

Can neuroimaging explain the underlying mechanisms of overeating when comparing obese to lean subjects?

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Abstract

Obesity is a condition caused by overeating, to a state where a person's weight is not proportional to his or her length anymore. The concept of overeating is not completely solved. Many researchers have tried to unravel the underlying mechanism that causes overeating using Positron Emission Tomography (PET) or functional Magnetic Resonance Imaging (fMRI).

Different research groups have used neuroimaging as a tool to come up with a piece of the story and they tend to agree that their answer is only part of the solution. Abnormal activation of brain areas of an obese person in response to eating is often in regions that are connected to emotion, control of behavior and reward. Important areas are the prefrontal cortex, dorsal insula, hippocampus, limbic/paralimbic areas, amygdala and a reduced amount of dopamine D₂ receptors in the striatum. The role of hormones is not taken into account in this paper, but is also important for understanding overeating.

The real mechanism is complex and more studies have to be done. Neuroimaging can be used to do further research.

Keywords: neuroimaging; obesity; positron emission tomography (PET); fMRI; satiation

1. Introduction

Heavy persons have existed for ages. In the old days, having a bit more weight was seen as a status symbol. Wealthy people could afford to eat what they want without having to work all day. Obesity was not considered a major concern. A person is overweight if his or her Body Mass Index (BMI) is more than 25 and obese if the BMI is more than 30. The BMI can be calculated by dividing the weight in kilograms by the squared length in meters. Nowadays, obesity is a big problem. Although obesity in itself is not a health problem, it is the cause of many; obese people have, among others, a higher risk of developing cardiovascular diseases or diabetes [18].

In rich countries, where (fast) food is largely available and people are always in a hurry, obesity is a big issue [9]. The time of three healthy, well-proportioned meals a day seems over for a lot of people. Food has to taste good, we do not stop eating until we are completely satiated and afternoon and midnight snacks are no exception.

Although many people can imagine the problem of obesity in rich countries, in developing countries, obesity is a problem as well [6]. People with a low income get obese due to a lipid-rich diet and not enough physical activities.

Luckily, this is not the case for every man and woman in the world; lean people still exist. A lean person stops eating when he or she has had enough and therefore does not eat more calories than he or she burns on a day.

A variety of researchers have looked at the differences between obese and lean people. All the studies were done using neuroimaging. Some of them involved tasting a little bit of liquid after a period of fasting [2, 3, 4, 5, 7 and 8], some researchers let subjects look at pictures of food [14, 16] and other studies concentrated on a particular brain area while taking scans, without performance of a task [1, 11, 20, 21].

What this paper doesn't cover is that some lean people or successful dieters might have the same problems as obese people, but are better able to withstand the urges to eat more, to maintain their figure. And there are people who have problems with their thyroid, which can cause a slower metabolism. A number of hormones, like leptin, that also play a role in the regulation of food intake fall outside the scope of this paper.

Lean people stop eating after they've had enough, so what is the difference between obese and lean people? Maybe obese people do not get a signal that their stomach is full. Perhaps they are addicted to food. Is there a reason why they can't control themselves? Can these questions be solved using neuroimaging? The main question in this paper is what are the differences in brain responses of

obese people compared to lean people? Afterwards is discussed whether the differences can explain why obese people overeat.

2. Modalities used

The modalities used in the mentioned studies are mainly Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI), both techniques are able to capture brain activity.

With PET, a radiotracer is used. In most of the cases this is labeled water, with a radioactive version of oxygen (^{15}O). A brain area that is more active uses more oxygen. The oxygen is transported to the area by blood. The labeled oxygen falls apart (because it is radioactive) sending out a positron. The positron collides with an electron and annihilate, while sending out two photons, in opposite directions. The PET scanner (a large ring around a participant) detects two photons on opposite sides of the ring. This way, the position of the event can be traced. What is measured is regional Cerebral Blood Flow (rCBF). As opposed to Cerebral Blood Flow (the blood supply of the brain in a given time), rCBF is the amount of blood going to a particular region of the brain. On the images, brain areas that were more active than others (thus where more oxygen was consumed) have a different color than less active areas.

With an MRI scanner, not only anatomical images can be made. Magnetic Resonance Imaging is also the basis for other techniques such as functional MRI or Diffusion Weighted Imaging (DWI). MRI itself uses the fact that the hydrogen protons in water molecules align when placed in a strong magnetic field. The human body contains a lot of water, in different concentrations. When a radio frequency pulse is given, the protons align in a different way, according to their position. After the pulse, the protons relax and return to their initial alignment. The main magnetic field is not the only magnetic field that plays a role in MRI; additional coils are used (preferably close to the region of interest) for the calculation of the position of the hydrogen protons.

The described technique is very useful for anatomical images, but there are numerous variations, like fMRI, which is capable of capturing brain activity. This MRI-based technique uses the so-called BOLD effect (blood-oxygen-level dependent). An active brain area needs more oxygen, this leads to an increased perfusion. As a result, the concentration of oxyhemoglobin (blood with oxygen) increases and the concentration of deoxyhemoglobin (blood without oxygen) decreases. This change in concentration can be measured locally, making it possible to create a functional image that shows brain activity.

Diffusion Weighted Imaging is a variation that is not able to show brain activity, but differences in water diffusion on a microscopic level. The contrast in a DWI image is accomplished by a difference in signal attenuation related to a variation in the diffusion coefficient of water molecules. The diffusion coefficient is different for various tissues. This method is used in one of the studies to measure the amount of intracellular and extracellular body fluid in obese and lean people [1].

3. What happens in the brain when you eat?

Before going into the matter of overeating, here is a short overview of what happens in the brain when food is consumed.

To maintain a healthy BMI, no more calories should be eaten than burned. To prevent overeating, a signal from our brains that tells us to stop eating will be convenient. This system of controlling human feeding behavior is not so simple; it is not just a signal. What happens exactly is not completely mastered yet, but there are indeed certain differences between obese and lean people, considering what happens when a meal is consumed.

What happens in the brain in response to a meal is that neuronal activity increases in the prefrontal cortex and decreases in the hypothalamus, insular cortex, orbitofrontal cortex, thalamus and hippocampal formation [11]. Moreover, brain dopamine should be released, to reward the properties of the food [12]. Together, this is what should make a person satisfied after eating a meal.

4. Table of studies

Table 1 shows what studies might answer the question posed in the introduction. The articles are grouped per type of experiment.

Table 1. Different studies comparing obese and lean people using neuroimaging

Author, year and title	What kind of experiment	Modality	Number of subjects	Differences between lean and obese people
DelParigi, 2005: Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast	Tasting a liquid meal after a 36-h fast.	PET (^{15}O -water)	21 obese, 20 lean subjects.	Obese people have a greater neural response to the sensory stimulation in the insular cortex.
DelParigi, 2005: Neuroimaging and Obesity: Mapping the Brain Responses to Hunger and Satiation in Humans Using Positron Emission Tomography	Tasting a liquid meal after a 36-h fast.	PET (^{15}O -water)	23 obese, 21 lean subjects.	After tasting a liquid meal, cerebral blood flow in obese people is higher in the prefrontal cortex and limbic/paralimbic areas than in lean people.
DelParigi, 2004: Persistence of abnormal neural responses to a meal in postobese individuals	Tasting and consuming a satiating meal after a 36-h fast.	PET (^{15}O -water)	11 postobese, 23 obese and 21 lean subjects.	When consuming a meal, successful dieters showed higher activation in the dorsal prefrontal cortex, dorsal striatum and anterior cerebellar lobe, while the orbitofrontal cortex was less activated.
Gautier, 2000: Differential Brain Responses to Satiation in Obese and Lean Men	Tasting a liquid meal after a 36-h fast.	PET (^{15}O -water)	11 obese, 11 lean subjects.	In obese men, increase in cerebral blood flow in ventromedial and dorsolateral prefrontal cortex, decrease in rCBF in limbic/paralimbic areas, caudate, precuneus, putamen and cerebellum. Neuronal activation of prefrontal cortex was greater in obese men. Neuronal deactivation was in obese men greater in limbic/paralimbic areas, temporal and occipital cortex and cerebellum.
Gautier, 2001: Effect of satiation on brain activity in obese and lean women	Tasting a liquid meal after a 36-h fast.	PET (^{15}O -water)	12 obese, 10 lean women.	Obese women respond to satiation with greater activation of the prefrontal cortex and greater deactivation of the limbic/paralimbic areas. Also a satiation-induced deactivation in amygdala and nucleus accumbens in obese women.
Le Duc Son, 2007: Less activation in the left dorsolateral prefrontal cortex in the reanalysis of the response to a meal in obese than in lean women and its association with successful weight loss	Tasting a liquid meal after a 36-h fast.	PET (^{15}O -water)	8 postobese, 9 obese and 10 lean women.	Obese women have less activation in the left dorsolateral prefrontal cortex. Women who used to be obese show comparable results with lean women.

Author, year and title	What kind of experiment	Modality	Number of subjects	Differences between lean and obese people
DelParigi, 2007: Successful dieters have increased neural activity in cortical areas involved in the control of behavior	Sensory experience of food and meal consumption.	PET (¹⁵ O-water)	9 successful dieters, 20 non-dieters.	Successful dieters are able to control inappropriate behavioral responses in response to meal consumption (they can restrain themselves from eating to much).
Stice, 2008: Relation of reward from food intake and anticipated food intake to obesity: an fMRI study	Intake of a chocolate milkshake compared to a tasteless solution.	fMRI	33 adolescent girls, of which 7 obese, 11 lean and 15 in between.	Obese girls show greater response in areas related to food reward. Activation of the striatum was decreased, which has a link to an impaired dopamine release.
Rothemund, 2007: Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals	Looking at pictures of food with different caloric content and energy density.	fMRI	13 obese, 13 lean subjects.	Obese subjects have a higher activation of the dorsal striatum while looking at pictures with high-caloric food. This abnormal activity is similar to that of alcoholics and drug addicts.
Stoeckel, 2008: Widespread reward-system activation in obese women in response to pictures of high-calorie foods	Looking at pictures of food with different caloric content and energy density.	fMRI	12 obese, 12 lean subjects.	Obese people have a higher activation in a large number of regions of the brain when looking at pictures of food with a lot of calories.
Wang, 2001: Brain dopamine and obesity	Measuring the availability of brain dopamine D ₂ receptors.	PET ([¹¹ C] raclopride)	10 obese, 10 lean subjects.	People with a higher BMI have less dopamine D ₂ receptors in the striatum.
Alkan, 2008: Diffusion-weighted imaging features of brain in obesity	Images were taken to examine distribution of fluid (extracellular /intracellular) in brain tissues	MRI, DWI	81 obese, 29 lean subjects.	Obese subjects showed an increased amount of fluid in locations of the brain that have to do with satiety and hunger.
Wang, 2006: Gastric stimulation in obese subjects activates the hippocampus and other regions involved in brain reward circuitry	Subject got an Implantable Gastric Stimulator (induces stomach expansion) metabolism was evaluated while IGS was on and off.	PET (FDG)	7 obese subjects (who had an IGS implanted for 1-2 years).	The region that was affected most by turning the device on was the hippocampal area. This area has influence on emotion, but also modulates dopamine release in the striatum.

5. Different kinds of studies

Table 1 shows several experiments (some of them are not so much different from each other and can be grouped, that is why the studies are discussed per type of experiment). There are roughly three kinds of studies that have been done, which are explained in the following sections.

5.1. Tasting a liquid meal after a period of fasting

This type of experiment was performed several times (mainly by the same research group). It involves both lean and obese people. Male and female subjects are tested (with the female subjects in the same phase of their menstrual cycle). The subjects did not eat for 36 hours (drinking water was allowed) to make sure they were not satiated (actually they were really hungry). In the experiments of DelParigi et al [2, 3, 4, 5], subjects first fill out the Three Factor Eating Questionnaire. This is a list of multiple choice questions about the control of eating behavior, disinhibition and hunger. Before and after consuming the liquid meal (a portion of 2 ml), a scan was made (if another scan was made, again, 2 ml of a liquid meal was consumed).

Although a lot of studies have been done, the results are very similar. They all use a PET scanner to measure regional cerebral blood flow (rCBF). If a certain region was active, more blood was needed, so a higher rCBF was measured.

In obese subjects, there is an increase in rCBF in the (dorsal) prefrontal cortex [2, 7, 8] after tasting the meal. The prefrontal cortex can have an inhibitory effect on food intake. This inhibition is accomplished by increasing the neuronal activity of the limbic/paralimbic areas, hypothalamus, caudate and putamen [7, 8]. This phenomenon can be seen on the scans, there is indeed less activation in these areas compared with lean subjects.

In addition to an increased activation of the prefrontal cortex, obese subjects have a greater neural response to the sensory stimulation in the insular cortex. The insular cortex has to do with (among others) food reward and emotion [4]. Because of this difference in activation, obese people may need more reward than lean people when they are eating (and therefore eat more). Successful dieters were able to control the need for more reward [5]. They show more neural activity in the dorsal prefrontal cortex, dorsal striatum and anterior cerebellar lobe, but less activity in the orbitofrontal cortex (3). This means that the cognitive control of food intake can be achieved by modulating neural circuits controlling food reward.

A different study by Stice et al [15], who compared the intake of some chocolate milkshake with a tasteless solution (scans with fMRI) has similar results. Participants with higher BMI show greater response in areas related to food reward. Activation of the striatum was decreased, which can cause an impaired dopamine release. This can all result in overeating, to make up for a lack of feeling rewarded.

A study by Le Duc Son et al shows contrasting results. This study shows that obese women have a decreased (not increased) activation of the left dorsolateral prefrontal cortex in response to a meal. This area has an inhibitory effect on inappropriate behavior, satiety and meal termination. Women who have successfully lost weight show the same activation in response to food intake as lean women [11].

5.2. Looking at pictures of food

The previous set of studies was about tasting food and measuring the amount of blood that flows to particular regions of the brain. Another way of finding out more about the differences between the brains of obese and lean people is to find out which brain areas are active when subjects look at pictures of food. This is done with fMRI and three sets of pictures. There were pictures of low-calorie food, pictures of high-calorie food and control pictures (with no food). Before scanning, subjects were asked how hungry they were, how likely they were to eat food now and how much they wanted to eat. The different kinds of pictures were alternated in blocks while in the scanner.

Obese subjects showed a higher activation of the dorsal striatum while looking at pictures with high-calorie food. This area is known for its role in planning and executive functions, but also involved in handling rewards and habit learning [14]. Looking at these high-calorie foods also activated regions associated with taste and motivation (anterior insula and lateral orbitofrontal cortex) and emotion and memory functions (posterior cingulate).

Another study [16] confirms this outcome. Obese subjects have a higher activation in multiple regions of the brain when looking at pictures of high-calorie foods, compared to lean subjects. This amplified response of the reward system could be responsible for the weight gain of obese people. This abnormal way of reward handling is similar to that of alcoholics and drug addicts and might have something to do with a lowered dopamine D_2 receptor availability, which will be discussed in the next section.

5.3. Other methods

In addition to studies where participants have to do a certain assignment (tasting a meal or looking at pictures), there are also neuroimaging studies where researchers look at differences in the brains of obese and lean people without letting them do a task. One of the studies is about a reduced amount of dopamine receptors in obese people [20], which has been indicated by several other researchers. Another study is about the distribution of fluids in the brain [1]. Finally, a somewhat different approach is a study that uses an implanted device in the stomach that can send a satiation signal to the brain [21].

To study the effect of dopamine release after food intake, the research group of Wang and Volkow et al made PET scans of the brain, without doing a particular task [20]. With a PET scan, not only rCBF can be measured. A radiotracer can also be used to determine the amount of dopamine D₂ receptors. The radiotracer binds to the D₂ receptors and the number of D₂ receptors in the striatum that are free to bind to the radiotracer can be calculated.

As predicted, people with a higher BMI have less dopamine D₂ receptors in the striatum. This effect can also be seen in people with an addiction to cocaine [19] or alcohol [10]. People with this impaired reward system are more likely to overcompensate for their lack of reward and (in case of food reward) thereby gain weight.

All described studies so far were done by either PET or fMRI. Another method is Diffusion Weighted Imaging (DWI). With this method, which is performed in an MRI scanner, differences in water diffusion can be made visible on a microscopic level. Obese people have more extracellular body fluid than intracellular compared to lean people [1], so to examine the distribution of intracellular and extracellular brain fluids, DWI is a very effective modality.

This study of Alkan et al [1] revealed that obese subjects showed an increased amount of extracellular fluid in locations of the brain that have to do with satiety and hunger, compared to lean people. Understanding these abnormalities might help understanding the underlying mechanism of obesity.

While all the other described studies include obese and lean subjects, there was another study that only involves obese people [21]. It is indeed a somewhat different study, because the subjects have had a device implanted for 1-2 years. It tries to understand the mechanisms of overeating in an alternative way.

The device is an Implantable Gastric Stimulator (IGS), which can stimulate the vagus nerve. This nerve can trans-

port a satiety signal to the brainstem. If this signal is sent by the IGS (instead of by the stomach itself), it can lead to satiety (without a full stomach), which leads to decreased food intake.

PET scans were made with the IGS on and off. The region that was affected most was the hippocampal area. This area has influence on emotion, but also modulates dopamine release in the striatum and inhibits the prefrontal area. Again, these regions have been associated with drug addiction.

6. Discussion

The different studies that have been done with obese and lean people all try to understand the underlying mechanisms of overeating. Obese people need more food than lean people to satisfy their body and mind. This effect is not due to one impaired region, it is a very complex network. There are a number of differences between obese and lean people that can cause an urge to eat more than necessary. First a summary of the found differences, followed by a discussion.

Abnormal activity in the prefrontal cortex and insular cortex can affect people's emotion, behavior control and reward system. Due to the lack of feeling rewarded or the lack of control, obese people tend to eat more. This kind of behavior can also be seen in drug addicts, who also can't control their craving for more drugs. More studies will have to prove whether people with this lack of control can be helped in case of obesity.

A reduced number of dopamine D₂ receptors can cause a reduced feeling of being rewarded, when eating food [20]. Eating more food to increase the amount of reward is obviously not the best solution. There are drugs that can increase extracellular dopamine, but they are addictive and anorexigenic [17]. Behavioral exercise to improve the release of dopamine has proved to be useful in animal research and might be interesting to test for humans [12].

Stimulation of the vagus nerve to simulate a full stomach leads to the feeling of being full [13]. After a meal, this signal should always be sent to the brain to let the brain know the stomach is full. If this signal does not reach the brain in time, the brain might think that there is still some room left. For people who have problems with this signal, an Implantable Gastric Simulator can be a solution.

The discussed studies are performed by only a few research groups and only a limited number of methods are used. The experiment where a liquid meal is consumed after a period of fasting [2, 3, 4, 5, 7 and 8] is always performed after a period of 36 hours. This is an extreme amount of time; af-

ter 36 hours, the subjects are hungrier than they usually are. Maybe a shorter period of time would have been sufficient as well. Doing the experiment after a night of fasting might give the same results. This shorter period is more comfortable for the subjects and is more similar to the state people are usually in when they are hungry.

Some of the results are not comparable. If DelParigi et al find a higher activation of the dorsal prefrontal cortex of obese people compared to lean people after consuming a meal [3] and Le Duc Son et al finds less activation of the left dorsal prefrontal cortex [11], is this contrasting or is this very specific for the left part of the dorsal prefrontal cortex? And while a lot of the studies are alike, the results differ. Taken together, a lot of regions account to the problem of overeating, some of which are only found in a few studies. More studies have to be done to find out which areas are the most important.

In this article, neuroimaging is only used to look at different responses in brain areas. The role of hormones is not taken into account. MRI can, however, also be used to study the effect of hormones, like leptin. Leptin is a protein hormone that plays an important role in regulating body weight and metabolism [13]. Combining the results of the discussed neuroimaging studies with different hormone studies can give a better insight in the concept of overeating.

In all the different studies, a lot of differences in brain responses were found between obese and lean people. Can the differences explain overeating? Every individual difference can cause a person to overeat and therefore become obese, but there is no universal difference that explains everything.

Although more studies have to be done, the eventual answer can probably be given by neuroimaging techniques. It is a good tool to take a look at the brain and can also be used to study the role that hormones play in overeating.

7. Conclusion

Neuroimaging can certainly help us getting more insight in the underlying mechanisms of overeating. There are several causes for an increased intake of food in obese people. Obese people have a different response in the prefrontal and insular cortex than lean people after eating, causing them to overeat. A reduced number of dopamine D_2 receptors in the dorsal striatum is also a cause for overeating. Yet the complete puzzle has still some missing pieces. If we understand these complex causes better, it might give us a chance to predict and maybe even prevent obesity. Neuroimaging has given us a better understanding of the differences in brain responses between obese and lean people, but it has not led to a complete understanding of why obese people overeat.

8. References

1. ALKAN, A ET AL. Diffusion-weighted imaging features of brain in obesity; *Magn Reson Imaging*. 2008 May;26(4):446-50. Epub 2007 Dec 11.
2. DELPARIGI, A ET AL. Neuroimaging and Obesity: Mapping the Brain Responses to Hunger and Satiation in Humans Using Positron Emission Tomography; *Ann N Y Acad Sci*. 2002 Jun;967:389-97.
3. DELPARIGI, A ET AL. Persistence of abnormal neural responses to a meal in postobese individuals; *Int J Obes Relat Metab Disord*. 2004 Mar;28(3):370-7.
4. DELPARIGI, A ET AL. Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast; *Neuroimage*. 2005 Jan 15;24(2):436-43.
5. DELPARIGI, A ET AL. Successful dieters have increased neural activity in cortical areas involved in the control of behavior; *Int J Obes (Lond)*. 2007 Mar;31(3):440-8.
6. DELPEUCH, F ET AL. Obesity and developing countries of the south; *Med Trop (Mars)*. 1997;57(4):380-8.
7. GAUTIER, JF ET AL. Differential Brain Responses to Satiation in Obese and Lean Men; *Diabetes*. 2000 May;49(5):838-46.
8. GAUTIER, JF ET AL. Effect of satiation on brain activity in obese and lean women; *Obes Res*. 2001 Nov;9(11):676-84.
9. GINTER, E ET AL. Adult obesity at the beginning of the 21st century: epidemiology, pathophysiology and health risk; *Bratisl Lek Listy*. 2008;109(5):224-30.
10. HIETALA J, ET AL. Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence; *Psychopharmacology*. 1994; 116: 285-90.
11. LE DUC SON ET AL. Less activation in the left dorsolateral prefrontal cortex in the reanalysis of the response to a meal in obese than in lean women and its association with successful weight loss; *Am J Clin Nutr*. 2007 Sep;86(3):573-9.
12. MACRAE, PG ET AL. Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats; *Psychopharmacology*. 1987; 92: 236-40.

13. PATEL, SB ET AL. Leptin: linking obesity, the metabolic syndrome, and cardiovascular disease; *Curr Hypertens Rep.* 2008 Apr;10(2):131-7.
14. ROTHEMUND, Y ET AL. Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals; *Neuroimage.* 2007 Aug 15;37(2):410-21. Epub 2007 May 18.
15. STICE, E ET AL. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study; *J Abnorm Psychol.* 2008 Nov;117(4):924-35.
16. STOECKEL, LE ET AL. Widespread reward-system activation in obese women in response to pictures of high-calorie foods; *Neuroimage.* 2008 Jun;41(2):636-47.
17. TOWELL, A ET AL. Behavioural microanalysis of the role of dopamine in amphetamine anorexia; *Pharmacol Biochem Behav.* 1988; 30: 641-48.
18. UREK, R ET AL. Obesity—a global public health problem; *Acta Med Croatica.* 2007 Apr;61(2):161-4.
19. VOLKOW, ND ET AL. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers; *Synapse.* 1993; 14: 169-77.
20. WANG, GJ ET AL. Brain dopamine and obesity; *Lancet.* 2001;357:354-7.
21. WANG, GJ ET AL. Gastric stimulation in obese subjects activates the hippocampus and other regions involved in brain reward circuitry; *Proc Natl Acad Sci U S A.* 2006 Oct 17;103(42):15641-5. Epub 2006 Oct 5.