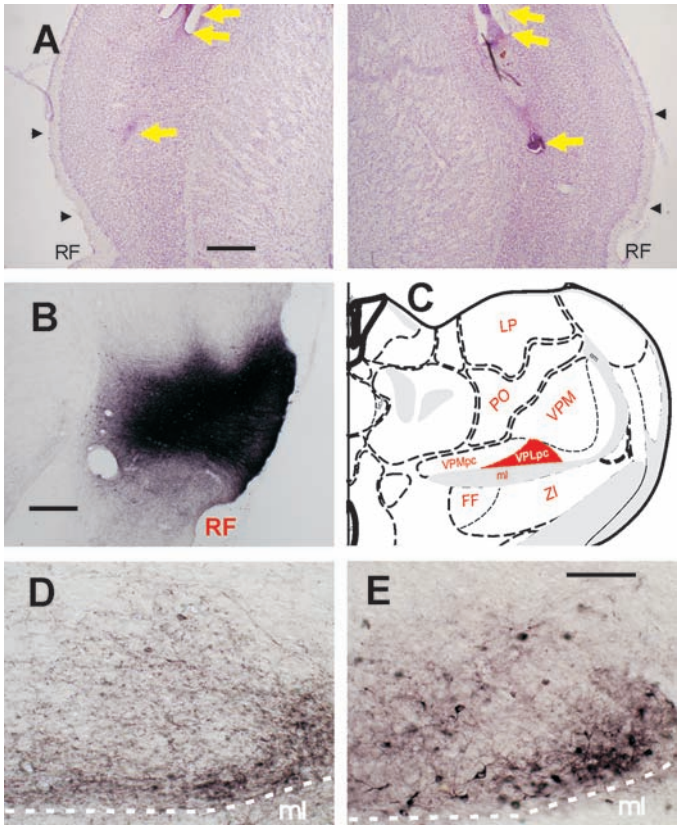

Fig. 4. Lidocaine injections targeted the posterior granular insular cortex. (**A**) Photomicrographs of a Nissl stained section showing the bilateral guide cannula (double arrows) and the injection cannula (single arrows) tracks, aimed at the posterior granular insular cortex (arrowheads). RF, rhinal fissure. (**B**) Photomicrograph of a BDA injection site into the posterior granular insular cortex, i.e., the same insular region inactivated with lidocaine. This small injection labeled axon terminals into the ventral posterolateral nucleus of the thalamus, parvicellular part [VPLpc, shown in red in (C)]. (**C**) Schematic drawing modified from Swanson's atlas (*7*) of the caudal thalamus. (**D**) Axon terminals and a few cell bodies in the VPLpc labeled from the granular insular cortex injection site in (B). Note the distribution of axons within the triangular VPLpc. (**E**) Photomicrograph of retrogradely labeled neuronal cell bodies into the VPLpc after an injection of CtB restricted to the posterior granular insular cortex. Scale bars are 0.5 mm for (A) and (B) and 100 μm for (D) and (E). Abbreviations: FF, fields of Forel; LP, lateral posterior nucleus; ml, medial lemniscus; PO, posterior complex; VPM, ventral posteromedial nucleus; VPMpc, ventral posteromedial nucleus parvicellular part; ZI, zona incerta.

urge to smoke (*19*). The role of this cortex in drug craving is also supported by several imaging studies showing activation of the insula, as well as other cortical and subcortical regions, in addicts with cue-induced drug craving (*20*–*22*). Remarkably, activation of the insular cortex was positively correlated with subjective reports of drug craving (*20*).

The conscious perception of interoceptive signals may be a general role of the insular cortex that explains why its inactivation blunts the urge to get a drug in addicted persons. It remains to be determined whether the insula anticipates the hedonic properties of the drug or whether the insula reports the aversive state associated with withdrawal that could be alleviated by amphetamine, as if it were a medicine. As J. Garcia has shown and discussed (*9*), when a flavored liquid like milk or grapefruit is given to animals recovering from malaise (induced for instance by LiCl), they will seek the flavor that is now predictive of a medicine. The insula is the cortical region that likely underlies conscious perception of the physiological state of the body (*23*), and it is in a key position to distribute this information to orbital, medial, and cingulate prefrontal cortices involved in decision-making (*24*) and to limbic structures involved in emotional responses (*17*, *25*). Interoceptive perception is modulated by attention (*26*), and, like exteroceptive systems, the insular cortex and the interoceptive thalamus (VPLpc) are connected to a particular sector of the thalamic reticular nucleus, a key structure in selective attention (*16*).

Damage to the insular cortex blocked the behavioral expression of a conditioned taste aversion (*27*, *28*), and, as shown here, its reversible inactivation blunted the behavioral responses to an unconditioned stimulus (LiCl) that induced malaise. Patients with insular cortex damage reported no decrease in food intake or desire to eat and no less pleasure in eating (*19*). These findings suggest that the insular cortex is reporting strong deviations from a "well being state," and if so then a low or absent insular cortex activity is interpreted by the brain as "feeling sound." However, insula activation has also been reported in relation to pleasant touch sensations or sexual arousal (*2*). Interestingly, whereas exposure to appetitive food stimuli increased the metabolism of many brain regions including the insula, only the activation of the right orbitofrontal cortex, but not the insula, was correlated with self-reports of hunger and desire for food (*29*).

Our results indicate a key role for the interoceptive insular cortex in the craving for drug in amphetamine-experienced animals and in the perception of malaise induced by lithium administration. Our results further suggest that the modulation of insula activity using noninvasive approaches (*30*) should be considered as a therapeutic target to alleviate the craving for drugs of abuse, as recently proposed by Bechara and colleagues (*19*) for nicotine craving, and, in a more general sense, to ease distressful interoceptive symptoms not related to drug craving.

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