

Insula function in anorexia nervosa

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Anorexia Nervosa

Anorexia nervosa is a morbid, emotionally and above all a life-threatening disorder¹. It is not a choice, but it is a psychiatric disorder, which belongs to the eating disorders¹. It is characterized by a relentless drive to lose weight and an obsession for thinness¹. This relentless pursuit for thinness can eventually lead to serious illness or can even be fatal¹. Despite this life-threatening characteristic the etiology of this disorder is still unknown¹.

There are two types of anorexia nervosa^{1, 2}. The first type is the restricting-type anorexia nervosa in which anorexic individuals lose weight just by dieting and restriction to eat^{1, 2}. The other type is the binge-eating/purging type of anorexia nervosa^{1, 2}. In this type individuals also restrict their food intake but this is periodically interrupted by binge eating and purging as in bulimia nervosa¹. Although anorexia nervosa and bulimia nervosa are both eating disorders and tend to be identical, especially the binge-eating/purging type, there are some fundamental differences. In bulimia nervosa, individuals regularly overeat and have a feeling they can not stop eating, which is followed by vomiting or the use of laxatives^{1, 2}. This has to be done at least twice a week for about three months contiguous to be diagnosed as bulimia, while this is less frequently in binge-eating/purging type anorexia or even absent in restricting type anorexia¹. Here anorexia nervosa will be referred as the restricting type anorexia.

Anorexia nervosa is one of the most homogenous psychiatric disorder as can be seen by the symptoms of this disorder³. The main symptoms of anorexia nervosa are a distorted body image, dieting despite being thin or even critically underweight and the perception of being overweight while being underweight with a tremendous fear to gain weight^{1, 3}. The age of onset falls in a narrow range, mostly in the early adolescence, and it has a relatively female specificity^{1, 3}. They have a powerful resistance to eat, but strangely enough they are continuously busy

with food and eating rituals to the point of obsession³.

Diagnosis of this disorder is difficult, because individuals with anorexia nervosa try to hide their disorder at all cost¹. Therefore, the diagnosis is mostly made when medical complications have emerged¹. To diagnose anorexia nervosa the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) criteria for anorexia nervosa can be used^{1, 3}. These criteria include^{1, 3}:

- Refusing to maintain a body weight of more than 85% of that expected.
- An tremendous fear to gain weight or become fat, even in a state of underweight.
- Disturbances in the way one's body weight and shape is experienced and denial the seriousness of the current body weight.
- The absence of at least three consecutive menstrual cycles.
- As said earlier, two types of anorexia nervosa can be distinguished the restricting type and the binge-eating/purging type. So anorexic patients can be different but still suffer from anorexia nervosa.

Anorexia nervosa is a prevalent disorder^{1, 2}. 1 of the 100 females in the United States alone develop anorexia and 1 of the 10 anorexia nervosa patients die from this disorder or other related complications^{1, 2}. The University of South Carolina estimated that about 3% of the young girls with age between ten and fifteen suffer from anorexia nervosa¹. This gives a staggering half a million girls with anorexia nervosa¹. Besides, the mortality rate is about twelve times higher than all other causes of death in this group of age, thereby showing the massive problems of anorexia nervosa¹. Because of the lack of effective and evidence based treatment in AN and because of the seriousness of this disorder, it is important to get more insights into mechanisms and pathophysiology of AN so better treatments can be developed¹⁻³.

Brain activities in anorexia nervosa

For over 50 years now it is known that starvation alters emotional and cognitive brain functions⁴. The study of Keys *et al.* was one of the first to find significant differences in concentration, decision making, comprehension irritability, depression, alertness and judgment during starvation⁴. Nowadays, recent studies with computed tomography and functional MRI (fMRI) have provided further insights in the neurobiology of starvation²⁻⁴. Most studies explored this neurobiology in starvation models like patients with anorexia nervosa^{2, 3}. Therefore, people got to know more about the brain functions and alteration in this disorder. Some of these recent studies showed for example brain atrophy in patients with anorexia nervosa, which was reversible as the patient gained more weight⁴. Others showed localized loss of brain volume as in the anterior cingulate cortex, while many emotional and cognitive alterations now have been associated with the disorder⁴.

Here, the alterations in activity of different brain regions in anorexia nervosa will be described, because these alterations are likely involved in the onset or maintaining of anorexia nervosa²⁻⁴. Therefore the structures and neurocircuits involved in anorexia nervosa will be briefly described.

Functional brain imaging with techniques like fMRI, single-photon emission tomography (SPECT) and positron emission tomography (PET) are important tools in today's research in anorexia nervosa⁴. They have been used to study the responses in brain activity after certain stimuli to evoke the symptoms of the disorder⁴. These stimuli give insights in the way the brain functions in such disorders. One of the studies reported a reduced activity in the insula, middle frontal gyri, occipital regions and the precuneus when the subjects were confronted with images of their own body compared with images of other's body parts⁴. The medial prefrontal cortex is found

to be more active in patients with anorexia nervosa when subjects were confronted with food and body image stimuli⁴. Other results showed a reduced activity in the right occipital cortex when patients were hungry⁴.

PET scans can reveal changes in the metabolism of tissues such as brain regions². In the nineties a series of PET studies has been performed². This revealed a global decrease in metabolism in the cerebral regions in patients with anorexia nervosa, compared with healthy controls². They suggested that the decrease in glucose consumption caused this decrease in the global cerebral metabolism². However, by looking at relative metabolisms they found a decreased metabolism in the parietal cortex, superior frontal cortex and an increased metabolism in the caudate nuclei, lateral inferior frontal cortex, thalamus and putamen². Later on, studies also found decreased metabolism in the anterior and posterior ACC in anorexic patients². Finally, after stimulation with high versus low calorie foods, PET studies revealed an increase bilaterally in blood flow of the temporal lobe in AN patients compared with healthy subjects².

SPECT studies use radioligands to study the perfusion through tissues of interest. These studies showed reduced perfusions in the left parietal cortex before treatment and increased perfusion in the temporal cortex after treatment^{2, 4}. Several other SPECT studies commonly found a decreased perfusion in the anterior cingulate cortex (ACC) and other nearby regions when compared with control subjects². This decreased perfusion in the ACC persisted even after gaining weight, suggesting a trait rather than a state marker². Finally, SPECT analyzes revealed a decrease in the perfusion of the right parietal cortex, the right inferior and superior prefrontal cortex and in the left inferior prefrontal cortex².

In the last years, changes in the receptor binding of dopamine and serotonin has become more interesting^{2, 3}. By using

radioligands to the dopaminergic and serotonergic components and comparing this with other groups, one could see changes in receptor binding². The most intensively studied receptor is the serotonin 2A receptor family (5-HT_{2A})^{2, 3}. Patients with AN seem to have reduced receptor binding of 5-HT_{2A} bilaterally in the parietal, occipital, and in the left frontal cortex when compared with controls^{2, 3}. In recovered AN patients, a decreased binding to 5-HT_{2A} could be seen in the parietal and temporal cortex and in the subgenual cingulate when compared with AN patients^{2, 3}.

fMRI studies use two different stimuli to provoke AN related symptoms in AN patients². Those two types are food stimuli and body images². Both types induced higher levels of anxiety, disgust and threat compared to healthy subjects². When AN patients were shown pictures of high and low calorie drinks, a more powerful signal could be seen in the anterior cingulate gyres, the left insula and the amygdala-hippocampal region when compared with healthy women². A larger study found increased activity in the ventral-medial prefrontal cortex and in the lingual gyres of the occipital cortex, while a decrease in activity could be seen in the parietal cortex². This study used images of savory and sweet foods.

The influence of satiety and hunger in healthy and AN patients has also been investigated². Researchers saw a reduced activity in the parietal cortex when AN patients were satiated and were exposed to images of savory and sweet foods compared with satiated healthy controls². However, when AN patients were hungry, a reduction in the activity of the right lingual gyrus could be seen in anorexic patients compared with controls². These results persists even after recovery². A recent provocation study with a sucrose-water mix showed a reduced activity in the insula, the ACC and ventral and dorsal striatum in recovered patients².

Other fMRI provocation studies use other's and one's own body images to investigate

one's body perception. When confronted with one's own distorted body image, an increased activity could be seen in the middle frontal gyrus and in the parietal cortex compared with controls². A recent study used an other kind of provocation stimuli. When using emotional salient words such as thin versus a neutral word, brain activation of the insula, frontal, temporal and parietal lobes and the medial frontal gyres could be seen². Fat versus an neutral word, however, showed an decreased activity of the prefrontal cortex, middle frontal gyrus and superior parietal lobe in AN patients compared with controls².

The parietal cortex is consistently involved in anorexia nervosa as revealed by functional brain imaging studies². Its activity is decreased at symptom provocation and receptor binding of 5-HT_{2A} is also reduced^{2, 4}. There seems to be no lateralization between both parietal cortexes, as one part reported left parietal activity differences while others reported right side differences². The alterations in the parietal cortex in AN patients are linked with an inadequate perception of their own body⁴. Despite being critically underweight they still believe they are overweight². It is thought that the parietal cortex is involved in integration of the proprioceptive and visual information of one's own body, which likely constructs a body image². Alterations in this integration could lead to a disturbed perception of the own body². A remarkable feature of AN is the fact that AN patients seem unaware of the existence of there disability². Investigators have linked this feature with lesions of the parietal cortex². Especially a lesion on the right parietal cortex could result in this feature².

Another important brain region in AN seems to be the anterior cingulate cortex². It showed a reduced activity in sucrose stimulation tests and an increase in activity during symptom provocation². Also in this region a reduced 5-HT_{2A} could be seen^{2, 3}. The ACC also seems to be involved in the

perception of body images just like the parietal cortex². Anorexic patients have a reduced activity in the ACC when confronted with their own body image². Furthermore, the ACC seems to be involved in the properties of food intake². Although it is thought to play a crucial role in AN, the ACC is also involved in many other psychiatric disorders as depressions or obsessive-compulsive disorders².

Although functional brain imaging studies have not been very consistent in the exact location, the frontal cortex may play one of the key roles in AN². Perfusion and metabolism reductions could be seen in different regions of the frontal cortex, including the superior frontal cortex, dorsolateral prefrontal cortex and orbitofrontal cortex². Furthermore, there is evidence of reduced 5-HT_{2A} receptor binding in the left frontal cortex². At last, different regions of the frontal cortex showed an increased activity during symptom provocation².

As described, the parietal cortex, anterior cingulate cortex and the frontal cortex are all involved in AN. However, investigators believe the insula plays the central role in AN³⁻⁵.

A study performed by Wagner *et al.* showed significant differences in the activity of the insula, a specific brain region involved in many functions including the perception of taste⁶. This study measured the perception of taste with a sweet and neutral taste by means of fMRI⁶. Women recovered of anorexia nervosa showed to have a reduced response in the insula and other related region's when given the sweet taste⁶. Besides, recovered women were not really able to give a good relation between how they judged the sweetness of taste and the activity of the insula⁶. The control group, however, did make this relationship⁶. Therefore anorexic patients could have difficulty with the recognition of taste or responding to the pleasure of food⁶. The insula is also involved in emotional regulation, so there may be an

alteration which causes the food to be aversive instead of rewarding⁶. Further image studies showed greater right insula and right lateral prefrontal cortex activity when anorexia patients had to estimate their body size and with satisfaction ratings for thinner images of the own body⁴. In line with this result, Friedrich *et al* found greater insula activity when anorexic patients were shown pictures of thin body parts⁴. Others found that the insula is a key structure in the interoceptive processing³. This includes various sensations as taste, the perception of pain, intestinal tension, itch, dyspnoea and temperature³. The integration of these interoceptive feelings are crucial for the establishing of the 'self' as it forms the link between cognitive and emotional processes and the current state of the body³. It has been thought that alterations in the interoceptive awareness might be a precipitating and reinforcing factor in this disorder³. Because of the role of the anterior insula in the interoceptive processing and the altered activities in the insula found in anorexic patients, individuals with this disorder may suffer from a altered sense of self being³. Many symptoms of anorexia nervosa could be related to the alterations in the interoceptive processing³. The distorted body image, failure to respond to hunger and the reduced motivation to changes may all be caused by these alterations³.

These studies support the theory that the insula has a central role in anorexia nervosa. Nunn *et al.* hypothesized that the insula serves as an integrative function for all other structures which are relevant to the functions of anorexia nervosa and therefore may be the central structure in this disorder⁷. Further they hypothesize that a rate limiting dysfunction of the neurocircuitry which is integrated by the insula accounts for the clinical manifestations of anorexia nervosa⁷.

This paper will look at the alterations in the brain structures in AN patients. Several brain structures will be discussed but central in this paper will be the insula, because the insula is

thought to be the central player in this disorder. Especially the role of the insula in several symptoms of anorexia nervosa will be described. Therefore, the functional anatomy of these structures will be described at normal healthy state, so that we can fall back later to the AN patients and see which structures and alterations are likely crucial for AN.

Functional connectivity of the insula

This chapter will deal with the insula, especially the connectivity of the insula with other brain regions. The insula is involved in a broad spectrum of brain functions, but it is also thought to be the center of psychiatric disorders as anorexia nervosa^{8, 9}. It is therefore important to investigate insula connectivity in resting state. Those results will give more insights in the neurocircuitry in which the insula plays a crucial role and can therefore be used to investigate neural disorders so ultimately better treatments can be developed.

The insula was first described by J.C. Reil, a German anatomist, in 1809¹⁰. The insula is also known as the insular cortex, Brodmann areas 13 to 16 or the island of Reil¹⁰. The insula is a separate lobe which is located deep inside the sulcus of the Sylvian fissure and it is hidden behind the frontal and temporal opercula¹⁰. Five to seven gyri can be seen on the surface of the insula¹⁰. These gyri converge inferiorly so that it looks like a sort of fan¹⁰. The insula can be divided in an anterior and a posterior part^{9, 10}. The separation is given by the main sulcus, the central insular sulcus¹⁰. In this central sulcus lies the main branch of the middle cerebral artery, which supplies the insula of blood¹⁰. When looking at the neuronal arrangement of the insula, three subdivisions can be identified⁸⁻¹⁰. These subdivisions are connected with regions of the frontal, temporal, parietal lobes but especially to the cingulate gyrus^{9, 10}. Compared with the macaque monkeys, the human insula is greatly enlarged, which suggests higher cognitive functions¹⁰.

The insula participates in a broad spectrum of processing, which include the processing of motor, sensory, pain, visceral motor and sensory, verbal, visual, auditory, olfactory and gustatory information, but it is also related to music, eating and the modulation of attention and emotion⁸⁻¹⁰. However, investigators have also shown that the insula

is involved in conditioning aversive learning, emotional and motivational modulation of pain perception, sleep, mood stability, language and stress induced immunosuppression¹⁰.

Since the invention of the functional MRI (fMRI) in 1990, it was possible to look and analyze different regions of the brain for connectivity in humans *in vivo*. fMRI measures the changes in blood flow which is related to neuronal activity in the brain or spinal cord. Since then fMRI has become the most used method for brain mapping due to its low invasiveness, no radiation exposure and availability. It has been long known that blood flow is closely related to neuronal activity. When more active, neurons use more glucose and switch to anaerobic glycolysis. This starts a local response which leads to increased blood flow and thereby an increase in oxyhemoglobin and deoxyhemoglobin. This is the marker of BOLD, blood oxygen level dependence, which can be detected by MRI and is used as MRI contrast. So fMRI measures changes in the concentrations of oxyhemoglobin and deoxyhemoglobin which is also known as BOLD. These changes can give information about the function of brain regions and connectivity. Because of the local response of increased blood flow due to a greater activity of the neurons, fMRI has a delay of about one to five seconds.

Taylor *et al.* investigated the functional connectivity between specific parts of the insula and sub regions of the cingulate cortex⁸. Their study included nineteen right handed and healthy individuals⁸. This study performed a resting state fMRI study so that anatomically distinct brain regions can be investigated at functional connectivity⁸. Resting state refers to the situation in which an individual lies in the MRI scanner without any tasks or stimuli⁸. Resting state fluctuations of BOLD signals may show patterns of synchronous activation or inhibition between different brain regions which then may give an indication for

anatomically and/or functional related brain regions⁸. Subjects were instructed to just lie in the MRI, think of nothing, keep the eyes closed but not to fall in sleep⁸.

They divided the insula in an anterior, middle and posterior region and determined whether BOLD fluctuations correlated with specific regions of the cingulate cortex⁸. Results showed the anterior insula was functionally connected with the posterior anterior cingulate cortex (ACC) and the anterior and posterior middle cingulate cortex (MCC)⁸. The mid and posterior insula was only connected with the posterior MCC⁸. They propose that the connections of the anterior insula are involved in affective salient monitoring, such as visuality, pain, touch and auditory information⁸. The connections linking the whole insula with the posterior MCC may function as a general action and salience system according to these authors⁸.

So with these results, Taylor *et al.* suggested a two system network between the human insula and cingulate cortex⁸. These are distinct systems with possible a different function⁸. The first network, linking the anterior insula with the posterior ACC and anterior MCC may be involved in the integration of interoceptive information with emotional salience forming a subjective representation of one's own current body state⁸. The other system, which links the entire insula with the MCC may have a more general function in salience and action⁸. It is likely to be involved in response selection, skeletomotor body orientation and environmental monitoring⁸.

A more recent study, performed by Deen *et al.* investigated functional subdivisions of the human insula⁹. In their study they also used resting state fMRI⁹. They subdivided the insula based on a certain technique called clustering analysis⁹. This revealed three insula subregions, the ventral anterior insula, dorsal anterior insula and the posterior insula⁹. These three subdivisions were investigated for connectivity with other brain regions⁹.

They showed that these three subdivisions all had distinct patterns of functional connectivity⁹. It was found that the posterior insula is functionally connected with primary and secondary motor cortex, while the greatest connectivity was found to be with the primary and adjacent secondary somatosensory cortex⁹. This pattern could also be found in the macaque monkey⁹. Previous studies in the macaque showed that the posterior insula had mutual connections with the secondary somatosensory cortex and supplementary motor area⁹. However, the macaque showed no extensive connections between the insula and the primary motor and somatosensory cortex⁹. The functional connectivity with these regions in the study by Deen *et al.* may reflect indirect connections between these brain structures or may result from the influence of a third region⁹.

The ventral part of the anterior insula was greatly connected with the posterior ACC, which is in line with the results of Taylor *et al.*^{8, 9}. The ventral anterior insula may play a role in the emotional processing⁹. Prior studies revealed that this part of the insula is cytoarchitectonically most related to the limbic cortex⁹. Besides, investigators found that the ventral anterior tended to be involved in peripheral physiological responses resulting from emotional experiences, such as the galvanic skin response or an increase heart rate⁹. Moreover, the anterior insula seems to be involved in pain and was the most probable place of insular coactivation with the amygdala⁹.

The more dorsal part of the anterior insula was found to be involved in a previous described cognitive network⁹. It showed connectivity with the dorsal ACC and both showed consistently sustained activity throughout the performance of cognitive tasks⁹. Furthermore, the dorsal anterior insula and ACC seem to be involved in decision making⁹. The more difficulty or uncertainty with a decision, the more these two regions are active⁹. So this network may be part of a system in which somatosensory

or interoceptive information in the posterior insula and/or emotional information in the ventral anterior insula may influence the decision making and behavior by the dorsal anterior insula⁹.

In contrast with the results of Taylor *et al.* and others, this study proposed a three system of functional connectivity in the insula, while Taylor *et al.* suggested a two part system^{8,9}.

Cauda *et al.* investigated sixteen right handed healthy individuals to research the connectivity of the human insula which was done in resting state¹⁰. As described earlier, functional connectivity of the human insula with the cingulate cortex was already investigated^{8,9}. However, connectivity with other brain regions remained unclear in humans. Therefore, Cause *et al.* studied the relationship of the human insula with other brain regions¹⁰.

The results of this study showed that the human insula can be divided in two functional parts which are both connected with a distinct neural network¹⁰. The ventral anterior insula is connected with the rostral anterior cingulate cortex, the middle and inferior frontal cortex and with the temporoparietal cortex¹⁰. The dorsal posterior insula, however, is connected with the dorsal posterior cingulate, sensorimotor, premotor, supplementary motor and temporal cortex and with several occipital regions¹⁰. Those two distinct neural networks probable have different functions¹⁰. The first network, that of the anterior insula, is involved in the integration of several cognitive, homeostatic, emotional and interoceptive functions¹⁰. The other network, that of the posterior insula, is most likely related to skeletomotor body orientation, environment monitoring and response selection¹⁰. Both networks are connected with other different networks but still function independently of those networks¹⁰.

Another finding of this study is the connectivity of the anterior insula with the thalamus within the neural network¹⁰. The

posterior insula or the posterior network does not seem to be functionally associated with the thalamus¹⁰.

However, previous studies revealed that the posterior insula did have projections to the thalamus¹⁰. In these studies, the posterior insula showed to be connected with the posteromedial and ventroposterior inferior thalamic nuclei whereas the anterior insula projects to the ventroposterior and ventrolateral thalamic nuclei¹⁰.

Furthermore, the insula receives input of different regions of the thalamus¹⁰. These include cell groups like the centromedian, ventroposterior medial, inferior and lateral nuclei¹⁰. These projections are also region specific, because the ventroposterior and centromedian nuclei project to the anterior insula, while the medial geniculate nucleus projects to the posterior insula¹⁰.

The study of Causa *et al.* also compared the left and right insula with each other¹⁰. The two sides seems to have slightly different patterns¹⁰. The right insula stops growing earlier than the left insula, while the left insula displays a larger surface compared with the right side¹⁰. The right anterior insula neural network showed stronger connections of the right anterior insula with the right ACC and several subcortical regions such as the thalamus compared with the left side¹⁰. The right posterior network also shows a right lateralization, although it is mild¹⁰. These results are in line with previous studies, explaining the right insula to play a central role in attention systems of the brain¹⁰.

Anatomic circuits of the insula

In 1977 investigators described an agranular field and a dorsally granular field in the insular cortex¹¹. The agranular field was rostrally subdivided into a ventral and dorsal agranular region¹¹. In 1987 this description was adjusted by Cechetto and Saper¹¹. Instead of an agranular and a granular field, they described a dysgranular field between the agranular and granular field, which also seemed to include a part of the dorsal agranular region¹¹.

The rostral agranular insular cortex (RAIC) is the most rostral part of the agranular insula. It is believed to play a crucial role in pain behavior¹¹. In order to get more information of the RAIC in pain behavior, Jasmin *et al.* investigated the afferent and efferent pathways of the RAIC¹¹. This was done with a tract-tracing study using anterograde and retrograde tracers. The anterograde tracers travel from the soma through the axons to the ends of the neuron. This is particular to see the efferent connections a certain area. A retrograde tracer travels in reverse, traveling from the ends of the neuron to the soma. These can be used to see the afferent connections.

In this study they used male Sprague-Dawley rats, which were injected with either Fluoro-gold or biotindextran amine¹¹. Fluoro-gold is one of the most used neuronal retrograde tracers and biotindextran amine can be used as an anterograde tracer. The sections were labeled using immunocytochemistry¹¹.

Efferent connections

Injection of biotindextran amine in the insula of rats produced labeling of different regions in the forebrain but also some labeling in the brainstem¹¹. As said earlier, biotindextran is an anterograde tracer, showing the efferent connections of the RAIC with other brain regions.

Cortex

Injection of biotindextran resulted in a heavy labeling in the lateral and ventrolateral

orbital cortices as can be seen in figure 1C¹¹. Furthermore, labeling could be seen in the anterior cingulate cortex, piriform cortex and in the nucleus accumbens¹¹. In the other hemisphere, heavy labeling was found in the RAIC. This was organized such that the ventral and dorsal parts of the RAIC projected respectively to ventral and dorsal parts of the homotypical cortices, which are the same structures but then on the other side of the brain¹¹.

Thalamus

Anterograde labeling was found in the medially located nuclei of the thalamus, while the laterally located nuclei were not labeled (Fig. 2A-D)¹¹. The labeled structures could also been found labeled on the other side, although in a lower degree¹¹. The nuclei which showed labeling were the paratenial, anterior, posterior paraventricular, centrolateral and the rhomboid nuclei and the nucleus submedius (Fig. 2B)¹¹. Figure 1D shows labeling in the parvicellular area of the ventral posterior nucleus (VPpc), meaning a area with small cells in the ventral posterior nucleus of the thalamus¹¹. Anterograde labeled fibers were also found in the lateral the lateral hypothalamus, as figure 2F shows¹¹.

Amygdala

The amygdala consists of several nuclei. Among these nuclei are the basolateral, cortical, medial and central nucleus. Labeled terminal fibers were found in different subregions of the basolateral nucleus, including the ventral, medial and anterior parts¹¹. Further labeling was found in the lateral nucleus and to a lesser extent in the central nucleus and the amygdalo-striatal transition area, a region between the amygdala and striatum (Fig 1F)¹¹.

Striatum

Figure 1E shows labeling in the dorsal and ventral caudate putamen, a part of the striatum. At the other site of the brain, labeling was could be seen in the nucleus accumbens, especially in the core parts but

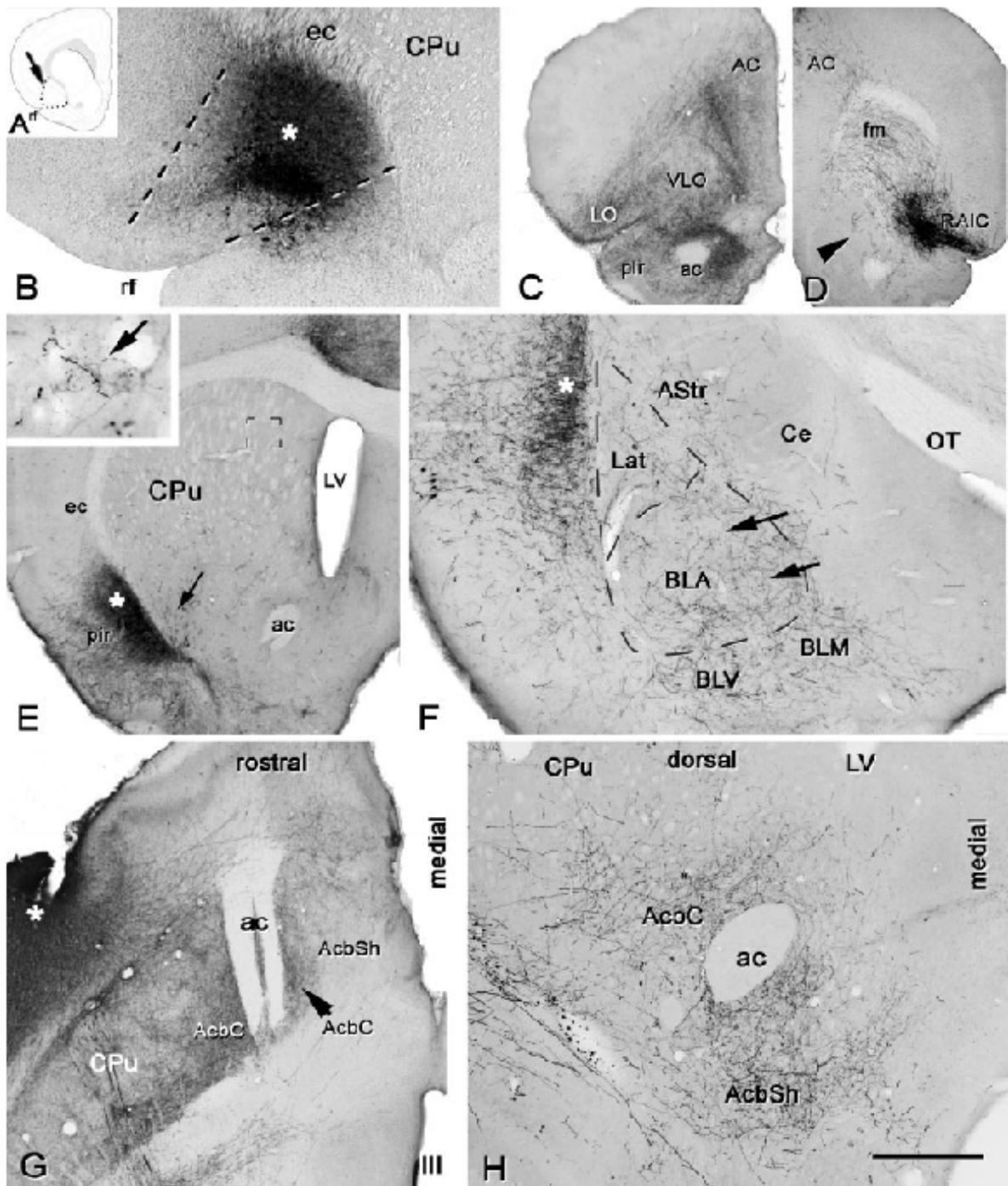


Figure 1. Forebrain labeling following injection of the anterograde tracer biotindextran amine into the RAIC. **A:** The area within the dots represents the area in which the tracer has been injected. **B:** * represents the injection site for biotindextran amine in the RAIC. Black coloring means labeling with biotindextran amine. Ec is external capsule, CPu is caudate putamen. **C:** Prefrontal cortex showing labeling in the lateral orbital (LO) and ventrolateral orbital (VLO) cortices. More labeling could be seen in the anterior cingulate cortex (AC), the piriform cortex (pir) and the nucleus accumbens medial to the anterior commissure (ac). **D:** Labeling was found in the contralateral RAIC and also in the contralateral dorsal AC and nucleus accumbens (arrowhead). **E:** The ventral and dorsal caudate putamen (CPu) are labeled. The area within the box in the dorsal CPu is enlarged in the inset. This shows labeling of the dorsal CPu. **F:** Labeling of the amygdala. Labeling is present in the lateral (Lat), basolateral anterior (BLA), basolateral ventral (BLV) and basolateral medial (BLM) amygdaloid nuclei. Only few labeling could be seen in the central nucleus (Ce) and amygdalo-striatal transition area (AStr). * represents the fibers of passage in the external capsule. **G:** * represents the injection site. Heavy labeling could be seen in the nucleus accumbens core region (AcbC) and in a lesser degree in the shell region (AcbSh) **H:** The AcbC and AcbSh both show labeling this time in a higher magnification.

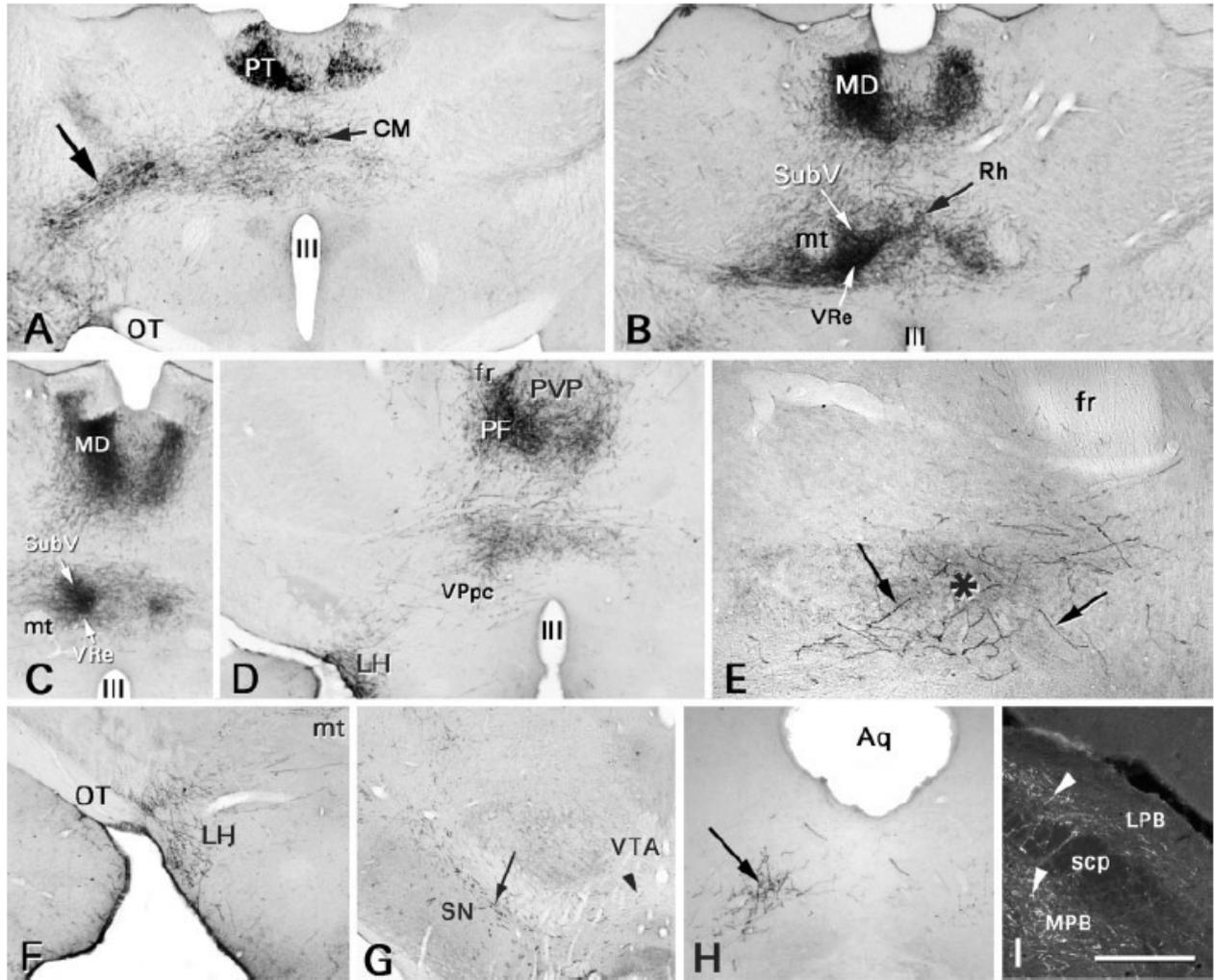


Figure 2. Diencephalon and brainstem labeling following injection of the anterograde tracer biotindextran amine into the RAIC. **A:** The thalamus is shown. The paratenial nucleus (PT) and the central medial thalamic nucleus (CM) are labeled. OT is optic tract, III is third ventricle. The arrow represents fibers coming from the striatal-amygdaloid junction and terminate on the medial thalamic nuclei. **B:** The mediodorsal nucleus (MD), rhomboid nucleus (Rh), ventral reuniens nucleus (VRe) and the ventral nucleus submedialis (SubV) are labeled. Mt is mammillothalamic tract. **C:** Other section showing labeled MD, VRe and SubV. **D:** Anterograde labeling was found in the lateral hypothalamus (LH), ventral posterior parvocellular nucleus (VPpc), parafascicularis thalamic nucleus (PF) and posterior paraventricular nucleus (PVP). Fr is fasciculus retroflexus. **E:** High magnification of the VPpc labeled with biotindextran amine (labeled fibers, see arrows) and CGRP (background staining, see *). CGRP staining identifies the VPpc. **F:** Labeling in the lateral hypothalamus (LH). **G:** Labeling was found in the substantia nigra (SN) and ventral tegmental area (VTA). **H:** Anterograde labeling in the ventrolateral (wings) of the dorsal raphe nucleus, which is indicated by the arrow. Aq is cerebral aqueduct of Sylvius. **I:** The lateral (LPB) and medial (MPB) parabrachial nucleus show labeling of biotindextran amine. Scp is superior cerebellar peduncle.

also, to a lesser extent, in the shell subdivisions (Fig. 1G,H)¹¹.

Brainstem and spinal cord

Labeling was present both in the ventral tegmental area and in the substantia nigra (Fig. 2G)¹¹. Previous studies had already found projections of the RAIC to the nucleus raphe magnus, one of the three raphe nuclei

in the medulla and the pericoerulear region, a region of neurons also found in the brainstem¹¹. On the other side of the brain, labeling was found in the ventrolateral (wings) of the dorsal raphe nucleus (Fig. 2F). Also the parabrachial area, an area in the brainstem which is involved in taste processing, is labeled especially the lateral

parabrachial nucleus (Fig. 2E). Labeling of the spinal cords was not found.

Afferent connections

Injection of Fluoro-gold in the insula of rats resulted in labeled regions across the brain¹¹. Fluoro-gold is a retrograde tracer and thereby showing the projections of other brain regions on the RAIC.

Cortex

Labeling of fluoro-gold was present in the lateral and ventrolateral orbital cortices and also in the anterior cingulate cortex and infralimbic region, a region in the medial prefrontal cortex (Fig. 4C)¹¹. The RAIC in the other hemisphere was also labeled with the retrograde tracer, as figure 4D shows¹¹.

Thalamus

Figure 4F-J shows the sections from rostral to caudal of the thalamus. The same with the anterograde tracers is this tracer not present in the lateral nuclei of the thalamus but exclusively in the medial nuclei. The thalamic nuclei which showed labeling were the centrolateral, central medial, posterior ventromedial parvicellular, mediodorsal, paracentral, parafascicular, paratenial, anterior and posterior paraventricular thalamic, reuniens, rhomboid and the submedial nucleus and the ventral reuniens (Table 1)¹¹. The nuclei showed ipsilateral much more labeling compared with contralateral¹¹. Retrograde labeling was seen in the lateral hypothalamus as can be seen in figure 5A¹¹. Only a few cells were found contralaterally¹¹.

Amygdala

The amygdala also showed labeling as can be seen in figure 4E. The anterior and ventral regions of the basolateral nucleus and the lateral nucleus showed the heaviest labeling¹¹. Further, labeling was present in the contralateral basolateral nucleus, the same nucleus but then on the other side, the anterior cortical and the basolateral posterior nucleus¹¹. The basomedial anterior, posterolateral cortical nuclei showed

labeling strictly on the ipsilateral side, the same side as injection¹¹. At last, some labeled cells were also present in the medial nucleus and lateral nucleus caudally¹¹. No labeled cells were present in the central nucleus¹¹.

Brain stem and spinal cord

A large concentration of retrograde tracer was found in the ventral tegmental area and substantia nigra, which is shown by figure 5B¹¹. Only a few labeled cells were present contralaterally in the substantia nigra¹¹. Another large concentration was found in the dorsal raphe nuclei (Fig. 5C), but the nucleus raphe magnus also showed labeling only in a lesser degree (Fig. 5E)¹¹. The parabrachial complex was also labeled mainly in the medial nucleus and less in the lateral nucleus¹¹. This is shown in figure 5D. Moderate numbers of retrogradely labeled cells could also be found in the paragigantocellularis nucleus and gigantocellularis nucleus¹¹. Finally, labeled cells were found in the nucleus of the tractus solitarius and in the medial and lateral reticular formation ipsilateral¹¹.

Figure 3 shows a summary of the anatomical connections of the RAIC. In this figure all the regions in which anterograde and retrograde labeling was found is presented.

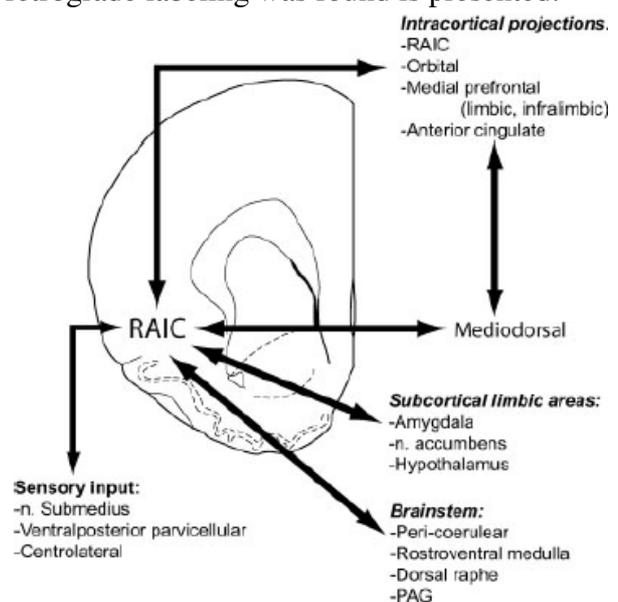


Figure 3. Schematic diagram of the principal connections of the RAIC.

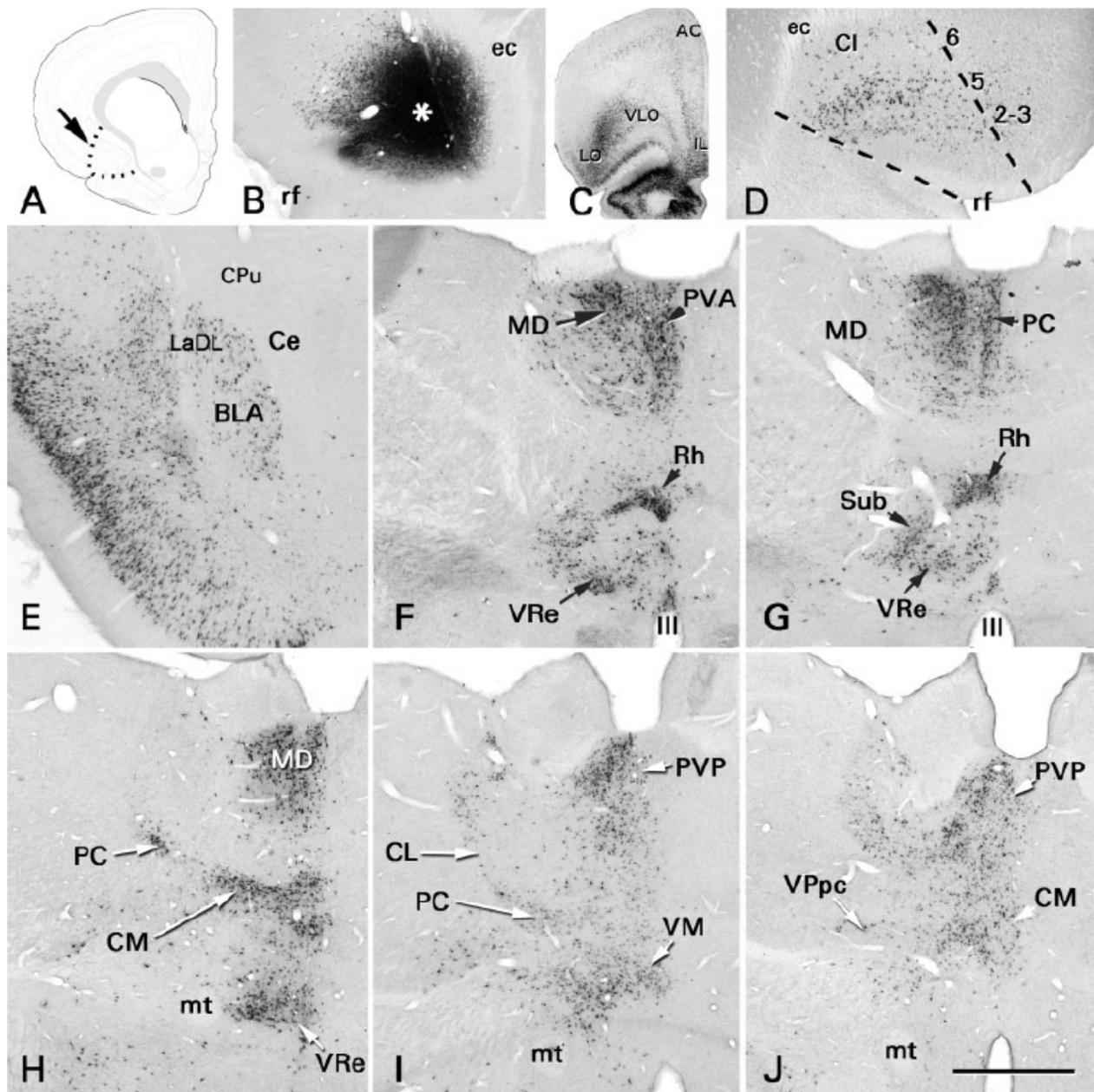


Figure 4. Forebrain labeling following injection of the retrograde tracer Fluoro-gold into the RAIC. **A:** The area within the dots represents the area in which the tracer has been injected. **B:** * represents the injection site of Fluoro-gold. Black coloring means labeling with Fluoro-gold. Rf is rhinal fissure, ec is external capsule. **C:** Labeling was found in the ventrolateral orbital (VLO), lateral orbital (LO), anterior cingulate (AC) and infralimbic (IL) cortices. **D:** Contralateral labeling of the RAIC. The broken lines represent the borders of the contralateral RAIC. The numbers represent the different lamina of the RAIC. CI is claustrum. **E:** Labeling of the basolateral amygdala (BLA), dorsolateral part of the lateral nucleus (LaDL) is shown. The central nucleus (Ce) is not labeled. CPU is caudate putamen. **F:** Retrogradely labeled cells in the thalamus are present in the mediodorsal nucleus (MD), the anterior paraventricular nucleus (PVA), the rhomboid nucleus (Rh) and the ventral reuniens nucleus (VRe) III is third ventricle. **G:** The thalamic regions MD, paracentral nucleus (PC), Rh, VRe and the nucleus submedialis (Sub) are labeled. **H:** Retrogradely labeled cells present in the MD, PC, VRe and central medial thalamic nucleus (CM). mt is mammillothalamic tract. **I:** Labeling of PC, contralateral thalamic nucleus (CL), posterior paraventricular nucleus (PVP) and ventral median nucleus (VM). **J:** The thalamic regions PVP, CM and ventral posterior parvicellular nucleus are shown labeled.

TABLE 1. Retrograde Labeling in the Thalamus After Fluoro-Gold Injection in the RAIC

Thalamic nucleus	Ipsilateral	Contralateral
Centrolateral nu (CL)	++	-
Central medial nu (CM)	++	-
Posterior ventromedial parvicellular (VPpc)	++	+
Mediodorsal thal nu (MD)	+++	-
Paracentral nu (PC)	++	+
Parafascicular nu (Pfc)	++	-
Paratenial thal nu (PT)	++	+
Paraventricular thal nu ant (PVA)	+++	+
Paraventricular thal nu post (PVP)	+++	-
Reuniens thal nu (Re)	++	+
Rhomboid thal nu (Rh)	++	+
Submedius nu (SM)	+	+
Ventral Reuniens (vRe)	+++	-

+++ : Heavy labeling; ++ : Moderate labeling; + : Slight labeling; - : Labeling not detected.

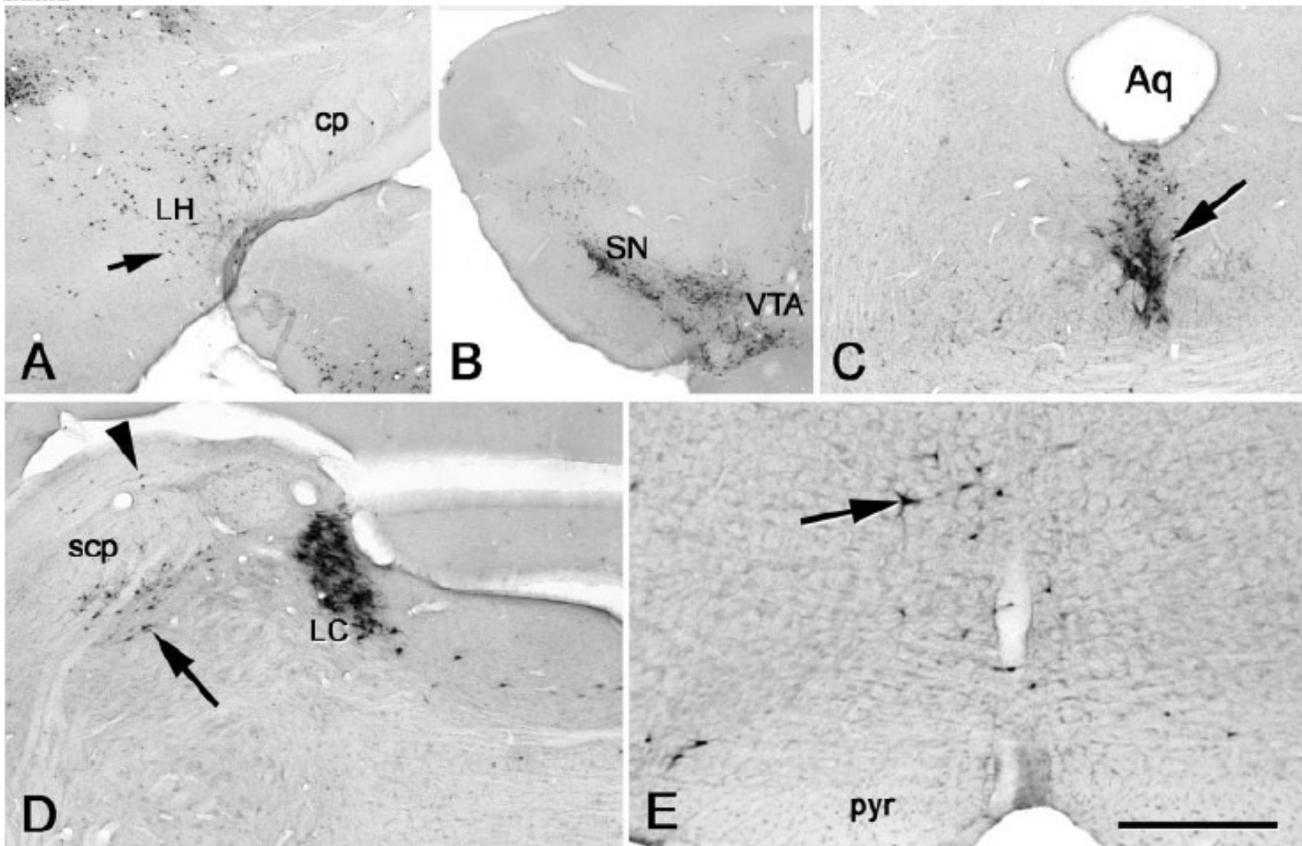


Figure . Hypothalamus and brainstem labeling following injection of the retrograde tracer Fluoro-gold into the RAIC. **A:** Labeled cells are found in the lateral hypothalamus (LH), also indicated by the arrow. Cp is caudate putamen. **B:** Retrograde labeling in the substantia nigra (SN) and ventral tegmental area (VTA). **C:** Retrogradely labeled cells in the dorsal raphe nucleus, also indicated by the arrow. Aq is cerebral aqueduct of Sylvius. **D:** Cells are labeled in the locus coeruleus (LC) and medial locus coeruleus (arrow). Sparse labeling is found in the lateral parabrachial nucleus (arrowhead). Scp is superior cerebellar peduncle **E:** Sparse labeling in the nucleus gigantocellularis, indicated by the arrow. Pyr is pyramid.

Connection of the insula with the cingulate cortex

The insula had a central role in previous chapters. However, these chapters showed a special relationship of the insula with the cingulate cortex, especially the anterior cingulate cortex. This relationship could be seen in terms of functional connectivity and anatomical. Indeed there is a growing base of evidence that these two regions have an special functional relationship¹². Besides, more understanding about this relationship may help to answer some important questions in this field. One of these questions is how subjective feelings originate from some sensory stimuli and how these feeling can influence cognition and behavior. This information may also be important for anorexia nervosa patients knowing that these patients also have problems with subjective feelings and behavior. In this chapter the anterior cingulate cortex will be briefly described as will the functional relationship between the insula and the cingulate cortex. Because a certain type of neurons may be important in this connection, they will also be described.

The anterior cingulate cortex (ACC) is the anterior part of a large gyrus lying in the middle of the cerebral hemisphere, which is the cingulate gyrus¹². This gyrus surrounds the corpus callosum, the structure that forms the connection between the left and right cerebral hemisphere¹². In the previous decades, investigators have proposed several anatomically models for the cingulate cortex, varying from a two part model to the current four part model developed by Vogt¹³. These include the anterior, medial, posterior and retrosplenial parts¹³. These parts can be divided in turn in several subdivisions¹³.

It is difficult to give one discrete function to the ACC¹². It is involved in a great variety of cognitive, emotional and behavioral functions^{12, 13}. However, the different subregions of the anterior cingulate cortex contain different projections, inputs and cytoarchitectonics¹². Therefore is it likely that these subdivisions have other

functions¹². In the latest four part model, the subgenual part for example heavily projects to brain regions which are involved in emotional, motivational and autonomic processing¹². These structures include the amygdala, hypothalamus and the periaqueductal grey, the grey matter surrounding the cerebral aqueduct in the brainstem¹². This suggest that the subgenual part of the ACC is important to produce emotion-related physiological and behavioral responses¹². The posterior part, however, may be involved in physiological pain stimuli and has little connections with subcortical area's^{12, 13}.

It has already been noted that studies reporting changes in activity in the insula, especially the anterior part, have also reported the same changes in activity in the ACC¹². This could be seen particular in studies involving certain aspects of affective processing¹². To investigate the function of these parts, the idea is that these two structures must be taken together¹².

In several studies over the past years, investigators found reciprocal connections between the insula and the cingulate cortex¹². Both the anterior insula and the ACC contain so-called 'von Economo' neurons^{12, 14}. It is believed that these neurons form the connections between these two structures⁵. As described before, Taylor *et al.* provided further evidence for this functional relationship by a fMRI study in which they discovered a two part insula-cingulate system⁸. The first connects the anterior insula with ACC and midcingulate cortex, while the second connects the posterior insula with the posterior midcingulate cortex⁸. However, Deen *et al.* found a three part insula-cingulate system, suggesting that these connections are not fully understood yet, but a relationship exists⁹. Anatomic studies provided the information that the insula and cingulated cortex do have heavily reciprocal connections which may be the von Economo neurons^{5, 12}. Therefore it is very likely that these structures directly exchange information⁵.

In 1929 von Economo studied the cytoarchitecture of the human cerebral cortex¹⁴. He described a certain type of neurons found in the anterior insula and the ACC¹⁴. Those neurons were large bipolar neurons with straight and twisted variants¹⁵. These neurons, also called 'spindle cells', are mostly called 'von Economo neurons' or VEN's¹⁵. The VEN's are significantly bigger compared with their neighbour neurons, the pyramidal cells¹⁴.

They are projection cells and have only one large dendrite, distinguishing them from the pyramidal cells, which have many small dendrites¹⁴. Their large size and dendritic structure may suggest that these neurons rapidly send basic information to other parts of the brain, while the pyramidal cells send more detailed information more slowly¹⁴. The exact connections of these neurons are not clear yet, but Graig proposed that it is these neurons which interconnect the anterior insula with the ACC both ipsilateral and contralateral⁵.

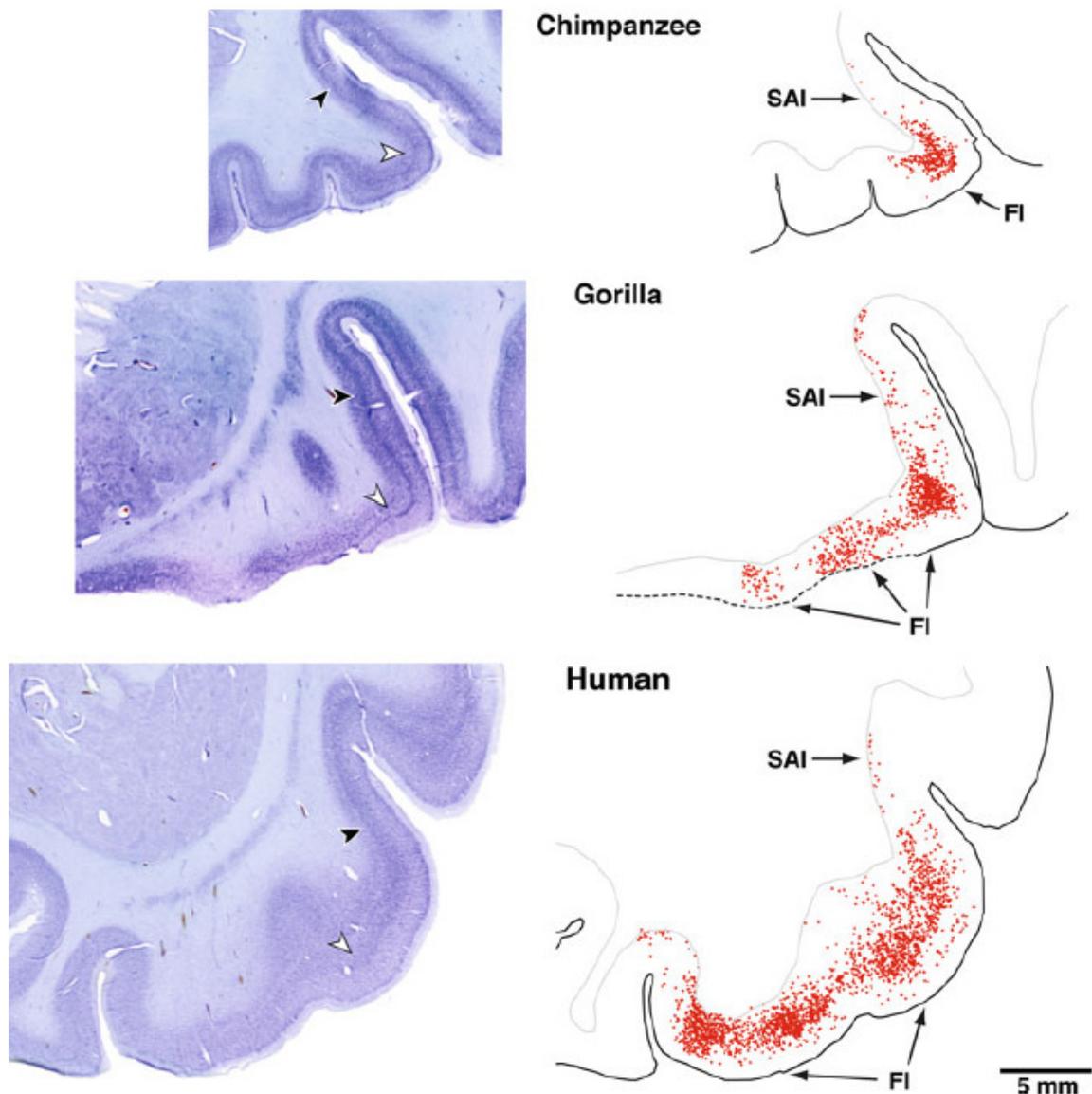


Figure 6. Distribution of VEN's in a chimpanzee, gorilla and human. The gorilla contains more VEN's than a chimpanzee, but a human in turn contains more VEN's than the gorilla. On the left side are photomicrographs of a section of the anterior insula in each individual. On the right side are the outlines of the anterior insula in which the location of VEN's are plotted. Each red dot represents a VEN. The black arrow represents the SAI, the white arrow indicates the FI. SAI is superior anterior insula, FI is fronto-insular cortex or anterior insula.

The VEN's are found in humans and in great apes such as gorilla's, bonobo's and chimpanzees but they are not found in other primates¹⁵. Recently they have also been found in whales and elephants and may be a specialization of a bigger brain size¹⁵.

Allman *et al.* investigated the VEN's in humans and great apes by using immunohistochemistry^{14, 15}. They found that a protein made by the gene DISC1, which is disrupted in schizophrenia, is particularly expressed in VEN's¹⁴. DISC1 has made rapidly major evolutionary changes in the evolutionary line leading to humans¹⁴. These changes may have caused a change in function since it now suppresses dendritic branching and may therefore be important in the development of the VEN's morphology¹⁴.

To see whether there are differences in VEN numbers in the anterior insula and ACC, Allman *et al.* studied the VEN's in humans, gorillas and chimpanzees¹⁴. They used an antibody, rabbit anti-DISC1, to stain the VEN's in the anterior insula in gorillas, chimpanzees and humans¹⁴. For each individual they took tree sections of the anterior insula and counted the number of cells¹⁴. They found that the chimpanzees had fewer VEN's in the anterior insula compared with the gorillas, which in turn had fewer VEN's compared with the humans¹⁴. This is illustrated by figure 6¹⁴. Each red dot represents 1 VEN¹⁴. The chimpanzee contained 354 VEN's, the gorilla 919 and the human 2415¹⁴.

The cytoarchitecture of the ACC and the location of VEN's in this region has been illustrated by von Economo and Nimchinsky *et al*¹⁴. This led to a comparison between the number of VEN's located in the anterior insula and ACC of great apes and humans (Fig. 7)¹⁴. This figure shows that humans do have significantly more VEN's in the anterior insula and ACC¹⁴.

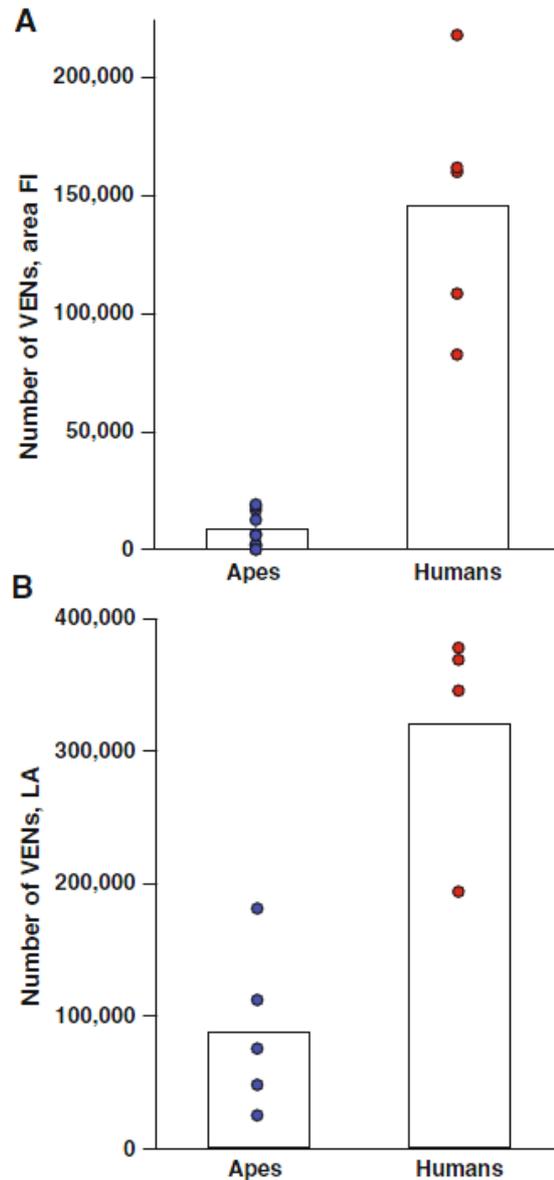


Figure 7. Number of VEN's in great apes and humans. The bars indicate the average number of VEN's. the blue and red dots represent the number of VEN's in a individual. A: Comparison of the number of VEN's in the anterior insula of apes and humans. Humans do have significantly more VEN's in the anterior insula compared with the great apes. B: Comparison of the number of VEN's in the ACC of apes and humans. Humans do have significantly more VEN's in the ACC compared with the great apes.

Allman *et al.* also found that many VEN's are present in aged humans and fewer VEN's are found in infants and the least are found prenatal^{14, 15}. Besides, the great apes also show significantly less VEN's in the anterior insula and ACC¹⁵. These results parallel the results of the mirror test, a measure of self-awareness⁵. Therefore, Graig proposed his theory that the VEN's in both regions form the connections between the anterior insula and ACC⁵. He believes the VEN's might enable fast and highly integrated representations of emotional situations and behaviours⁵. Overall the joint action of the anterior insula and ACC might function as a system important in the forming of self-awareness and appropriate behaviours⁵. Patients with frontotemporal dementia lose emotional awareness and self-conscious behaviours, which is correlated with the degeneration of VEN's supporting the idea of Graig⁵.

A recent study by Di Martino *et al.* found a negative correlation between resting state functional connectivity of the anterior insula and ACC and autistic traits in neurotypical adults¹⁶. Thus, less functional connectivity was related to autistic traits, which suggests this system plays a role in self-related processes^{12, 16}. With regard to this self-related processing, Damasio had already developed a model in which the insula played the central role in the generations of subjective feeling states¹⁷. In this model the posterior insula receives somatosensory information from afferent pathways which is then integrated by the posterior insula and is then transferred to the mid and anterior insula parts¹⁷. Those parts will integrate that information with other conscious information to produce a self-awareness and feeling¹⁷. A definition of 'self' is difficult to give, leading to more and more unanswered questions and confusions, but Damasio uses: 'a mental representation corresponding to our innate, moment to moment feeling and subjective awareness¹⁷. The afferent somatosensory information which the insula receives to form the self-awareness comes

from exteroceptive and interoceptive senses¹⁷. Exteroceptive senses include mechanical contact (touch), chemical contact as taste and smell and telesensing as vision and hearing, while interoceptive senses include pain, temperature, viscera, muscles and the vestibular system¹⁷. Combined these senses produce the interoception, which is explained by Graig as the sense of the internal milieu⁵. Several studies provided direct evidence for the theory that the insula is the crucial structure for interoception⁵. For example one functional neuroimaging study used as index for interoception the ability to accurately judge the timing of one's own heartbeat⁵. This study found that activity of the right anterior insula predicts the accuracy of the judgements in the heartbeat test⁵. Another result was the increased activity of the ACC during the attention for the internal physical state, which is in line with the insula-cingulate functional relationship⁵.

The evidence for the link between interoception and emotional experience in the insula-cingulate system is provided by another set of studies¹². High density elektroencefalografie was used to study electrophysiological responses to emotional stimuli¹². It was found that the anterior insula and ACC are crucial structures in the responses to such stimuli¹². The investigators translated this as that the activity in the insula represents the bodily state as a feeling and that the ACC is important to translate these feelings and emotionally reactions into different autonomic responses¹².

Further evidence has come from fMRI studies, showing that both structures are important in heart rate responses after emotional facial expressions and responses after errors made in a Stroop-type test¹². The stroop-type test is a test in which the reaction time is important. Several colour names are or aren't printed in the same colour as the name said. It takes more time to name the colour when the word and colour are different compared with both the same colours.

The anterior insula and the ACC appear to be the components of a system based on the awareness of the self¹². In this system the anterior insula receives somatosensory information, which can be named as interoception, while the ACC is the output centre¹². This means that this system is involved in the integrated awareness of the emotional, cognitive and physiological state¹². The anterior insula receives sensory information and integrates the information into a self-awareness¹². It is then projected and processed in the ACC, which may ultimately select and prepare autonomic responses for inner and outer events¹². These events may be motor-responses but may also be a modulations of sensory processing or a modulation of the cognitive setting¹². Although the exact connections of the VEN's are not precisely known, they might form the connection between the anterior insula and ACC^{5, 14}. Therefore, it might be these cells, which are crucially involved in the formation of self-awareness and autonomic responses.

Functionality of the insula

The insula is implicated by a broad spectrum of functions¹⁸. Just like the anatomic connections of the insula are very complex, so are its functions¹⁸. Its functionality has been mainly investigated by imaging studies and lesion studies. Here, several possible functions associated with the insula will be described.

lesions showed more frequently atrial fibrillations, ectopic beats, inverted T-waves and atrioventricular blocks compared with left insular lesions (Table 2)²⁰.

This lateralization is also described by Graig, in which a model is described which states that emotional processing and sympathetic and parasympathetic afferents are processed

Table 2 ECG abnormalities and insular involvement in 179 patients with acute stroke, and ECG abnormalities and side of insular lesion in 42 patients with acute stroke and a unilateral insular lesion

	Insular involvement (n = 43)*	No insular involvement (n = 136)	χ^2 p value	Right insula (n = 17)	Left insula (n = 25)	χ^2 p value
Atrial fibrillation	6	15	0.772	4	1	0.009
Sinus tachycardia HR > 120 bpm	15	16	0.001	8	6	0.118
Sinus bradycardia HR < 45 bpm	7	18	0.802	3	4	0.896
Atrioventricular block	7	21	0.969	4	2	0.029
Ectopic beats > 10%	19	35	0.032	12	7	0.016
Prolonged QTc	4	7	0.470	1	3	0.736
ST depression	9	15	0.136	5	3	0.060
ST elevation	5	2	0.011	4	1	0.148
Inverted T wave	10	17	0.144	6	3	0.040
Isoform T wave	0	11	0.067	0	0	-

Data are number of patients.
*Including one patient with bilateral lesions.

Autonomic functions

Autonomic functions have been linked with the insula for several decades now¹⁹. Especially the cardiovascular modulation by the insula has been extensively studied¹⁹. Christensen *et al.* studied the effect of insular lesions on ECG abnormalities. They included 162 patients with a cerebral infarction and 17 patients with intracerebral hemorrhage²⁰. 43 of the 179 patients were diagnosed with acute insular lesion based on CT scans 5 to 8 days after the onset of the stroke²⁰. 25 patients were diagnosed with left insular damage, 17 with right insular damage and 1 patient with bilateral damage to the insula²⁰. By means of ECG any cardiac abnormalities were measured²⁰. The study found several ECG abnormalities with insular damage²⁰. Sinus tachycardia with heart rate >120 bpm, ectopic beats >10% and ST elevation were significantly more present in patients with insular damage (Table 2)²⁰. Besides involvement of the insula in autonomic functions, there also seems to be a lateralization of insular involvement²⁰. Patients with right insular

in a various way by the left and right insular cortices²¹. Evidence on which his model is based come from several studies²¹. The asymmetry already begins in the periphery with sympathetic and parasympathetic nerves²¹. For example, the left and right nervus vagus and nervus splanchnic differentially innervate organs in the thorax and abdomen²¹. Furthermore, left vagus stimulation, tasting foods and cardio respiratory manipulations leads primarily to left anterior insula activity²¹. Conversely, thermal sensations, pain and sensual touch leads to right anterior insula activation²¹. Therefore, Graig proposes that the left anterior insula is activated especially by parasympathetic functions, like taste and bradycardia, while the right anterior insula is activated by sympathetic functions like pain and thermal sensations²¹.

Taste and gustatory perception

The insula is associated with receiving, perception and integration of taste¹⁸. The anterior insula seems to play a role as a primary gustatory cortex as it shows

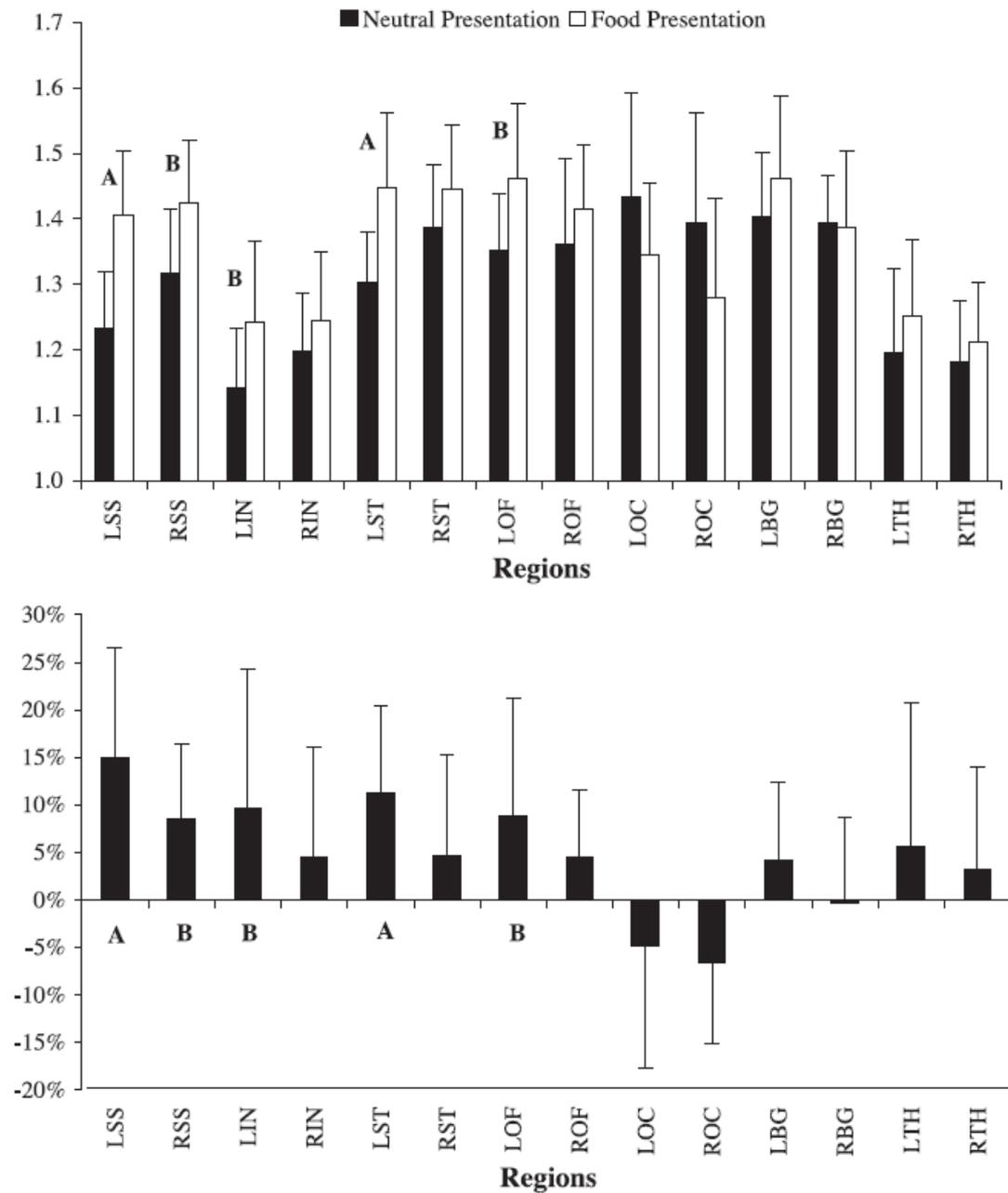


Figure 8. Relative changes in brain metabolism between food presentation and neutral stimuli. LSS: left somatosensory cortex; RSS: right somatosensory cortex; LIN: left insula; RIN: right insula; LST: left superior temporal cortex; RST: right superior temporal cortex; LOF: left orbitofrontal cortex; ROF: right orbitofrontal cortex; LOC: left occipital cortex; ROC: right occipital cortex; LBG: left basal ganglia; RBG: right basal ganglia; LTH: left thalamus; RTH: right thalamus. The largest increases can be found in the left and right somatosensory cortex, the left insula, the left superior temporal cortex and the left orbitofrontal cortex. A, $P < 0.005$; B, $P < 0.05$.

increased activity during food stimuli and because it has connections to the ventro-posterior-medial thalamic nucleus¹⁸. Wang *et al.* studied the response of the human brain during the presentation of food stimuli

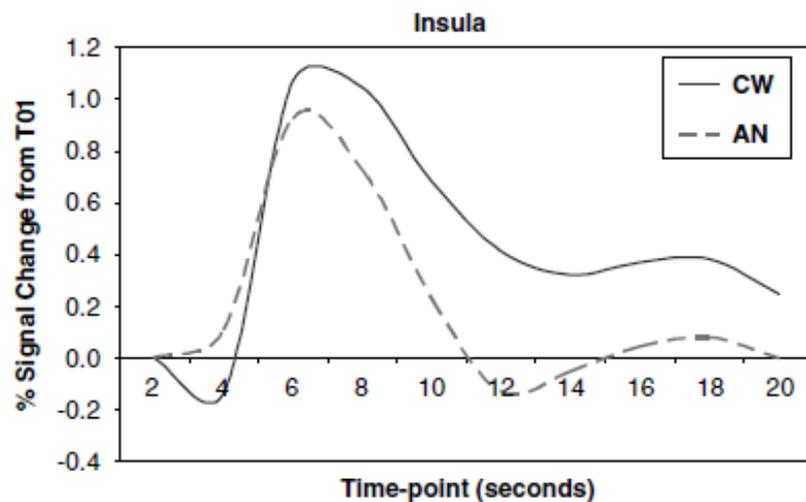
using PET scan²². Their study included twelve right-handed individuals²². Subjects were scanned with a PET scanner using 2-deoxy-2[18F]fluoro-D-glucose (FDG)²². They found that food presentation leads to a

significant increase in whole brain metabolism compared with neutral presentations²². Besides the increase in the whole brain metabolism, also specific brain regions were significantly increased during food presentation²². After normalization by whole brain metabolism, the left anterior insula showed one of the largest increases in metabolism with an increase of $+9.6 \pm 14.6\%$ $\mu\text{mol}/100\text{g}/\text{min}$ (Fig. 8)²². Other brain regions with large increases included the left and right postcentral gyrus (primary somatosensory cortex), left superior temporal and left orbitofrontal cortex (Fig. 8)²². This study shows that especially the left anterior insula is activated during food presentation²². These findings are consistent with the idea that the anterior insula has a role as primary gustatory cortex, together with the postcentral gyrus^{18, 19, 22}.

striatum and the orbitofrontal cortex showed no significant reductions in response to food stimuli in recovered AN patients⁶. Furthermore, recovered AN patients did not show a relationship between the activity of the insula and the pleasantness of the sucrose taste⁶. Moreover, they did not show any relationships with the activity in brain regions and the pleasantness of the taste, while the control group showed positive relationships for the insula, ventral and dorsal putamen and ACC⁶. Therefore reduced insula activity has been associated with a reduced sense of taste^{6, 18}.

There are also several insular lesion studies reporting gustatory deficits. Pritchard *et al.* performed a study in 1999 in which they examined the gustatory capacity of patients with insular damage²³.

Figure 9. AN patients do have an reduced response to food stimuli in the left and right insula compared with matched controls. The time course of BOLD signal has been displayed as a mean of the 16 recovered AN patients (AN) and the 16 control women (CW) for taste-related response in the insula.



Further evidence is provided by a study by Wagner *et al.* The propose of the study was to see whether individuals recovered from restricting-type AN respond differentially to sucrose, sweet taste, and water, neutral taste in the cortical regions of taste⁶. They used fMRI to investigate the brain response to the administration of food in individuals with AN⁶. Results showed that recovered AN patients had reduced responses to food stimuli in the left and right insula compared with matched controls (Fig. 9)⁶. In addition, the striatum and the ACC showed similar results, while the amygdala, atrioventricular

Six patients with unilateral insular damage were compared with three patients with non-insular damage and eleven matched controls²³. A magnitude estimation task and a taste recognition task were performed to assess an individuals taste intensity²³. Results showed that patients with insular lesions showed taste deficits in either of both tests²³. Further results showed that right insular damage impairs taste quality identification on the right side of the tongue but not on the left side, while left insular damage resulted impairment of taste quality identification on both sides of the tongue²³.

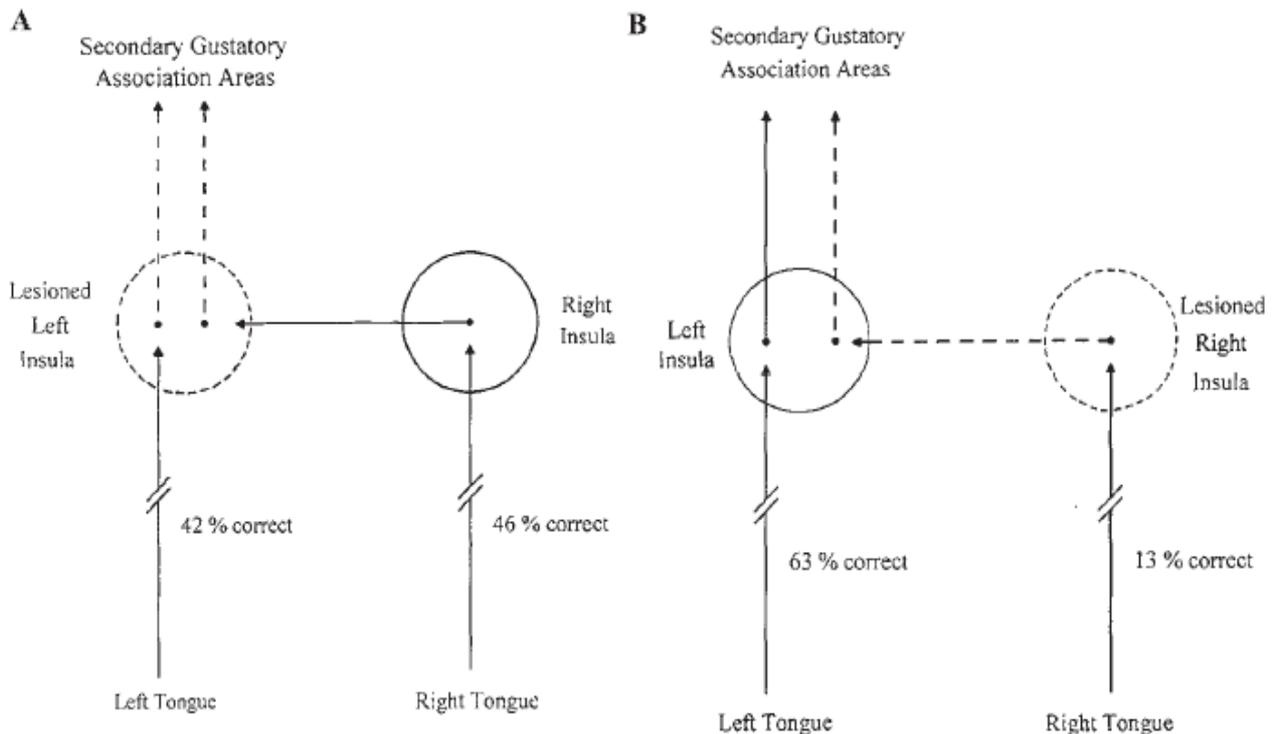


Figure 10. Schematic presentation of the pathway for the transmission of taste information from the tongue to the cortical areas. A: Left insular lesions causes a bilateral taste quality identification impairment of the tongue. This suggests that taste information of both sides of the tongue are being processed by the left insula, before being projected to the secondary gustatory areas. The % correct indicate the impairment. B: Right insular damage causes impairment of taste quality identification on the right side of the tongue, but not on the left side. This indicates that taste information on the right side of the tongue passes the right insula before crossing to the left insula.

These data suggest that taste information from both sides of the tongue first pass the ipsilateral insula with the information of the right insula projecting to the left insula before projecting to secondary gustatory brain regions (Fig. 10)²³.

Pain

The insular cortex has been associated with pain processing because the insula is often bilaterally activated during noxious stimuli²⁴. It is believed that afferent nociceptive information is transferred from the second somatosensory cortex to the posterior insula followed by transmission to the anterior insula²⁴. The reciprocal connections of the insula with the amygdala, prefrontal cortex, ACC, parahippocampal gyrus and the second somatosensory cortex allows the insula to play a central role in the integrating the sensation of pain^{18, 24}.

Recent fMRI studies have reported that the posterior insula is activated during innocuous cooling and noxious heat stimulation of the skin²⁵. Furthermore, muscle and viscera pain have also been associated with posterior insula activation²⁵. Lesions in the posterior insula have also been associated with alterations in pain perception. Some studies reported reduced pain effects and appropriate responses to pain, while anterior insula lesions seem to cause a greater pain tolerance contralateral¹⁹.

Craving

Studies investigating drug addiction and craving have mainly focused on the limbic structures like the amygdala, ventral striatum and mesolimbic dopamine systems¹⁹. However, the insula has become more interesting in the last years¹⁹. In a study by Contreras *et al.* the effect of inactivation of

the interoceptive insula on drug craving was investigated²⁶. The insular cortex of amphetamine-experienced rats was reversibly inactivated and the rats were tested using the place preference test²⁶. This test uses a two compartments with one white compartment paired with amphetamine or saline administration and one black room paired with saline administration²⁶. The rooms were connected with each other²⁶. Results showed that drug-naïve rats and saline-treated rats in the white compartment spent significantly more time in the black compartment in the first test, because of their preference for dark places²⁶. When treated with amphetamine in the second test, the amphetamine rats spent more time in the white compartment compared with the saline-treated rats²⁶.

white room again as preferred compartment²⁶. To make sure that this behaviour was specifically due to insula inactivation, the primary somatosensory cortex was injected with lidocaine and saline was injected into the insula of amphetamine-treated rats²⁶. These rats still spent more time in the white compartment²⁶. These results showed that inactivation of the insula in rats caused impairment of craving for drugs²⁶. Therefore, the insula is likely to be involved in craving for drugs. (Fig. 11)²⁶. This is supported by a study investigating the effects of insular lesions on smoking addiction. They predicted that insular damage would disrupt cigarette addiction²⁷. In this study, performed by Naqvi *et al.* 19 cigarette smokers with brain damage involving the insula were investigated²⁷.

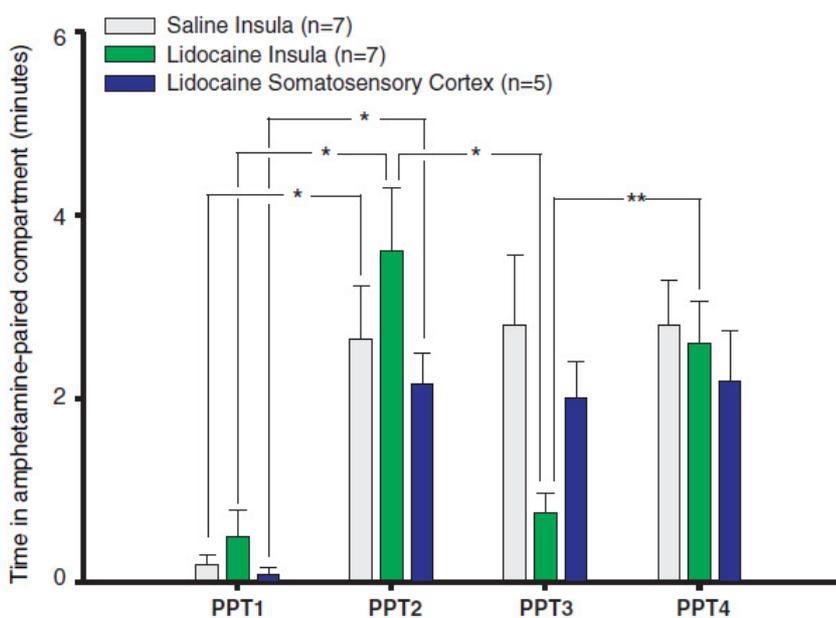


Figure 11. Amphetamine-treated rats changed their preference for the white compartment when the insular cortex was reversibly inactivated in a place preference test. The figure shows the time spent in the white compartment which was paired with amphetamine administration. Drug-naïve rats mostly chose the black compartment in the first test (PPT1). When treated with amphetamine in the second test (PPT2), the three groups prefer the white room. In PPT3 each rat was injected with lidocaine or saline in the insula or somatosensory cortex. Only rats with lidocaine injections in the insula changed preference to the black room. In PPT4, when the effect of lidocaine was gone, the lidocaine-injected rats preferred the white compartment again. The bars represent SEM. N is number of rats. *, $P < 0.001$; **, $P < 0.004$.

In the third test, amphetamine-treated rats were injected with the Na^+ channel blocker Lidocaine bilaterally in the insula²⁶. This blocks cortical structures reversibly for about 20 minutes and is used to inactivate the insular cortex²⁶. With insular inactivation, the amphetamine-treated rats returned to the black compartment and spent more time there compared with the white room which was paired with amphetamine administration²⁶. In the last and fourth test, inactivation of the insular cortex was over and amphetamine-treated rats chose the

Six patients with left insular damage and thirteen with right insular damage²⁷. This group was compared with 50 matched controls with brain lesions not involving the insula²⁷. They performed multiple logistic regression analysis which showed that patients with either left or right insular lesion are significantly more likely to disrupt their smoking addiction compared to non-insular lesions²⁷. Similar results were found when the left or right insular were examined separately²⁷. The results also showed that patients with right insular damage are more

likely to stop the addiction compared with left insular damage, although this was not significantly shown as the sample sizes were too small²⁷. Together, this indicates that smoking patients with insular damage are likely to stop smoking easily and immediately²⁷. Besides, they retain their stopped status, because smokers with insular lesions are very likely to not experience craving to smoke after quitting²⁷. Therefore, this study also suggests that the insula plays an important role in craving²⁷.

Experience of disgust

The insula is strongly associated with emotional processing^{18, 19}. One of these emotions includes disgust^{18, 19}. Animal and human studies over the last decades have given strong evidence to reinforce this association¹⁹.

fMRI studies in humans have shown that the insula is involved in the processing of disgust by facial expressions related to disgust²⁸. Phillips *et al.* used fMRI to investigate the neural substrates of disgust perception²⁸. They used pictures of facial expressions, which were shown at two intensities: 75% and 150%²⁸. Results indicated that subjects which were shown facial expressions of disgust had a significant increase in insula activity, mainly in the anterior insula²⁸. Thereby, activation in the anterior insula was significantly greater with a 150% intensity compared with the 75% intensity²⁸.

In addition, Wicker *et al.* conducted an fMRI study in which 14 right-handed males were exposed two times to a movie in which individuals smelled the contents of a glass with disgusting, pleasant or neutral substances²⁹. In this movie the individuals showed their facial expression of the respective emotions²⁹. The activation during the facial expression of disgust and pleasure were compared with that of the neutral expression²⁹. In the other test the subjects needed to inhale disgusting or pleasant

odorants²⁹. In this case, both odorants were compared with resting state activity²⁹.

The results of the olfactory test showed that the amygdala was activated during disgusting and pleasant odorants²⁹. The activation areas in the amygdala overlapped (Fig. 11A)²⁹. This finding is in accordance with other studies²⁹. The insula, however, showed also activation during disgusting and pleasant odorants but without an overlap in the activation area²⁹. The disgusting odorants activated the anterior insula on both sides, while the pleasant odorants activated the posterior insula only in the right hemisphere (Fig. 11B)²⁹.

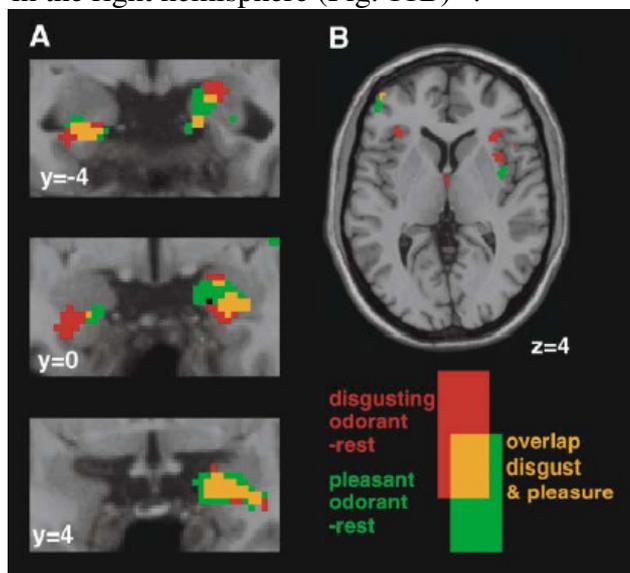


Figure 11. Results of the olfactory test. A: Coronal sections of the amygdalae showing activation during odorants. Green indicates activation during pleasant odorants, red indicates activation during disgusting odorants. And orange means the overlap between these two activation. Especially the right amygdala shows great overlap in activations. B: Axial slice showing the activation in the insula in response to odorants. Activity is bilateral and anterior in the insula during disgusting odorants and is more posterior and right-sided during pleasant odorants. There is almost no overlap.

The visual test results showed that the insula is mainly activated during the observation of disgust²⁹. More importantly, there seemed to be areas of overlap between the activation during the observation of disgust and the smell of disgust (Fig. 12)²⁹. These areas

included the left anterior insula and the transition zone between the insula and the inferior frontal gyrus²⁹. Another small overlap was found in the right ACC²⁹. The amygdala was not activated during the observation of disgusted facial expressions, which was a similarly result as previous reports²⁹. Further research indicated that the amygdala is more important in the emotion of fear than in disgust²⁹. However, the insula seems to be crucially involved in the experience of disgust²⁹.

Roman *et al.* conducted a CTA study in which the effect of insular lesions on CTA were investigated³³. They used rats which were divided into a SHAM and insular lesion group and were subdivided into a familiar condition and novel condition group³³. The conditioned stimulus was sodium saccharin³³. To evoke a conditioned taste aversion, lithium chloride injection was used³³. The results showed that pre-exposure to saccharin attenuated the forming of a taste aversion, which is indicated by the SHAM-

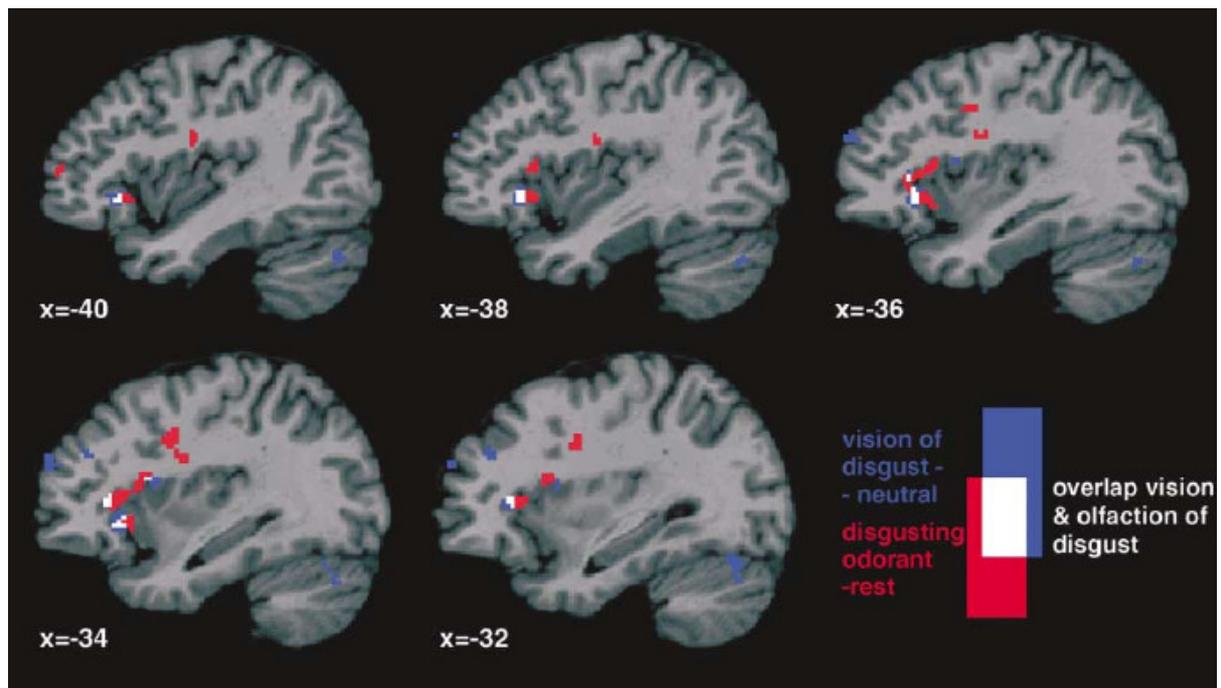


Figure 12. The overlap of the activity during disgusting odorants and the observation of disgusting facial expressions is illustrated. Red color indicates activity during exposure to disgusting odorants, blue indicates the activity during the observation of disgusting facial expressions and white indicates the overlap between these two kinds of activity. The left anterior insula, the transition zone between the insula and the inferior frontal gyrus and a small area in the right ACC are colored white.

Another kind of disgust is conditioned taste aversion (CTA)¹⁹. This is a phenomenon in which a subject associates a certain taste or food with a negative feeling³⁰. It is mainly used in animal studies in which the taste aversion is caused by the ingestion of food made toxic or disgusting and causes any kind of sickness to the subject³¹. When this aversion is made, the subject associates the food with illness and prevents it from eating the food again³². CTA is used in animal studies to artificially develop a taste aversion³³.

familiar rats³³. These rats showed an latent inhibition effect compared with SHAM-novel rats, which developed the CTA much quicker (Fig. 13)³³. The latent inhibition effect is the phenomenon that when a stimulus, like a taste, did not had any consequence in the past, it takes longer to learn the aversion to it³³. Furthermore, familiar insular lesion rats developed a CTA at the same slow rate as the novel insular lesion rats did (Fig. 13)³³. Besides, both insular lesion groups acquired the CTA more slowly compared with the SHAM groups³³.

These results support the idea that the insula is an important factor in the experience of disgust by learning a taste aversion but also that the insula is involved in the detection and recognition of taste novelty³³.

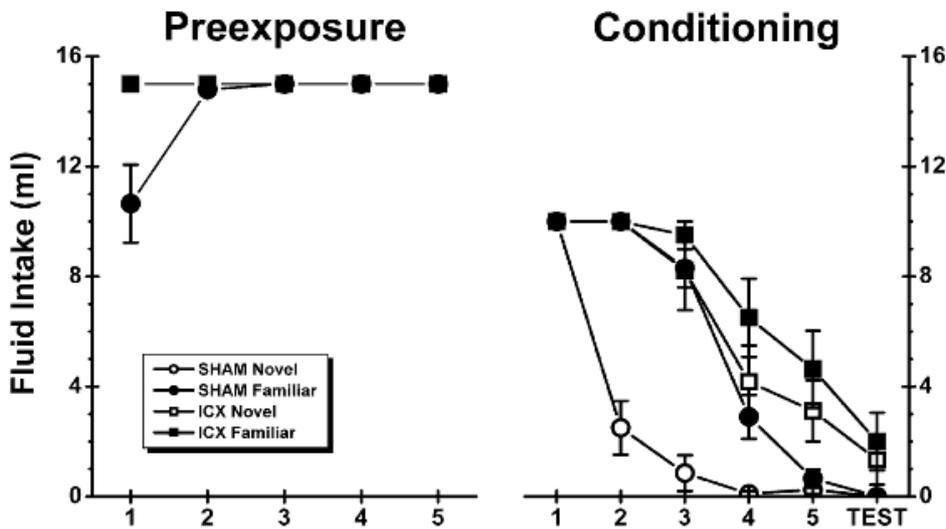


Figure 13. Saccharin intake by SHAM rats and rats with insular lesions (ICX) depicted as SEM. The pre-exposed rats showed similar saccharin intake, so differences in the conditioning are not caused by differences in pre-exposure to saccharin. SHAM-familiar rats developed CTA more slowly compared with SHAM novel rats, showing latent inhibition. ICX rats developed CTA more slowly compared with SHAM rats, but ICX novel and ICX familiar did not show differences in developing CTA when comparing each other. This suggest that the insula is crucially involved in the development of CTA and the recognition of disgust.

Insula impairment related to AN

The insula is a central structure which communicates with regions like the amygdala, striatum, frontal cortex, somatosensory cortex and parietal cortex⁷. The insula may be dysfunctional in AN patients and therefore the functional circuitry of the insula with other structures is impaired. This dysfunction may lead to the different characteristics commonly seen in AN.

Autonomic functions

Patients with eating disorders, like anorexia nervosa (AN) patients do have many disturbances in their autonomic nervous system³⁴. These disturbances are often associated with impairments of the insula, although the origin and pathogenesis of these abnormalities are not fully understood yet³⁴.

Several studies described a low arterial blood pressure in AN patients compared to healthy controls, while others showed that these patients have typically a heart rate lower than 50 beats per minute, also called bradycardia³⁴. Furthermore, several cardiovascular abnormalities have been found in AN patients like T-wave inversion, thinning and mass reduction of the left ventricle, voltage decrease, QT-prolongation and a reduced ejection fraction and minute blood volume³⁴. This is in line with the results of ECG studies, reporting ECG abnormalities in patients with insula impairments as described before.

Nunn *et al.* stated that the insula inhibits activity from the amygdala and its efferent projections to inhibit the sympathetic nervous system and that the insula can stimulate the parasympathetic nervous system⁷. In the model of Graig, the left insula is particularly involved in the parasympathetic system, while the right insula is important in the sympathetic system²¹. Disturbances in one or both insular cortices or the balance between these regions could lead to the intense anxiety and increased sympathetic nervous system activity, which

are often seen in patients with AN, especially during food stimuli⁷.

Taste and gustatory perception

The insula and especially the anterior insula has been associated with a role as primary gustatory cortex¹⁹. Over the years, multiple evidence has emerged showing that sweet and high fat stimuli are processed differently in persons with AN compared with healthy controls³⁵. Several studies have reported AN patients that dislike or avoid high fat foods but also AN patients dislike or avoid foods with many carbohydrates³⁵.

As described before, Wagner *et al.* found that patients with AN responded differently to sweet tastes compared with healthy controls⁶. They also did not show a relationship between the activity of the insula and the pleasantness of the sucrose taste⁶.

Evidence from lesion studies support the idea that the insula is involved in taste perception and may function as a primary gustatory cortex. These studies found that insular lesions result in the impairment of taste recognition and taste quality²³. The left insula is particularly important in this system as it receives taste information from the right insula and is then projected to the secondary gustatory cortex²³.

It is likely that the insula plays an important role in the impairment of taste perception in AN patients. Its presumable role as primary gustatory cortex and the taste impairments during insula dysfunction support this theory. As Wagner *et al.* found, AN patients have a reduced insular activity during stimuli with sweet tastes. A reduction or impairment in insular function or activity can therefore be important in the reduced sense of taste, which is commonly reported in AN patients¹⁸.

Pain

An increased pain threshold is an less well-known characteristic of AN⁷. There have been several studies which investigated pain thresholds in AN patients, with most of them reporting increases in pain thresholds³⁶.

Several possible explanations have already been proposed. These include a generalized peripheral neuropathy resulting from chronic malnutrition and an elevation in endogenous opioids, which is hypothesized to contribute to AN³⁶. Opioids are ligands for the Opioid receptors which are involved in pain perception. Other explanations include that it is the distorted body image which results in the higher pain threshold or the personal traits that are common in AN may cause this phenomenon, such as perfectionism, restraint and the need to control everything³⁶. However, other results indicate that these explanations are not very likely, although they can not be excluded³⁶. The dysregulation of the serotonin system has been proposed as a possible explanation. Serotonin activity has been associated with differences in pain perception and thresholds³⁶. Further research is needed to test this hypothesis.

Because the insula is often associated with pain perception, the insula could also have an important role in the elevated pain thresholds seen in AN patients. Especially the posterior insula seems to be involved in pain perception⁵. fMRI studies have shown that the posterior insula is activated during noxious stimuli, while posterior insular lesions resulted in reduced pain responses^{24,19}. Lesions of the anterior insula have been associated with a greater pain tolerance contralateral¹⁹.

All together, insula impairments may result in the elevation of pain thresholds. This leads to a relative insensitivity to pain, which is one of the characteristics of AN patients.

Craving

The intense dietary restriction leads to all sorts deficits and the body may compensate these deficits by craving for food³⁷. The serotonin system may be important in this craving³⁷. This system is involved in satiety and especially carbohydrates satiety³⁷. When people consume more carbohydrates, more tryptophan, the precursor of serotonin, is

available and more serotonin will be produced³⁷. This will lead to satiety³⁷.

The insula is also a region of interest when craving is investigated. Several studies have reported that the insula is involved in drug or smoke craving as described before^{26, 27}. People with a smoke addiction and an insula lesion are more likely to stop their addiction and do not experience craving anymore²⁷. An other study showed that insular inactivation leads to reduced craving for drugs like amphetamine²⁶.

Because of its role in drug and smoke craving, the insula may also be involved in the craving for food in AN patients. Impairments in the insula could lead to a reduced craving for food, so that AN patients are more able to resist their hunger and to keep dieting.

Experience of disgust

Disgust is one of the earliest emotions developed³¹. It is involved in the distaste of things that are potentially dangerous and can not be eaten³². It is reported that AN patients have a feeling of disgust towards foods, eating and the perception of their own body³². Because of the disgust towards foods and their own body, AN patients may have a strong system that enables them to restrict their food intake and strengthens the idea that they have to be thinner.

The insula may also be involved in this emotion as researchers reported increased insula activity during the perception of disgusted facial expressions²⁹. Also the experience of disgust by olfactory tests leads to insula activity²⁹.

Another form of disgust, conditioned taste aversion (CTA) is also related to the insula³³. Insular lesions resulted in a longer time to learn a taste aversion, showing that insula inactivity results in a reduction of the experience of disgust³³.

Taken together, it is likely that the insula plays a role in the experience of disgust. Increased activity results in disgust, while less activity results in a relative disgust insensitivity. Increased activity in the insula may therefore result in the elevated disgust

towards foods and one's own body, which is commonly noted in AN patients.

Although the insula may play a central role in the pathology of AN, there is still more research needed to make sure that the insula is really so important and to fully understand the mechanisms of insular pathology in AN patients.

All taken together, it can be concluded that the insula plays one of the most crucial roles in the pathology of AN. Due to its many functions it can be responsible for many of the symptoms of AN patients. Many studies have already found abnormalities in the insula of AN patients which may account for the taste deficits, elevated pain thresholds, disgust for food and less craving. Although it is not fully proven that impairments of the insula are accountable for many symptoms in AN, the autonomic nervous system, self-awareness, pain, craving, taste perception and disgust are all associated with insula (dys)function. An other important brain

structure is the ACC, which seems to work in close relation with the insula. The VEN's may be the connecting neurons between these structures and further research is needed to investigate their real function. Figure 14 summarizes the anatomical and functional connections of the insula with different parts of the brain.

With the results described before here, new hypothesises can be made. Because taste is closely related to appetite and eating, further research could focus on taste abnormalities in AN patients.

Based on the information described here, it is likely that the insula has a reduced activity in patients with AN during taste stimuli compared to healthy patients. A study in which the response of the insula to food and taste stimuli is investigated can therefore be suggested.

In this study, AN patients can be compared with recovered AN patients and healthy controls. The healthy women (controls) may

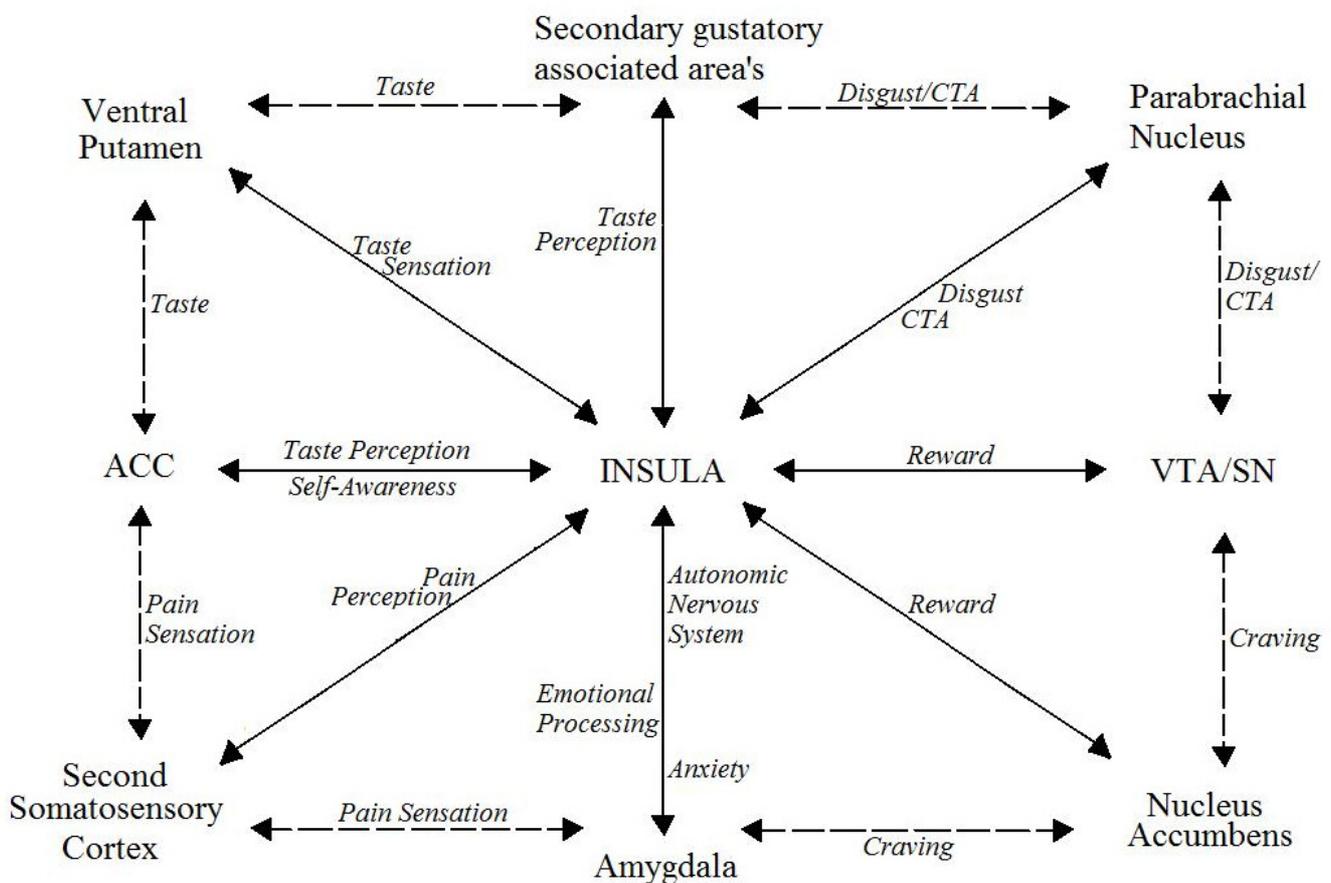


Figure 14. Schematic diagram of the anatomical and functional connections of the insula, which presumable play an important role in AN.

not have any desire to lose weight and must have a normal BMI (20-25), because this may influence certain brain activities. With the recovered AN group, one can hopefully get more information about the state or trait of certain abnormalities.

To make sure that the recovered patients are really recovered and are not likely to fall back into AN again, the recovered patients need to be healthy for at least a year and they need to have a normal BMI. Each group needs to contain about 15 to 20 individuals.

Subjects may be stimulated with different sorts of tastes such as sweet, neutral, sour, salt, bitter and high fat foods. AN patients dislike sweet tastes and high fat foods and therefore the insula may show a reduced activity during these tastes compared with the other tastes and the healthy controls. To see whether the whole taste system or just the aversive tastes are affected, the salt, sour, bitter and neutral tastes are incorporated in the test. It is likely that AN patients do not have an aversion against salt, sour and bitter tastes and therefore the insula may be more active during the ingestion of these tastes. After each taste individuals need to rinse their mouth to prevent habituation of tastes. By applying different intensities of the tastes, one can see whether taste intensity is related to brain activity. It may also be interesting to include two different sorts of sweet taste. The first will be a sweet drink with sugar and the second will be a sweet drink without sugar. This may give the information whether AN patients dislike the sweet taste or just the sugar.

Insular activity can be measured by means of fMRI. Before the test one can ask the subjects to try all the tastes and score the intensity and liking. People can have differences in the intensity of tastes. It is likely that a lower intensity also leads to a lower brain activity, therefore the concentrations of the tastes need to be different in order to get the same intensities. During the fMRI tests, subjects are not told which taste they will get in order to rule out

the brain activities found during the anticipation of food ingestion. This way the results show only the activity in the brain due to the taste. One can also let the subjects score the liking of the taste to see whether liking has changed due to the fMRI test. Patients with AN show increased levels of anxiety during food stimuli. If the anxiety levels of the subjects are scored and the activity of the amygdala is measured, one can see whether anxiety differences are related to insula abnormalities.

Although there is evidence that the insula plays the central role in AN patients and that the insula is mainly responsible for the symptoms found in AN patients, the evidence is mainly based on activity studies. Activity studies can give lots of information and can give an indication of the causes of abnormalities. However, in this case the insula has many connections with regions all over the brain. Therefore, abnormalities in the frontal lobe for example can affect the activity of the insula, while the insula is actually not damaged at all. So the results and associations between the insula and AN symptoms described in this paper can actually be caused by abnormalities of other brain regions. Because of its many functions and connections, its role is to integrate all that information, therefore it is difficult to perform a task or test in which the insula is specifically activated.

Besides, the results of the lesion studies are not fully reliable, because the lesions, for example strokes, do not damage only the insula, but also damages other brain regions. So results of these studies can not be fully assigned to the insula. This means that lesion studies can give a lot of information but can not be used to prove the hypotheses. Only specific lesions can be used to fully prove a hypothesis.

Another problem is that activity or lesion studies do not give information about why the insula is damaged in AN patients. It is important to know which regions cause the disorder but more crucial is to know why

these regions cause the disorder and how abnormalities in these regions can cause the symptoms of anorexic patients. More insights in the pathophysiology is needed to get more information about the cause of AN.

Research in animal models may be the key to get more understanding about the causes of the disease. The insula of starved or anorexic rat models can be investigated for differences in the cytoarchitectonics or gene expressions. Starvations will lead to loss of weight and loss of tissues. This may also influence gene expression and the cytoarchitectonics. It is therefore important to look at different grades of starved and anorexic rats to see whether the differences are correlated with weight loss and starvation.

One of the test that can be performed is a microarray in which gene expressions of cells in the insula of starved and anorexic rats can be compared with the gene expression in the insula of healthy rats. This can give an indication of which genes may be influencing AN so that the function of these genes can be further investigated.

The insula has many anatomical connections with other brain regions. These anatomical connections may be different in AN patients compared with controls. Studies could investigate in animal models whether differences in anatomical connections may be involved in AN. Starved and anorexic rats can be compared with control rats so that differences in these connections can be found. In this case it is also important to know that differences in the connections are not due to loss of weight, as loss of weight leads to reduced brain volumes.

Others may focus on the communication of brain regions due to neurotransmitter release. Neurotransmitters like serotonin and dopamine play an important role in the functions and communication between regions. Because it is very likely that a wide variety of brain structures are involved in AN and these communicate with each other mainly through neurotransmitters, there may

be some crucial differences between neurotransmitter communication in AN patients and controls. Also here it is important to make sure that differences are not caused by starvation and weight loss only.

The insula and the ACC are working in close relation with each other and may be responsible for the development of the 'self' and self-awareness, which is dysfunctional in AN patients. If the connections between the insula and ACC in rats are broken, one may see the differences in behavior in this group compared with other rats. This behavior may be related to self-awareness processes.

As described before, higher primates and humans have the so called von Economo neurons (VEN), which are believed to be the neurons connecting the insula and the ACC with each other. Primates may have more self-awareness because they are cognitive higher animals. Tests in which connections between in the insula and ACC are broken may also be performed in the higher primates to see differences in behavior which is likely more related to behaviors in humans. In this case the VEN's can be important to investigate. By damaging the VEN connections one can get more insights in the function of VEN's. If these neurons are really the cells which connect the insula and ACC with each other, some differences in behavior related to insula-ACC function has to be found.

Another test related to the VEN's may be to investigate the organization and gene expression of the VEN's in starved or perhaps even anorexic primate models. If the VEN's are important in AN, some differences had to be found. Again, this can be done with micorarrays and immunohistochemistry as VEN's can be labeled by DISC1.

These tests may be some of the many investigations, which has to give more information about the pathological mechanisms of AN. Ultimately, we hope that these investigations can lead to a successful therapy.

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