Effects of Glucose on Cognitive Performance and Brain Activation: a fMRI Study

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December 2010

Abstract

Background: Glucose is the primary fuel for the brain. The role of glucose in the performance of cognitive tasks been extensively studied over the years. The possible and desirable facilitative effects of glucose on cognitive performance have been explored in various ways including behavioural and psychological tests and by studying brain activation patterns on brain imaging. The literature is also dotted with anecdotes of detrimental effects of acute severe hypoglycemia on cognition. However, the possible deleterious effects of recurrent hypoglycemia, in people with diabetes treated with insulin, remains divisive.

The body of work which this thesis forms part of was to look at the effects of hypoglycemia on cognitive performances and simultaneous brain activation patterns (Appendix 1) by inducing experimental hypoglycemia (clamps).

Rationale: The study was set to examine the effects of changes in blood glucose on memory function and upon accompanying patterns of brain activity. It was also aimed to identify memory differences between non-diabetic subjects, and patients with type 1 diabetes (T1DM) with and without exposure to severe hypoglycemia. Thus, prior to embarking on complex clamp studies in people with diabetes, the cognitive performances and brain activation patterns of a small dose of oral glucose were studied in non-diabetic healthy individuals. This study would serve as a critical prelude to an exploration of the effects of altered glucose levels on memory performance in diabetic subjects.

Topical work by Farooqi et al (Farooqi, et al., 2007) showing modulatory effects of leptin on the nucleus accumbens and appetite regulation, prompted the study of glucose load on a further cognitive domain, namely brain activation patterns in response to images of foods.

The first part of the body of this thesis is made up of the study of the cognitive performances and the brain activation patterns of a small dose of oral glucose in healthy volunteers. The second part details the study of brain activation patterns on viewing food imagery under influence of glucose and sweetener.

Hypotheses: Previous work has shown an effect of glucose on neurocognitive functioning. The hypothesis was that cognitive performance on long-term verbal memory tasks would be enhanced following 25 g of oral glucose. This would be mirrored by differences in brain activation patterns.

For the food task, the hypothesis was to see increased brain activation in limbic regions of the brain on viewing food pictures following ingestion of the control drink, aspartame. This state would be perceived as the fasted state and thus activate the hedonic circuitry on presentation of food (images). Activation in the limbic regions would be dampened following 25 g of oral glucose.

Methods: A controlled randomised single-blind cross-over study was conducted on 13 healthy right-handed individuals. Brain activation patterns using functional magnetic resonance imaging and cognitive performance (verbal memory tasks, continuous performance testing and working memory) were studied following 25 g of oral glucose.

Following the cognitive tasks, in parallel, the effects of the glucose load on a further cognitive domain, namely activation in response to images of foods was also examined.

Results: Despite being sufficient to cause a small but significant rise in blood glucose and insulin, there was no significant effect of the glucose load on either cognitive performance or brain activation for either of the cognitive tasks.

In contrast to the lack of effects seen on the tasks above, significant differences in brain activation was observed with the glucose load, with areas of the limbic region, being increasingly activated in response to food images following glucose ingestion. This was in contrast to our original hypothesis.

Conclusions: The study was short of demonstrating a facilitative effect of glucose on cognitive performance partly due to loss of vital performance data on the verbal memory task, which has been shown to be glucose-sensitive. In addition, one could speculate that failure to observe a glucose effect may be because the tasks were not sufficiently demanding.

Though the expectation was to see dampened activation in the hedonic brain areas post-glucose, the contrary was found. This unexpected finding could be a part of a “feed-forward” control mechanism with an evolutionary advantage to reinforce/ increase food intake when appropriate. The contrast between the latter food task and the former cognitive tasks suggest that this is not just a general fuel effect but rather a specific “post-ingestive” influence to increase activation in hedonic areas of brain when presented with food.

The novel findings shed light on the mechanisms controlling appetite and highlight the need for further research in this field.

Results from this study were presented as an oral presentation at the European Association for study of Diabetes (EASD) Conference, Amsterdam in 2007.

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Acknowledgements

It is only when I sat to write this section of my thesis that I realized that this piece of work would not have been completed without significant contribution from my volunteers, colleagues and family. To all of them, I offer my gracious and humble gratitude.

In particular, I extend my special thanks to my supervisor and mentor Dr Mark Evans. I consider myself fortunate to have had the opportunity to work alongside Mark, who is one of the best teachers I have come across. Mark has not only been an incredible source of knowledge but has also been a great source of inspiration and encouragement. His constant backing and patient attitude has been a catalyst for the completion of this work.

I am grateful to Dr Paul Fletcher. Without his expert knowledge in the field of functional magnetic resonance imaging (fMRI), this project would have not seen materialised. I acknowledge and appreciate his significant contribution to this work.

I am thankful to Dr Sandra Sünram-Lea who performed the vital preliminary tests leading up to the research project and for the helpful suggestions along the way. I also acknowledge the contributions of my contemporaries and various members of the team who facilitated the smooth running of this project. I also extend my heartfelt gratitude to Dr Sadaf Farooqi, a mentor and a friend through all the ups and downs of my research journey.

I am thankful to Prof. Malcolm Adams, my Supervisor from University of East Anglia (UEA) for his thoughtful comments and support during my research tenure.

I was fortunate to have become a mother (twice!) during this period. I cannot underestimate the number of times I have been pardoned by my daughters for my absence at home. I also owe it to my husband, Naveen, who not only put up with my long working hours, but also did my share of work at home and family, which kept me going.

Lastly but not the least, without the willingness and enthusiasm of my volunteers, this piece of work would not have seen the light of day. To all of them I say a big thank you.

I am grateful to:

JDRF: Juvenile Diabetes Research Foundation, for funding my research with their generous grant.

Wolfson Brain Imaging Centre (WBIC), Cambridge, where the participants were scanned.

Department of Biochemistry, Addenbrooke's Hospital, Cambridge, I am thankful to Mr. Keith Burling for analyzing my samples.

Declaration of sources consulted for completion of work

Dr Mark Evans: Prepared the original research proposal, secured funding from Juvenile Diabetes Research Foundation (JDRF) and obtained the initial ethics approval. Dr Evans was also involved in overseeing and conducting the experiments and critical appraisal of the work.

Dr Paul Fletcher: Developed and analyzed the cognitive tasks. Dr Fletcher helped set up and assisted with the final analyses and reporting of the fMRI images.

Dr Ashwini Swamy: Advertised, recruited and consented volunteers. Prepared for and conducted the experiments. She coordinated the day-to-day running of the experiments with the imaging team, Clinical Research Facility (CRF), volunteers and the researchers. Dr Swamy was also responsible for the administrative procedures related to the experiment (purchasing equipment and consumables, reimbursing volunteers etc.). She successfully applied for minor amendments (twice) to the ethics committee. She collected and collated data. She conducted the pre-analytic processing steps on the fMRI images including setting up contrasts for final analyses. She analyzed the biochemical data, referenced the literature and wrote the thesis.

Dr Sandra Sünram-Lea: Performed the initial 'sweetness-match' test at Lancaster, UK and was a collaborator in the study.

Mr. Keith Burling: Performed the hormonal assays.

Chapter 1: Introduction

This chapter outlines the scope of the research project, the hypothesis and aims of the study. It also explains how the body of work presented in the thesis contributes towards the research project.

Glucose from the blood stream is the main fuel supporting brain metabolism and function. Acute hypoglycemia results in cognitive impairment, with deterioration in a number of cognitive processes including memory. Although cognition generally recovers rapidly after acute hypoglycemia, the long-term cumulative effects of recurrent moderate episodes of hypoglycemia are unclear. There have been conflicting reports in the literature linking a history of severe hypoglycemia with memory problems. Recent studies in rats suggest that the effects of recurrent hypoglycemia on memory may be different at different blood glucose levels.

The aim of the project was to look at the effects of changes in blood glucose on memory in both non-diabetic volunteers, and subjects with type 1 diabetes without and with a history of problems with recurrent severe hypoglycemia. This was done by using detailed cognitive assessments of memory performed in combination with functional magnetic resonance imaging (fMRI) at different blood glucose levels. The first part of the study looked at cognitive performance with fMRI performed on non-diabetic volunteers on 2 occasions (randomised, single blind study design) following a drink of either glucose or sweetened water. The results from this study form the body of the thesis.

I then went on to study diabetic subjects twice in random order, once at hypoglycemia and once at euglycemia using the hyperinsulinemic clamp technique. I also studied an additional group of 12 non-diabetic subjects on a single occasion using a similar hyperinsulinemic clamp protocol at hypoglycemia. Outcome measures were memory performance and brain activation under different glucose conditions compared between groups. Results from this study do not form part of this thesis. The original proposal has been included in the appendices (*Appendix 1*) for reference.

In the latter part of the thesis the effects of glucose on the brain activation patterns on viewing images of food have been described. This study was carried out in view of the topical facilitative effects of leptin on the hedonic regions of the brain (Farooqi, et al., 2007). The hypothesis was that glucose had a modulatory effect on the hedonic regions of the brain which in turn was involved in regulation of appetite. In particular, a small dose of oral glucose would dampen appetite and thus reduce the activation of brain regions to pictures of food. Furthermore the expectation was to see differences in brain activation patterns between appetitive vs. bland food pictures.

Thus this study contributes to the existing literature on effects of oral glucose load on cognitive performance. It additionally delineates the brain regions recruited during performance of these tasks under different metabolic conditions (glucose vs. sweetener). The second part of this work looking at effects of glucose on hedonic circuitry of the brain would shed new light on the current understanding of appetite regulatory controls.

At the outset, the literature looking at effects of glucose on memory functions and brain activation patterns during performance of cognitive tasks have been

reviewed. Following this the literature looking at contemporary knowledge and understanding of the processes involved in the control of appetite have been reviewed.

1.1 Brain glucose metabolism

The brain is an extremely active organ consuming 20% of the body's oxygen and receiving 15% of its cardiac output (Sokaloff, 1989). Glucose constitutes the principle fuel for brain metabolism, with 120 g of glucose per day being oxidized in the brain to liberate energy (Sieber & Traystman, 1992). Thus presence of adequate levels of glucose in the blood stream is paramount for the smooth functioning of basic cell functions and regulation of critical metabolic processes such as body temperature, energy balance and thirst, for example. Glucose is also vital for the execution of more complex and sophisticated tasks of memory and learning. The effects of glucose on performance of cognitive functions have been studied for decades with variable conclusions. The cerebral regions involved during performance of cognitive tasks have been studied using modern brain imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to name a few.

It has been known for several years that glucose has an impact on the performance of some of these functions of brain (P. E. Gold, 1995b). Memory enhancement following glucose administration has frequently been studied by neuropsychologists and has been variably shown to be enhanced, reduced or unaffected by glucose. On the other hand other studies have shown detrimental effects of low blood glucose on memory performances in people with diabetes suffering from hypoglycemia.

1.2 Glucose and memory

1.2.1 Hypoglycemia and Memory:

The best studied glucose manipulation with respect to memory is hypoglycemia. Low blood glucose levels (< 3.0 mmol/l) have been shown to have short-term effects on various aspects of cognitive performance, including memory as described below.

Before experimental hypoglycemia became an accepted investigative tool in diabetes, expert clinical observers noted impairments of cognitive functions despite clear consciousness during hypoglycemia (A. A. Fletcher, Campbell WR, 1922; Wilder, 1943). Type 1 diabetes (T1DM) is a form of diabetes which develops when the insulin-producing cells in the body have been destroyed and the body is unable to produce insulin. It accounts for 5-15 % of all patients with diabetes and is treated by daily insulin injections (Diabetes UK, www.diabetes.org.uk). Inherent to treatment with insulin is the adverse effect of developing hypoglycemia. The deleterious effects of acute hypoglycemia on the brain are well known to T1DM patients and their carers and families, with low blood glucose levels frequently resulting in slowed and dampened thought processes, and even lethargy or frank coma if levels fall low enough (Gaudieri, Chen, Greer, & Holmes, 2008). Cognitive functions including orientation and attention, perception, memory (verbal and non-verbal), language, construction, reasoning, executive function and motor performance (Fisher, 2007) can be variably affected by hypoglycemia. Hypoglycemia tends to affect the performance in certain select domains of cognition whilst sparing the others. This is partly explained by the different areas of the brain involved in processing of

these various functions and their inherent resistance to hypoglycemic effects. Early studies (P. N. Russell & Rix-Trott, 1975) have shown that fine motor co-ordination, mental speed, concentration and some memory functions were disrupted at blood glucose levels of about 3.0 mmol/l. Although experimental hypoglycemia does not exactly mimic natural episodes of hypoglycemia experienced with people with T1DM, psychological tests like the four choice reaction time, Stroop and trail making tests which test attention, concentration, psychomotor skills and mental tracking , have been shown to be sensitive to acute hypoglycemia (Evans, Pernet, Lomas, Jones, & Amiel, 2000). The four choice reaction time (where a mental decision of some kind is needed before reacting to a stimulus) is affected at higher blood glucose concentrations more than simple reaction time (Heller & Macdonald, 1996). Studies have also suggested that certain aspects of memory is resistant to the acute effects of hypoglycemia, with performance on a variety of memory tasks being unaffected by a moderate decrease in blood glucose (Mellman, Davis, Brisman, & Shamoon, 1994; Meneilly, Cheung, & Tuokko, 1994). Certain mental functions, such as simple motor tasks (speed of tapping) and sensory skills and speed of reading words aloud are relatively spared during hypoglycemia (Fisher, 2007). In summary, tests which involve rapid responses and which are more cognitively complex and attention-demanding tend to show impairment during hypoglycemia (Deary, 1993).

1.2.2 Hyperglycaemia and Memory

1.2.2.1 Acute hyperglycaemia

Clinically less apparent are the effects of acute hyperglycaemia on neurocognitive functioning. A number of studies have demonstrated that intraperitoneal glucose administration improves cognitive performance in rodents (P. E. Gold, 1991, 1992; P. E. Gold, Vogt J, Hall JL, 1986; Wenk, 1989; NM White, 1991). A possible mechanistic explanation for this comes from elegant microdialysis measurements of rat brain glucose have shown that hippocampal extra-cellular fluid (ECF) glucose levels fall during maze testing (a spatial working memory task), suggesting that metabolic demands associated with cognitive performance in the hippocampus are limited by glucose supply, even in non-diabetic rats at euglycemia. The fall in ECF glucose can be prevented by (intraperitoneal) administration of glucose, correlating with enhanced performance on maze testing (McNay, Fries, & Gold, 2000).

In humans, the memory-improving action of glucose has been studied for more than 20 years. The memory-improving effect of glucose on memory was originally discovered by Lapp (Lapp, 1981) in young high school students. She showed that giving a mixture containing 450-g carbohydrate over a 1-h period improved word learning of high school students. Similarly studies have shown that experimentally raising blood glucose levels enhances certain aspects of memory performances. Initial studies of effects of glucose on cognition appeared to show that glucose specifically enhanced memory functions for those individuals with memory deficits. However, it is now recognized that the efficacy of glucose as a cognitive enhancer extends to a variety of populations, including healthy young adults, healthy elderly subjects and subjects with severe

cognitive deficits. Studies performed by Sünram-Lea et al have shown that oral glucose administration enhances memory, in particular declarative long term memory (LTM), in healthy non diabetic humans (Gonder-Frederick, et al., 1987; Messier, Gagnon, & Knott, 1997; Sunram-Lea, Foster, Durlach, & Perez, 2002). Declarative long term memory (LTM) is memory for events or materials (such as word lists or stories) which can be consciously brought to mind and described some time after the materials were originally learned, and after sufficient delay or competing mental activity has elapsed for the information to be no longer held in short-term memory (STM) (E. Tulving, 1972). Declarative LTM , namely episodic memory, is essentially memory for past events or materials and there is clear evidence that it is highly dependent upon the hippocampus with the left hippocampal region differentially mediating memory of verbal materials and the right hippocampal region mediating non-verbal (e.g. spatial) memory (Aggleton & Brown, 1999).

1.2.2.2 Chronic hyperglycaemia

Finally, although not a classical end-organ for diabetic damage, there is also evidence that chronic hyperglycaemia may adversely affect the brain, resulting in “diabetic encephalopathy” (Ferguson, et al., 2003; Ryan, Williams, Finegold, & Orchard, 1993). These results are probably partially confounded by hypoglycemia. It is seemingly paradoxical that acute glucose treatments enhance cognitive function, while chronic glucose consumption may lead to degeneration of neural and other tissues across the neural and other tissues cross the life span via metabolic stress following production of free radicals (D. L. Korol, 2002).

However, as mentioned above, processes through which acute rises in blood glucose enhance cognition may act by providing precursors for neurotransmitter synthesis or other neurochemicals from glycolysis and may not solely rely on oxidative metabolic actions of glucose (D. L. Korol, 2002).

Rodent studies have demonstrated increased apoptosis in the hippocampus of BB/ Wor rats- a model of T1DM accumulating over time with diabetes (Li, Zhang, & Sima, 2002).

A number of studies have suggested that T1DM subjects perform less well than non-diabetic patients on a variety of cognitive assessments, although it is worth emphasizing that in general the differences were relatively subtle (Brands, Kessels, de Haan, Kappelle, & Biessels, 2004). Suggested mechanisms for this have included oxidative stress, microvascular disease and the effects of brain insulin signalling. Naor et al (Naor, Steingrüber, Westhoff, Schottenfeld-Naor, & Gries) compared the performance of two groups of 20 type 2 diabetic patients who showed initial poor metabolic control (HbA1c >10%). They were divided into two groups with one being the intensive treatment group and the other the regular treatment group. The intensive treatment group improved for most cognitive measures, across the assessments. In the regular treatment group, performance either remained unchanged or even decreased across the assessments.

1.3 Brain glucose levels

Although intracellular levels of glucose are tightly controlled, extracellular levels of glucose fluctuate with blood levels. The glucose content of the brain extracellular fluid has been measured in several rodent experiments.

Microdialysis and in vivo voltametry provide similar estimates of extracellular glucose, a value of 0.35 mmol/l (Lowry, O'Neill, Boutelle, & Fillenz, 1998). Experiments examining the impact of hyper- and hypoglycemia on glucose extracellular content have yielded variable results in the absolute values of extracellular glucose. Conventional thinking has been that brain extracellular glucose levels are about 20-30% of the blood glucose levels in the physiological range. Brain activity in general is unaffected by the variation in the brain extracellular glucose levels (Claude Messier, 2004) (except in case of significant hypoglycemia), increases or reductions in brain extracellular glucose following changes in blood glucose levels are unlikely to affect overall brain function and are also unlikely to produce changes in neuronal glucose uptake because this uptake is driven by the neuron's activity, not by blood glucose concentration. On the other hand, increased blood glucose may facilitate glucose uptake in brain regions where extracellular glucose levels are overly decreased by either high neuronal uptake or by poor transfer of glucose from endothelial cells to brain extracellular space. In another experiment, changes in hippocampal extracellular glucose levels in rats during performance of a memory task were examined (McNay & Gold, 2002). Y maze spontaneous alternation is a behavioural test for measuring the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. The hippocampus is typically thought to be involved in the performance of this task. (D. S. Olton, & Samuelson, R.J, 1976). The level of glucose (injected intra-peritoneally) in the extracellular fluid of a given brain area decreased substantially when a rat was performing a memory task for which the brain area was necessary. In another experiment, the decrease in extracellular

glucose was reduced when the animals performed a three-arm alternation task, which was less difficult and presumed to require less processing (McNay, et al., 2000), suggesting that task difficulty induced a larger decrease in extracellular glucose. In animals undergoing the three-arm alternation task, the hippocampal extracellular glucose decrease was minimal and injection of 250 mg/kg glucose did not increase alternation behaviour. In a different experiment, the changes in hippocampal extracellular glucose levels in young and old F-344 rats were examined during a four-arm alternation task (McNay & Gold, 2001). It was found that the alternation task was associated with a 12% decrease in hippocampal extracellular glucose levels in young rats while old rats showed a 48% decrease. The injection of 250mg/kg glucose abolished these decreases and also increased the number of alterations, indicating better memory. These results suggest that efficacy of glucose transfer to the extracellular space in the hippocampus is reduced with aging. Glucose transporter 1 (GLUT 1) facilitates the transfer of glucose across cell membranes. Thus deficit in GLUT 1 transfer of glucose from the blood to the brain with aging could explain the deficiency in transfer of glucose into the hippocampus and that slightly increasing blood glucose levels is sufficient to overcome this deficit. Alternatively insulin sensitive glucose transporters such as GLUT 4 (which mediate passive diffusion of glucose through the blood brain barrier) are enriched in the hippocampus, thus glucose administration and/or impairments in glucoregulatory mechanisms are thought to exert the most profound effects on medial temporal regions (Owen, Finnegan, Hu, Scholey, & Sünram-Lea, 2010).

1.4 Neuropharmacological basis of central actions of glucose

The neurobiological mechanisms of glucose's action on memory are not completely delineated. Changes in available glucose may alter local cerebral metabolism (Ragozzino, Hellems, Lennartz, & Gold, 1995), may modify neurophysiological properties of neurons via changes in ion channel activity (M. R. Stefani, Nicholson, & Gold, 1999), and/ or may interact with the output of neurochemical systems (Ragozzino, Unick, & Gold, 1996), perhaps by providing substrates for the synthesis of various neurotransmitters. An ATP-sensitive potassium channel is a type of potassium channel on cell membranes that is gated by ATP (adenosine triphosphate). Intra-septal injections of glucose and the direct K-ATP channel blocker glibenclamide enhance spontaneous alternation performance in the rat, and attenuate the performance-impairing effects of the putative K-ATP channel opener morphine (M. R. Stefani, et al., 1999).

1.4.1 Glucose acting as a fuel

As discussed earlier, microdialysis studies in rats suggest that systemic glucose can support hippocampal glucose levels during memory task performance (McNay, et al., 2000). There are a number of pathways by which circulating glucose can support cerebral metabolism. First, is that it enters the brain parenchyma through GLUT1 glucose transporter present in the endothelial cells lining the blood vessels. Glucose is then transported out of the endothelial cells into the brain extracellular fluid and a substantial portion of glucose is transported from the brain extracellular fluid too astrocytes that have processes (end feet) that surround capillaries via an isoform of GLUT1 transporter. Glucose in the astrocytes can be stored as glycogen that can again be broken down into glucose and then transported back into the extracellular fluid either as glucose or

as lactate (a product of glycolysis) and into the neurons via GLUT3 glucose transporter (Claude Messier, 2004). Thus, glucose provides energy to neurons either by direct transfer of glucose from blood vessels to extracellular fluid to neurons or by an intermediate transfer of glucose through astrocytes and by metabolism of glucose into lactate in neurons and transfer into neurons. Another factor known to control glucose uptake by the brain is cerebral blood flow. As blood flow increase, blood glucose concentrations remain high and may facilitate glucose entry into the endothelial cells by keeping a high concentration gradient between the two compartments (Claude Messier, 2004; Zonta, et al., 2003).

1.4.2 Glucose altering neurotransmitter release and/or channel activity

Increases in glucose uptake during neuronal activation are related to increased synaptic function. Several key neurotransmitters in the brain are directly dependent on exogenous glucose for their synthesis. This includes two of the main excitatory neurotransmitters in the brain, glutamate and acetylcholine as well as an inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) (Kaufman, Houser, & Tobin, 1991; Schousboe, et al., 1993). Thus synthesis of neurotransmitters may drive a portion of changes in glucose uptake and utilization kinetics seen during neuronal activation (Claude Messier, 2004).

Gold et al (P. E. Gold, 1992; P. E. Gold, 1995a) examined interactions between glucose and drugs directed at specific neurotransmitter systems, to study how glucose might affect brain processes. In rodent studies using systemic injections, findings indicate that glucose ameliorated the effects of many drugs, including reversing impairments in learning and memory produced by opiate and γ-aminobutyric acid (GABA) receptor agonists, and cholinergic (both muscarinic and nicotinic) and glutaminergic (N-methyl-D-aspartate, NMDA) antagonists.

Thus glucose interacts with several drugs, crossing many neurotransmitter domains. Further experiments using microinjections of glucose and drugs into specific brain targets in rats have been used to try and define mechanisms by which glucose might act on the brain. Gold et al. have identified two brain injection sites where glucose has effects on learning and memory, namely the amygdala and the medial septum (P. E. Gold, 1995b). Injection of morphine, an opiate agonist into the amygdala, before training in an avoidance task impaired later memory for that training experience; co-injections of glucose, also directly into the amygdala, attenuated the morphine-induced impairment (Ragozzino & Gold, 1994a). The medial septum or the septohippocampal system appears to participate in learning and memory for spatial tasks and other situations in which relations between stimuli are important features of the learned responses (Eichenbaum, 1992; McDonald & White, 1993). Scopolamine, an anticholinergic drug has been used to produce cholinergic blockade of which one prominent effect is to produce antegrade amnesia (Wesnes, Anand, Simpson, & Christmas, 1990), thereby impairing initial memory acquisition, secondarily impairing memory retrieval and especially delayed free recall (Meador, et al., 1993). Intraseptal injections of morphine, muscimol, scopolamine, and propranolol all impair spontaneous alternation performance when injected into the medial septum. Glucose injections into the medial septum attenuate deficits in spontaneous alternation performance after intraseptal injections of morphine, but glucose does not attenuate the effects of either scopolamine or propranolol under the same conditions. Further, glucose metabolism through pyruvate may contribute to the mechanisms by which glucose regulates brain functions (M. R. Stefani, Ragozzino, M.E., Thompson, P.K., hellems,K., Lennartz, R.C., Gold,

P.C., 1994). It has also been suggested that glucose's actions on memory may be mediated by one or more outputs from the medial septum to the hippocampus. This has been demonstrated by co-injections of glucose (either systemic or intraseptal) fully blocking the morphine-induced decrease in release of acetylcholine in the hippocampus (Ragozzino, Wenk, & Gold, 1994b). This suggests that the memory-impairing effects of morphine and the memory-enhancing effects of glucose are paralleled by decreases and increases, respectively, in the release of acetylcholine, a neurotransmitter important to memory formation. Glucose has reversed amnesia resulting from scopolamine pre-treatment in young adult rodents (Croul, 1986). Glucose has also been shown to attenuate amnesia in rats given scopalmine, a cholinergic antagonist (Wenk, 1989). In a series of studies (Ragozzino, et al., 1996), it was found that while rats were learning spatial information, acetylcholine release in the hippocampus, a structure believed to play a role in learning and memory, was elevated compared with output while the rats sat quietly in their cages. In addition, peripheral injections of glucose enhanced performance in the learning task and in the same rats potentiated the output of acetylcholine from the hippocampus in a dose-dependent manner. Moreover, glucose injections failed to increase acetylcholine output when the rat was in its own cage, suggesting that animals must be engaged in learning activities before glucose enhancement of neurotransmitter function can be observed.

The results from the rodent studies reflect the human studies in that subjects appear to require sufficient task difficulty, and perhaps neural activity, for cognition-enhancing effects of glucose to be observed (D. L. Korol & Gold, 1998). Glucose has been found to resemble the effect of cholinergic agonist,

when 25 g glucose was given to healthy young women, some enhancing effects were found in delayed recall of items from four tasks of the California Verbal Learning Test (Delis, 1987) compared to those taking a saccharin drink or water (Winder & Borrill, 1998).

Thus the evidence suggests that glucose modulation of cognitive performances is at least to some extent carried out via alterations in the levels of brain excitatory neurotransmitter acetylcholine.

1.5 Interaction with Oxygen

In addition to glucose, oxygen has been studied for its memory enhancing properties, due to dependency of glucose metabolism upon oxygen supply. The brain's utilization of glucose parallels blood flow and oxygen consumption at rest (Sokoloff, et al., 1977). Energy expenditure increases with the difficulty of a working memory task (Backs & Seljos, 1994). The dependency of memory on these substances has also been illustrated by a number of studies which demonstrate the reduction in cognitive functioning that can occur when the supply of glucose or oxygen is abnormally low (Crowley, et al., 1992; Clarissa S. Holmes, 1986). Various studies have shown memory enhancement following inhalation of natural or hyperbaric oxygen (Crowley, et al., 1992; Edwards & Hart, 1974; Moss & Scholey, 1996). Thus it has been postulated that increasing the oxygen supply would in itself increase the opportunity for glucose utilization. The increase in memory consolidation following oxygen administration could be due to the oxygen's direct or indirect (via glucose) effect on modulating the cholinergic system (Moss & Scholey, 1996). In a study conducted by Winder et

al., oxygen enhanced participants' long-term (but not short-term) memory for names with faces and shopping list items, while glucose (50 g, pre-task) alone appeared to have no significant effect on short (immediate) or long-term memory (8 min) in either task. On the other hand, oxygen with glucose group recalled significantly more names and faces than those given glucose alone. However there was no significant difference between those given oxygen alone (Winder & Borrill, 1998). Thus the effects of glucose and oxygen on memory performance were not additive and no more than that found with oxygen alone.

1.6 Peripheral actions of Glucose

A number of experiments have suggested that the primary action of glucose in modulating memory might be in the periphery. The vagal afferents terminate in the nucleus of tractus solitarius and the dorsal motor nucleus. Secondary projections from these structures project to the amygdala (Claude Messier, 2004) and the nucleus accumbens shell (Kerfoot, Chattillion, & Williams, 2008) and modulate memory processes.

Coeliac ganglion lesions in rodents that effectively block most of the efferents of the liver have been shown to abolish the effect of large doses of glucose on memory (NM White, 1991). Others have shown that vagotomy produced an attenuation of the memory-enhancing effects of several peripherally injected drugs (James F. Flood & Morley, 1988; J. F. Flood, Smith, & Morley, 1987; Nogueira, Tomaz, & Williams, 1994; C. L. Williams & Jensen, 1993).

Further, stimulation of the vagus nerve in humans has been found to either improve (Clark, Naritoku, Smith, Browning, & Jensen, 1999; Sackeim, et al., 2001) or impair (Helmstaedter, Hoppe, & Elger, 2001) cognitive functions.

However these results are difficult to interpret as they involved either epileptic or treatment-resistant depressive patients. In one study, it appeared that the improvement in cognition produced by vagal stimulation was associated with the reduction of depressive symptoms (Sackeim, et al., 2001), suggesting a nonspecific effect of the stimulation through the improvement of depressive symptoms.

1.7 Role of Epinephrine

Epinephrine is a hormone secreted under stressful conditions and during arousal situations. Epinephrine has been shown to facilitate memory and other brain functions via its action of releasing glucose from peripheral stores (liver) into the circulation (P. E. Gold, 1995b). Although epinephrine does not enter the brain in large amounts (P. E. Gold, 1995a; Wenk, 1989), it may modulate brain functions by brain stem mechanisms, such as by activating neurons of the nucleus of the solitary tract. This suggested that glucose would mimic the effects of epinephrine on memory However; the neurobiological mechanisms of action of epinephrine on memory are less well defined. Gold et al (P. E. Gold, 1991) have shown that similar to epinephrine, posttraining injections of glucose enhanced later memory for the avoidance response in rats. Similar to epinephrine, glucose's effects on memory were time-dependent and were most effective when glucose was administered immediately after training, with no effect if glucose was administered 1 hour posttraining.

Although the effects of glucose mirror those obtained with epinephrine in most respects, one important difference is that glucose's effects on memory are not attenuated by pre-treatment with systemically administered adrenergic

antagonists (P. E. Gold, Vogt J, Hall JL, 1986). These results are consistent with the view that glucose effects on memory are subsequent to epinephrine actions at hepatic adrenergic receptors to liberate glucose stores into the circulatory system. It has also been noted that systemically administered adrenergic receptor antagonists block the effects on memory of many treatments, but not that of glucose.

Emotionally significant, stressful or arousing events can play an important role in the regulation of memory. Acute emotional arousal results in activation of the hypothalamo-anterior pituitary - adrenocortical - axis (HPA) and the sympatho-adrenal (SAM axis). Most evidence concerning regulation of memory formation is for adrenaline. Presentation or encoding of emotionally arousing material not only increases subsequent memory performance, but also raises plasma glucose levels (Blake, 2001; Brandt, Sünram-Lea, & Qualtrough, 2006). It has also been observed that glucose facilitation (post 25g oral glucose) does not emerge for material that already benefits from memory advantage by the arousal mechanism, as the emotional material would inherently raise the blood glucose levels (Brandt, et al., 2006).

1.8 Role of Insulin

It is important to emphasize that there is a close correspondence between the rise in blood glucose and the rise in blood insulin following an oral glucose load and it is hard to dissociate the effect of glucose from that of insulin in the mediation of the memory-improving action of ingested or injected glucose. Studies in rodents have shown the ability of small doses of insulin (0.4-0.8 units/kg) to reverse the amnesia produced by a 2mg/kg scopolamine injection (Blanchard &

Duncan, 1997; Claude Messier, 2004) and intracerbrovascular (intraventricular) injection of insulin can facilitate memory (Park, Seeley, Craft, & Woods, 2000). In humans, euglycemic clamp, in which glucose levels are held constant and insulin levels rise, has been shown to improve memory (S. Craft, et al., 1999).

1.9 Cognitive performance and glucose

There is considerable evidence that modest increases in circulating glucose concentrations enhance learning and memory processes in rodents and humans. It has now become clear, that several factors influence the possibility of observing a gluco-facilitatory effect on cognition. Some of these are, age, dose of administered glucose, timing of glucose administration with respect to task performance and also the type of cognitive task administered.

1.9.1 Age

The effects of glucose on cognitive performance vary with different ages.

Rodent Studies:

In an experiment involving aged and young mice, both young and aged mice had a spontaneous alternation scores near 70%. Spontaneous alternation in mice and rats refers to the natural tendency of the rodents to spontaneously choose alternate arms in a Y- or a T- maze. It relies on the ability of the animal to remember which arm it had entered on a previous occasion to enable it to alternate its choice on a following trial. It is considered as a reliable test of spatial working memory devoid of fear, reward or reinforces. In this experiment, when allowed to alternate freely; the rats chose a new arm approximately every 15 s. If a 1min delay was imposed between arm choices, young mice still alternated at ≈

70% rates, but aged mice exhibited a deficit in spontaneous alternation performance, alternating near chance levels (50%). If the aged mice received glucose injections before testing, their alternation scores were near the 70% values seen in young mice (P. E. Gold, 1995b).

Human Studies:

Hall et al. (Hall, Gonder-Frederick, Chewning, Silveira, & Gold, 1989) tested the effects of glucose on generally healthy, elderly (60-80 years old) subjects. Similar findings were documented by the group showing facilitation of performance following a 50 g oral glucose drink in elderly, non-diabetic individuals on the Wechsler Memory Scale (Gonder-Frederick, et al., 1987). The findings indicated that performance was better after ingestion of glucose (50g) than saccharin (27 mg) on a test of memory for narrative prose (free recall of information contained in a short paragraph). Manning et al. (C. A. Manning, Hall, & Gold, 1990) found that glucose ingestion before testing improved retention for contextual (narrative prose) and non-contextual (word list) verbal material (selective reminding task) by 12% relative to performance after saccharin in elderly subjects. Glucose had no significant effect on performance of non-memory neuropsychological tasks such as attention, motor speed, or overall IQ. Gold et al (P. E. Gold, 1995b) found that glucose did not enhance performance on a test of implicit (i.e. memory that occurs without subject awareness, thus not requiring deliberate recollection) verbal memory while, at the same time, enhancing memory for words assessed as explicit (i.e. memory requiring deliberate recollection) declarative memory. This findings concurs with the notion that implicit memory involves a separate memory store (Schacter, 1991), one apparently less sensitive to manipulations of circulating glucose

concentrations. Enhancement of memory in elderly has also been reported by Craft et al (S. Craft, Murphy, C., Wemstorm, J., 1994), including evidence suggesting insulin responses might contribute to glucose's effects on cognition. Riby et al. reported that glucose specifically boosted episodic memory functioning following 25 g of oral glucose in 60-80 years old participants (Leigh Martin Riby, Meikle, & Glover, 2004). They did not find any interaction of task difficulty with the glucose effect and neither a general increase in arousal to account for the glucose effect. Thus they summed that glucose selectively impacted on hippocampal function which was known to decline with ageing. Enhancement with glucose in healthy elderly individuals is greatest on tests that reveal age-related cognitive deficits, deficits that perhaps emerge during tasks that are particularly demanding (D. L. Korol, 2002).

An order-effect has been observed in the within-subjects study designs, in that superior performance was seen in the second testing, relative to the first testing session, irrespective of treatment condition (M. A. Smith & Foster, 2008). This emphasizes the importance of the random order study design employed in the current experiments.

Also perhaps relevant to the current data, in a variety of laboratories, observations of glucose facilitation in young adult human subjects have been inconsistent. A failure to observe facilitation in college-age subjects might result from a variety of factors, including task difficulty, that is, the investigators chose cognitive tests to observe a relative deficit in the older subjects to facilitate the enhancement of performance, leaving the performance level in the younger subjects sufficiently high to unmask any improvement (D. L. Korol & Gold, 1998).

There was no influence of glucose on the average working memory span nor were any effects seen on the face and word recognition task (requiring delayed forced-choice recognition). In simple terms, working memory span is the longest list of items that a person can repeat back in correct order immediately after presentation on 50% of all trials. This has been explained in detail by Baddeley et al. (A. D. Baddeley, & Hitch, G.J. , 1974). These differences in observations were attributed to difficulty in precisely identifying the cognitive attributes most affected by glucose and the importance of adjusting task difficulty to enable demonstration of glucose effects across cognitive domains. Lapp (Lapp, 1981) reported that subsequent to ingestion of a carbohydrate rich meal (which elevated blood glucose concentration), healthy adolescents outperformed a fasted control group of adolescents on a paired-associate learning task. These findings have been argued as possibly reflecting the negative effects of fasting on memory, rather than the positive effects of elevated blood glucose (Doniger, Simon, & Zivotofsky, 2006). In another study of eighteen 19 to 25 year old human males, Azari reported no significant effect of glucose administration upon memory performance (Azari, 1991), and Cormier et al failed to observe a glucose-related facilitation effect on memory in either young or elderly participants (Cormier, 1993). No differential effect of glucose was found when university students had to remember high-imagery words versus the more difficult low-imagery words (Messier, Desrochers, & Gagnon, 1999) (but glucose did facilitate the memory for the order of words in a list, a task that is quite difficult (Awad, Gagnon, Desrochers, Tsiakas, & Messier, 2002)).

It is thought that in a learning situation where the subjects are challenged with a difficult task**,** stress hormones could interact with the action of glucose on

memory, by either producing an additive facilitating or impairing effect on memory or contributing to increased variability because subjects do not necessarily react the same way to stressful stimuli (Claude Messier, 2004).

In another study, young healthy normal adults ingested 30 g and 100 g of glucose solutions in a random double-blind triple crossover design. Thirty minutes post-glucose, subjects were shown nouns on a computer monitor and then administered recall and recognition memory tests. There was no effect of glucose on memory tests and plasma glucose measures did not correlate with memory test scores (Azari, 1991). Other studies have failed to demonstrate the facilitative effect of glucose on memory (Means & Edmonds, 1998; Means, Holsten, Long, & High, 1996; Messier, Pierre, Desrochers, & Gravel, 1998).

By contrast, Hall et al. found that the extent to which glucose enhanced memory in young participants was less than that seen in their elderly counterparts, but that an effect was nevertheless observed (Hall, et al., 1989). Smith et al (M. A. Smith & Foster, 2008) studied the effects of a 25 g oral glucose load vs. placebo on verbal memory in healthy adolescents. In this study, ingestion of glucose laden drink or a sweetness matched placebo, participants were required to perform a secondary hand movement task during encoding a supraspan word list (CVLT II). CVLT (Californian Verbal Learning Test) is a well established neuropsychological test of verbal memory. It involves a ‘shopping list’ of items for the days of the weeks belonging to different food groups (e.g fruits, herbs, meat etc.). Participants are then tested for recall with several repeated trials and also records if the participants make use of the category information to facilitate the recall process. Thus this test allows the examiner to test various components of verbal memory. They observed improved memory performance on short delay

cued recall, long delay free recall and long delay cued recall post glucose ingestion, in healthy young adults under conditions of divided attention at encoding. In another study involving 18 subjects (5 women and 13 men) who were undergraduates at Virginia University, with a mean age of 20 years, 50 g glucose (vs. 35 g saccharin) significantly enhanced performance on both immediate and delayed recall of a narrative prose passage. Subjects recalled ≈ 35% more meaningful items correctly after glucose than after saccharin consumption. In this experiment, glucose tended to improve attentional processes by 8%, though observations of positive glucose effects on attention have been inconsistent (D. L. Korol, Lexecen, F.J., parent, M., Ragozzino, M.E., Manning, C.A., Gold, P.E., 1995). Several other studies have demonstrated glucose facilitating effects on cognition (Foster, 1998; L. M. Riby, et al., 2006; S. Sünram-Lea, Foster, Durlach, & Perez, 2001; Sunram-Lea, et al., 2002).

These studies indicate that glucose selectively tends to influence (facilitate) hippocampal based cognitive functions which are known to decline with age in elderly populations. Thus as would be expected, glucose facilitatory effects were observed for tasks which are hippocampally dependent (e.g. episodic memory). Glucose facilitation in younger individuals (e.g. college students) has been variable. This could be attributed to lack of cognitive decline in this age group with performances already at ceiling levels, emphasizing the importance of selecting the correct level of task difficulty in this age group.

1.9.2 Glucoregulation

Further studies have been conducted looking at the effects of efficiency of glucoregulation (i.e. the ability of the body to cope homeostatically with a glucose challenge) following an oral glucose load on cognitive performances

(Awad, et al., 2002; Messier, 2005; Wenk, 1989). Older adults with poorer glucose regulation have been shown to have most profound glucose induced cognitive enhancements (Hall, et al., 1989; Kaplan, Greenwood, Winocur, & Wolever, 2000; Messier, Tsiakas, Gagnon, Desrochers, & Awad, 2003). Other studies on older adults, have suggested that glucose memory facilitation effect is more pronounced in those individuals exhibiting relatively better glucose regulation (S. Craft, Murphy, C., Wemstorm, J., 1994; Kaplan, et al., 2000; Messier, et al., 1997; Leigh Martin Riby, et al., 2004).

Task difficulty may play an important role in our ability to observe improvements with glucose. Consistent with this idea, patient populations with cognitive deficits such as Alzheimer's victims exhibit an enhancement by glucose more robust and broader in extent than that seen in healthy elderly (S. Craft, Zallen, & Baker, 1992; C. A. Manning, Ragozzino, & Gold, 1993). Similar findings have been demonstrated in young adults with Down's syndrome (C. A. Manning, et al., 1993).

Young adult males with poor glucose regulation have been observed to demonstrate superior paragraph recall, subsequent to glucose ingestion, relative to ingestion of saccharin control drink (S. Craft, Murphy, C., Wemstorm, J., 1994). This is in contrast to inferior performance on verbal episodic memory task in poor glucose regulators subsequent to glucose ingestion relative to ingestion of a saccharin control drink, in younger individuals – an effect that is ameliorated if glucose is consumed prior to memory encoding (Messier, et al., 1999).

These contrasting effects on memory in good and poor glucoregulators have been addressed by Smith et al (M. A. Smith & Foster, 2008). They suggested that rather than glucoregulatory efficiency *per se* determining susceptibility for

glucose facilitation of memory, it may actually be that a glucose memory enhancement effect is observed only when blood glucose concentration is located within an optimal range to induce memory facilitation effect, as has been alluded to before (Parsons & Gold, 1992). The lack of facilitation in young adults may result from a variety of possibilities including an absence of a loss of function, leaving no room for improvement, or similarly the use of tasks that are too easy, yielding no cognitive deficit upon which to improve. If this is the case, any benefits of glucose on human memory in the young are most likely to be observed only when participants are engaged in sufficiently demanding cognitive tasks.

The contrasting results from various studies suggest that the effect of gluco-regulation on cognitive performance currently remains uncertain.

1.9.3 Dose of glucose

Rodent Studies:

A number of animal studies have characterized the dose-response effect of glucose on memory. In general, animal studies showed facilitating effects at doses of 100mg/kg or of 2g/kg (N. White, 1993) but doses as low as 10-30 mg/kg were also shown to modulate memory (Kopf & Baratti, 1996; Rodriguez, Horne, Mondragon, & Phelps, 1994) as were doses as high as 4g/kg (Messier & Destrade, 1988). Contrary to conventional wisdom, regional extracellular glucose levels fluctuate widely with behaviour, with reductions in specific brain areas appearing soon after animals were placed in a learning situation that relied on that specific brain structure. (McNay & Gold, 2001). Their results imply that neurons are not always saturated with glucose and that normal brain functioning may put a demand on glucose stores. Moreover, severity of the glucose depletion

correlates with the difficulty of the task: higher the cognitive load of the task, the greater the reduction in extracellular glucose (McNay, et al., 2000). Providing systemic glucose restores depleted brain levels of glucose in both young and old rats during maze learning and reverses age-and task related deficits in performance of the task. Regions that are highly activated may drain available stores; on less complex tasks brains of young animals may be able to sustain the supply of fuel, whereas those from old animals may have difficulty maintaining adequate levels for optimal cognitive functioning (Rachael T. Donohoe & Benton, 1999b). As task difficulty rises, demands on cerebral glucose may increase, even in healthy young brains (Scholey, Harper, & Kennedy, 2001).

Different doses of glucose are thought to modulate cognitive effects dependant on different brain structures. For example, in a maze task on rodents, 2g/kg (but not 100mg/kg) glucose injection posttraining resulted in glucose improved memory for the association of light and food. This test which did not require the animals to remember the previously visited arms but only to learn the light-food association is referred to as a reference memory task (O'Keefe & Dostrovsky, 1971), and is thought to depend on structures such as the caudate nucleus. In another maze experiment involving rats (win-shift), rats were trained to a criterion of four correct choices; when a rat reached a criterion, it was injected with glucose and tested for retention of the training 18 h later. Results showed that both the 100mg/kg and 2g/kg glucose dose improved the performance of the rats in this win-shift procedure. This task is thought to require the involvement of the hippocampus and is referred to as the working memory task (D. S. Olton, Wible, & Shapiro, 1986; M. G. Packard, Hirsh, & White, 1989; M. G. Packard & Teather, 1997). Thus, these results suggest that the low and the high optimal

doses of glucose improve memory that depends on the hippocampus while the higher optimal dose is specific for memory that depends on the striatum (Claude Messier, 2004).

Animal literature further suggests that different doses of glucose can influence different types of memory. Research in rats showed that the dose-response curve for the effect of post-training glucose injection seems to be bimodal, as peaks were found both at 100 mg/kg and 2,000 mg/kg (NM White, 1991). It was suggested that these doses may represent the action of glucose on two brain structures; the dorsal striatum (caudate nucleus and putamen) and the hippocampus. The dorsal striatum has been shown to be associated with response learning (habit memory) and the hippocampus with 'cognitive' learning (cognitive memory) (M. G. Packard, et al., 1989; M. G. Packard, White, N.M., 1990). White (NM White, 1991) demonstrated that hippocampally dependent memory was enhanced by glucose doses at both 2,000 mg/kg and 100 mg/kg. However striatal dependent enhanced learning was only observed at higher dose, and this effect as thought to be mediated by amygdala-hippocampal interaction. Thus lower doses fail to activate amygdala-hippocampal interactions or do so in a manner that favours cognitive learning and memory (hippocampus dependent). Low or extremely high plasma glucose levels (out of the normal physiological range) after footshock training were associated with poor memory performance and moderate plasma glucose levels were associated with better memory performance (P. E. Gold, Vogt J, Hall JL, 1986). Thus glucose levels less than or greater than the optimal dose of (100mg/kg) have not enhanced memory in rodents (Azari, 1991).

Human Studies:

In humans, doses of 25 to 75 g have been shown to be effective, which corresponds to doses of 300 mg/kg to 1g/kg for a 75-kg human. In studies in human participants given doses between 10mg/kg and 1g/kg, memory facilitation was observed only for the 300 mg/kg dose (Messier, et al., 1998). Moreover doses of 2g/kg produced nausea in many human subjects (Claude Messier, 2004). In general, lower doses of glucose (25g) appear to be more effective in young human adults while higher doses (50-75 g) are more often found to improve memory in older human adults (Claude Messier, 2004). 25 g glucose dose has shown cognitive enhancement on a number of measures including verbal recall and recognition and spatial working memory (Sunram-Lea, Owen, Finnegan, & Hu, 2010a) as has 60 g of glucose (Sunram-Lea, et al., 2010a). Studies comparing glucose effects on cognition using different doses of glucose have found that though blood glucose levels were slightly higher following administration of 60g glucose compare to 25 g glucose, this did not reach significance over the study period (Azari, 1991; Owen, Finnegan, Hu, Scholey, & Sünram-Lea, 2010b). At other times, same researchers (Sunram-Lea, et al., 2010a) have found significant differences in blood glucose levels between these glucose doses over a similar time period. These variable results have been attributed to reflect differences in the glucoregulatory control within the sample (Owen, et al., 2010b).

Implicit memory (learning) has been shown to be facilitated following 60 g glucose (and not 25 g glucose) (Owen, et al., 2010b), whereas other studies have found no effect of glucose administration on implicit memory performance in healthy young and older adults (C. A. Manning, Parsons, M.W., Cotter, E.M.,

Gold, P.E., 1997). This suggests that higher glucose dosages may be able to exert effects on brain areas other than the medial temporal lobe. The medial temporal lobe, basal forebrain and diencephalon support the formation of new explicit memories but do not appear to contribute to the formation of new implicit memories (Squire & Zola-Morgan, 1991). Areas of the brain pertaining to tasks such as repetitive priming (and formation of implicit memories) are thought to involve the occipital, posterior temporal cortices and basal ganglia (Keane, Gabrieli, Fennema, Growdon, & Corkin, 1991; E. Tulving & Schacter, 1990).

It has been suggested that effects of glucose on human memory have an inverted-U dose-response curve; the glucose doses that are optimal for memory are those producing blood glucose concentrations near 8.9 mmol/L. This equates to an oral dose of 25 g in elderly human participants (Parsons & Gold, 1992) as an optimal dose for memory enhancement.

Thus in general, lower doses of glucose, i.e. 25 g has shown to have facilitatory effects on cognitive performance in younger individuals in most studies. On the other hand, higher doses of 75 g of glucose tends to show improvement in cognitive performances in older individuals probably reflecting an element of poor glucose transfer across the cells with aging, as alluded to before. Thus there does not seem to be a single dose of glucose that would potentiate cognitive performance reliably. Various factors including baseline or inherent cognitive ability seem crucial to observing a difference.

1.9.4 Priming

Priming refers to increased sensitivity to certain stimuli due to prior experience. Studies showing reliable glucose facilitation of long-term memory performance following 25 g glucose have generally used repeated exposure to the to-be-remembered material (Foster, 1998; Sunram-Lea, et al., 2002). Similarly, there are reports showing no differential glucose enhancement of memory following single exposure to the to-be-remembered material following 25 g glucose (Scholey & Kennedy, 2004). Practice and repetition aid in learning episode and the memory trace is strengthened through repetition of stimuli. Moreover in order to reveal a glucose effect of materials more shallowly encoded, a greater glucose load is required (Owen, et al., 2010b). A study by Sunram-lea et al. showed that glucose administration (25 g) appeared to facilitate recognition memory (a component of long-term memory) that was accompanied by recollection of contextual details and episodic richness but had no effect on the proportion of recognition responses made through participants' detection of stimulus familiarity (S. I. Sünram-Lea, Dewhurst, & Foster, 2008). Facial recognition task has been shown to be resistant to glucose induced enhancement in various studies (Mitchell M. Metzger & Flint, 2003; Owen, et al., 2010b), suggesting that facial recognition is particularly stable and robust and thus not as sensitive to dietary changes compared with some other memory domains (Owen, et al., 2010b).

1.9.5 Types of carbohydrate

A number of different sugars have been tested for their impact on memory. In addition to glucose, fructose has been tested extensively and it was found that post-training injections of 2g/kg fructose improved memory (Messier & White,

1987; Rodriguez, et al., 1994). Focus has been given to elucidating whether glucose acts through provisions of energy or acts as a specific signal for other neuronal processes. If glucose enhancement was through the supply of readily useable energy, then substituting other carbohydrates with glycemic indices similar to the glucose load should be as effective as glucose alone. Thus studies looking at commonly eaten breakfast food (ready to eat cereal) have been compared with glucose drink for its cognitive effects (D. L. Korol & Gold, 1998). In a study conducted by Korol et al , blood glucose response was identical for the cereal (providing 50 g of available carbohydrate) group and the 50 g glucose (in lemonade) group, but cognitive enhancement was not identical.

Other studies have looked at the combined effects of glucose and other macronutrients like fat and proteins on cognitive performance. In one study, young participants received either a 25 g glucose/ sweetener drink with either a full fat/fat-free yogurt (S. I. Sünram-Lea, Foster, Durlach, & Perez, 2004). Participants receiving a glucose drink in conjunction with fat-free yogurt displayed higher blood glucose levels and better performance on short-and long-delay recall of the word list compared with the other group. Thus the group concluded that foods with a relatively fast glucose absorption rate are able to significantly enhance the encoding and long-term retention of novel memory materials in healthy young adults. Furthermore this group found that it was the individuals' absolute blood glucose rather than the degree of change from baseline level that mediated the effect of glucose administration on their memory performance. This effect was achieved at 25 min.

Kaplan et al have argued that glucose may not be special in improving memory, because they demonstrated that consumption of the same amount of

carbohydrates (50 g) as a high-GI food (potato) or a low-GI food (barley) produced similar cognitive enhancing effects (Kaplan, et al., 2000) on hippocampally based long-term verbal declarative memory.

Aspartame, a sweetener has been extensively used in various experiments as a control for studies looking at glucose effects on cognition. The active ingredient of aspartame is phenylalanine. Phenylalanine crosses the blood-brain barrier and may influence CNS neurotransmitter levels (Pardridge & Oldendorf, 1977). Since only large doses of aspartame (15-20 mg/kg) disrupt normal CNS function (Elsas, 1988), doses of 5-6 mg/Kg, commonly used in studies probably do not influence normal CNS function (Azari, 1991). Further aspartame alone or in combination with carbohydrates does not seem to affect memory in normal humans (Lieberman, 1988).

These studies hint that glucose may not be exclusive in its effects of enhancing cognitive performance. However the effect of different forms of carbohydrates on cognitive performance has not been extensively studied and the data on the topic is slim, making it difficult to draw any reasonable conclusions. Nonetheless, as far as aspartame is concerned it does not seem to have any known significant neurological effects in doses commonly used in experimental studies.

1.9.6 Pre- and post-trial effects

One of the main differences between animal and human studies of the effect of glucose on memory is that most animal experiments used posttraining glucose injections.

Posttraining administration of glucose is thought to reflect an action of glucose on memory processes that take place after learning about a new task or a new

situation (consolidation process). On the other hand, pre-training glucose administration could possibly interact with attentional, perceptual and other cognitive processes taking place during and after a new learning experience.

However, Gold et al showed that both pre and posttraining administration of glucose in humans improved memory for a paragraph recall task (C. A. Manning, Parsons, & Gold, 1992). Thus glucose appears to act on learning, memory, and retrieval of information, as the treatment is effective when given either pretraining (C. A. Manning, et al., 1990), posttraining (C. A. Manning, et al., 1992) or just prior to recall (C. A. Manning, Stone, Korol, & Gold, 1998).

The duration of memory facilitation following a bolus of glucose has also been studied. Sünram Lea et al examined long-term memory facilitation and observed that glucose facilitation persists 24 h after glucose administration (Sunram-Lea, et al., 2002) but not after 1 week of glucose administration (Owen, et al., 2010b). However, the later study utilized a memory task, involving repeated recall and thus exposure to the stimulus, ensuring a deep memory trace, suggesting trace strength as an important factor to ensure greater protection against increasing decay and / or increased interference over time. In another study, elderly subjects learned a narrative prose passage, then received lemonade (glucose or saccharin) in a counter-balanced crossover design) and were tested for memory 24 h later (C. A. Manning, et al., 1992). Because the subjects had received no treatment before hearing the paragraph, and were tested the next day well after blood glucose concentrations had returned to baseline, the enhancement of memory by glucose under these conditions did not reflect glucose-induced differences in sensory-motor function or attentional processes at the times of training or testing. Furthermore, the findings indicated that the effects of glucose on memory

outlasted the increases of circulating glucose concentrations, that is, the enhanced memory remains improved in a retrograde enhancement fashion, reflecting improved storage or retardation of forgetting processes. In other words, these findings imply that the administration of glucose participates in a chain of events which proceed after target information has been encoded into memory (P. E. Gold, 1992).

Thus glucose has been shown to have a facilitatory effect under both pre-training and post-training circumstances in humans indicating its influence on various steps involved in memory formation.

1.9.7 Time of the day

Blood glucose levels in response to a glucose load are about 1.7- 2.8 mmol/l higher that during the morning (Van Cauter, Polonsky, & Scheen, 1997). Evidence also suggests that insulin secretion is higher in the morning than in the evening (Van Cauter, et al., 1997). Cognitive testing using the CVLT with 25 g glucose drink either in the morning after a 24-h fast, in the morning after 2-h fast (with controlled breakfast) or in the afternoon after 2-h fast with controlled lunch content, produced blood glucose patterns different from those observed in the morning and afternoon. Memory facilitation was slightly better after a 2-h fast but there was little difference between morning or afternoon effects after a 2-h fast (S. Sünram-Lea, et al., 2001).

Thus the time of the day has not been convincingly shown to influence memory performance though slight variation in glucose levels has been observed reflecting physiological diurnal hormonal variation.

1.10 Glucose and Cognitive Domains

Memory is defined as an organism's ability to store, retain and recall information and experiences. Within the long – term memory, memory can be differentiated into two kinds of processes (i) active information processing that is isolated in time and (ii) processes or mechanisms that maintain and consolidate information over extended periods of time. Encoding and retrieval are active processes that occur at relatively specific points in time; encoding refers to the initial processing of information that potentially instantiates a memory trace, and retrieval refers to newly evolved processing that results from, and often requires access to, prior encoding episodes. Somewhere between these two sets of active processes occur the more temporally distributed processes involved in storage and consolidation, the mechanisms that convert the otherwise transient encoding event into a more enduring form (R. L. Buckner & Koutstaal, 1998).

Memory also encompasses a variety of dissociable processes believed to be mediated by distinct brain systems. It can be divided into explicit or implicit memories and may be further categorized based on types of information that are stored, for example, visual, spatial, verbal or auditory types of information. Glucose facilitating effects are observable for appetitive or aversive tasks and also for tasks where spontaneous exploration is used as the motivator (Hughes, 2002, 2003; Messier, et al., 1997). The cognitive nature of the task is a critical factor in determining whether a significant glucose facilitation effect is observed (Foster, 1998).

1.10.1 Encoding

Tests that measure episodic memory have been commonly examined to test glucose effects on memory. Other tasks facilitated by glucose include memory

for movements (Scholey & Fowles, 2002), visual memory for drawings (S. Sünram-Lea, et al., 2001) or faces (M. M. Metzger, 2000). Faster reaction times and better performance in a target detection task were found in children given 25g glucose (David Benton, 1990; David Benton, Brett, & Brain, 1987). In this experiment, after taking a drink containing either glucose or a placebo, subjects performed a task that involved watching a screen for 25 min, during which they had to press a space bar when one of four digits appeared. Over the duration of the task the performance of both groups improved, but in those taking the glucose drink the improvement occurred significantly earlier. In the second part of this experiment subjects performed a task for 20 min requiring hand-eye coordination. The taking of a glucose-containing drink was not associated with a change in performance on this task. In the healthy elderly, evidence suggests that glucose has its largest effect upon declarative memory (P. E. Gold, 1992). Similarly Hall et al. found in elderly participants that glucose administration significantly enhanced performance on the Wechsler Memory Scale. Wechsler Memory Scale is a commonly used neuropsychological test designed to measure different memory functions (verbal and performance indices) in a person. In the younger participants , glucose facilitated memory performance on the forward digit- span test only, performance on which was also facilitated in the elderly (Hall, et al., 1989). Messier et al. (Messier & Gagnon, 1996) argue that glucose preferentially improves performance on complex verbal declarative memory tests such as the Wechsler Logical Memory Scale. Benton and Owens, despite reporting no overall treatment effects, did note a significant correlation between blood glucose values and the number of words recalled in a short-term memory task. This effect was found irrespective of the initial blood glucose levels of

participants and specifically did not relate to pre-test hypoglycemic status. The observed correlation indicates a positive influence of glucose rather than merely the negative effect of fasting (David Benton & Owens, 1993).

Lapp reported that the lists of words were more easily learnt by participants with high blood glucose measures (>7.2 mmol/l) compared with those with low blood glucose levels (<4.4 mMol/l)(Lapp, 1981). Similarly, Sünram- Lea et al. have demonstrated significantly improved performances post glucose on the delayed recall components of the CVLT, and on short and long delay cued recall tasks. These glucose facilitation effects were preserved when individual differences in resting blood glucose concentration and immediate recall were partialled out. They did not find glucose facilitation effect on the digit span test nor on the reproduction of the Rey-Osterrieth figure, even though performances on these tests were not close to ceiling levels. Thus they suggested that the cognitive nature of the task was the critical factor in determining whether a significant glucose facilitation effect is observed (Foster, 1998). Moreover, the saccharin and the water groups in the latter study were not significantly different from each other on any of the memory tasks.

Looking at the effect of glucose administration (50 g) on non conscious or implicit memory, Manning et al. (C. A. Manning, Parsons, M.W., Cotter, E.M., Gold, P.E., 1997) found no effect of glucose administration on both aspects of memory (explicit and implicit) in healthy adults. In older adults, explicit memory performance was improved, whereas no effect was observed on a measure of implicit memory in that population.

Recognition memory is a subcategory of declarative memory. Recognition memory can be further discriminated by memory retrieval processes (either by

recollection or familiarity processes) (Aggleton & Brown, 1999; Eichenbaum, 1992, 1994). Recollection and familiarity are sometimes referred to as remembering and knowing, respectively (Medina, 2008). Recollection is the retrieval of details associated with the previously experienced event. In contrast, familiarity is the feeling that the event was previously experienced, without recollection. Thus, the fundamental distinction between the two processes is that recollection is a slow, controlled search process, whereas familiarity is a fast, automatic process (Jacoby, 1991). Recent research has shown that the proportion of 'remember responses' is significantly greater following glucose administration (S. I. Sünram-Lea, et al., 2008). Previous research has demonstrated that when glucose is administered prior to an encoding event, long-term memory facilitation is observed following a short delay (30 min) and 24 h after glucose administration (Sunram-Lea, et al., 2002). Studies done by Fletcher and others have used fMRI to show activation of the hippocampal system during memory tasks such as encoding of faces, words, scenes or objects (Bernard, et al., 2004). The amount of hippocampal activity at the time of encoding predicts how well that item is subsequently remembered (Brewer, et al., 1998; Kirchhoff, Wagner, Maril, & Stern, 2000; A. D. Wagner, et al., 1998) termed the subsequent memory effect or the difference due to memory (Dm) effect (Paller, Kutas, & Mayes, 1987).

Fletcher et al. have previously demonstrated differences in brain activation seen at different levels of encoding, with deep encoding during learning trails resulting in left prefrontal cortex and medial temporal lobe activation (Paul C. Fletcher, Stephenson, Carpenter, Donovan, & Bullmore, 2003). Similarly this study attempted to investigate the brain regions that were differentially sensitive

to glucose administration under different encoding conditions (deep versus shallow encoding).

Another study looked at glucose effects on cognition in young healthy undergraduates females from the University of Manchester (Foster, 1998). In this study, after an overnight fast the influence of a 25 g oral dose of glucose on cognition was tested, against saccharin and water. There was significant glucose facilitation effect upon performance of long-term verbal free and cued recall tasks which did not vary with test delay, but correlated with significantly with blood glucose levels. Further, no-glucose related facilitation was observed on tests involving short-term memory or long-term non-verbal memory. Thus the researchers suggested that the memory facilitation effects of glucose may be fractionated in young adults and that glucose may enhance retention in and/or retrieval from long term memory (Foster, 1998). Manning et al have reported no effect on measures of digit-span or visuospatial memory following a glucose drink, whereas they reported significantly improved memory for remembering a story and for word list recall (C. A. Manning, et al., 1992). Benton et al. have argued that the time to search for and retrieve items from memory (a proposed reflection of levels of attention, alertness and motivation) were associated with blood glucose concentrations (David Benton & Sargent, 1992).

1.10.2 Working Memory (WM)

Baddeley and Hitch suggested a working memory model, in which memory is an active set of processes (i.e. short term, not long-term memory). It is suggested that when we first perceive something it is ‘worked on’ in working memory. This is called encoding. Memories have to encoded before they can be stored in long term memory. The model has various components, each processing different

types of information. It has a master controller called the ‘Central Executive’ which receives and sends information to the slave systems. These include the ‘phonological loop’ (holds speech based information), viso-spatial sketch-pad (processes visual information) and more recently the episodic buffer (combines information from the phonological loop and viso-spatial sketch-pad into a single representation) (A. D. Baddeley, 2000; A. D. Baddeley, & Hitch, G.J. , 1974).

Ingestion of glucose has been shown to facilitate the performance of the serial sevens task during which participants have to subtract 7 from 100, then subtract 7 from the result and so on (Kennedy & Scholey, 2000). However, glucose did not improve the easier serial threes task. Similarly, glucose facilitated a fluency task (executive component of the working memory task) in which participants had to generate as many words as possible starting with three letters with a low occurrence at the beginning of a word (R. T. Donohoe & Benton, 1999a). Glucose had no effect when the three letters began words with a higher occurrence. Glucose also improved the difficult versions of Porteus mazes but not the easy ones (Rachael T. Donohoe & Benton, 1999b). In another study, there was no effect of 25 g of oral glucose on the digit-span test, a type of working memory task, in young healthy individuals (S. Sünram-Lea, et al., 2001). In a study by Manning et al. glucose (50 g) did not improve short-term memory as measured by performance on a digit span (Wechsler, 1987) in a group of healthy elderly volunteers aged 62-84 years (C. A. Manning, et al., 1990).

Messier et al. tested the effects of 50 g of oral glucose in 93 healthy non-diabetic 55-88 years old individuals on cognitive functioning. They found progressively worse glucoregulation predicted poorer performance on measures of working memory and executive function i.e, on the Arithmetic, Digit Span Backward,

Letter-Number Sequencing, Spatial Span Forward, Spatial Span Backward (trend), and Modified Brown-Peterson tasks (Messier, Tsiakas, Gagnon, & Desrochers, 2010). In a study involving people with type 2 diabetes, hyperglycaemic clamp to maintain plasma glucose levels at 16.5 mmol/l, resulted in deterioration in the performance of working memory tasks namely, the digit span forwards and backwards and the letter/number sequencing (Sommerfield, Deary, & Frier, 2004). A group of young females failed to show facilitation of performance on the Brown-Peterson task following 50 g of oral glucose when compared to placebo. Fasting was associated with poor performance and the glucose drink improved their performance, however it failed to do so in those who had consumed breakfast (Martin & Benton, 1999). Thus currently the evidence suggests that working memory is less likely to be manipulated by a glucose load.

The working memory task was included in this experiment to ensure that any changes observed in the key tasks were not simply a reflection of general working memory effect. One of the other objectives of the study was to observe the brain regions recruited in the performance of this task.

1.10.3 Continuous Performance Test (CPT)

A Continuous Performance Task/Test, or CPT, is a psychological test which measures a person's sustained and selective attention and impulsivity. Sustained attention is the ability to maintain a consistent focus on some continuous activity or stimuli and is associated with impulsivity. Selective attention is the ability to focus on relevant stimuli and ignore competing stimuli. This skill is associated with distractibility. Benton et al have reported improved CPT reaction time to

glucose (David Benton & Owens, 1993; C. S. Holmes, Hayford, Gonzalez, & Weydert, 1983) and improved attention (D. Benton, Owens, & Parker, 1994; C. S. Holmes, et al., 1983). Holmes et al reported attentional deficits and slower performance of fine-motor skills in individuals who were either hypo- or hyperglycemic (C. S. Holmes, et al., 1983).

The CPT task was administered as a non-memory cognitive task in this study.

Thus so far, the memory task most consistently shown in various studies to be facilitated by glucose administration is the episodic memory involving encoding and its retrieval. Furthermore plethora of studies demonstrates that the glucose facilitation of cognition is not universal but selective to certain domains of cognition, predominantly that dependant on hippocampal activation.

1.11 Task Difficulty

Task difficulty can involve simultaneous performance of two tasks, increase in cognitive load or attention switching.

A series of experiments examined the impact of glucose when participants had to perform two tasks simultaneously. In one study (Sunram-Lea, et al., 2002), participants who received either glucose (25g) or aspartame were tested under four conditions. For the first condition, subjects had to perform a motor sequence with their hand during the presentation of a 20-word list. They also had to keep track of the number of words so that they could alternate every 5 words between two motor sequences. Another group of subjects had to type a four-letter sequence on a computer keyboard. Finally, an additional group of subjects was presented with a 20-word target list (California Verbal Learning Test) by a male voice and 20 distractor words were presented by a female voice. Results showed

that the interference tasks, particularly the hand and keyboard tasks, interfered significantly with performance and that glucose attenuated these deficits, particularly the verbal list learning task, and serial sevens task.

The effects of a glucose drink on participants' performance of a serial subtraction task (computerised Serial Sevens), a somatically matched control task (key-pressing), a short interval Word Memory task and a Word Retrieval (Verbal Fluency) task were assessed by Scholey et al. The change in blood glucose during the demanding computerised Serial Sevens was compared to the change occurring during the key-pressing control. Glucose consumption significantly improved performance on Serial Sevens, with a trend for improved performance on Word Retrieval and no effect on the Word Memory task. Compared with the control task, Serial Sevens resulted in a significant reduction in blood glucose in both drink conditions. This accelerated decay was significantly greater following glucose than placebo. Thus there appears to be a reciprocal relationship between falling glucose levels and cognitive performance, particularly under conditions of cognitive demand (Scholey, et al., 2001).

Glucose has also been shown to improve performance on tasks involving attention switching. Gagnon et al. studies the effects of glucose ingestion (50 g) vs. placebo on different attentional tasks in fasting healthy older adults (60 years) after 12 h of fasting. Participants were tested on neuropsychological tests of attention (trail A and B, modified Stroop) and on a computerized dual-task. They found that participants in the glucose group were faster than the placebo group to complete the switching condition of the modified Stroop test and showed a smaller dual-task cost in the divided attention task, suggesting that glucose ingestion appeared to momentarily enhance attentional performances in tasks

requiring switching and dividing attention (Gagnon, Greenwood, & Bherer, 2010).

Another experiment compared the effect of glucose (25g), saccharin and water. Participants had to learn two lists of words, one of which was presented while they performed hand movements (fist, chop, slap) and the sequence of hand movements changed every fifth word. The glucose group remembered the word list better at the immediate and delayed free recall but no effect of glucose was found for the list when no motor interference was present. Recognition performance for both lists was equally good in all groups, possibly indicating that memory storage as such was unaffected by glucose but that glucose only improved free recall performance under the interference condition (Foster, 1998). Thus the level of difficulty and presence of interference increase the likelihood of observing the effect of glucose on cognition in young people. Robust glucose facilitation of memory has usually been observed in studies in which a concurrent task was carried out during encoding, suggesting that the possible "depletion" of episodic memory capacity and/ or glucose-mediated resources in the brain due to performing a concomitant task might be crucial to the demonstration of a glucose facilitation effect (Sunram-Lea, et al., 2002). Though it has been suggested, that with sufficient task difficulty, glucose ingestion can enhance performance on specific cognitive tests in individuals considered cognitively healthy (D. L. Korol, 2002), other studies failed to demonstrate glucose facilitation (Riby 2004) with increasing task difficulty.

Thus in general, more the cognitive demand, more are the chances of unmasking the gluco-facilitative effects on cognitive performance.

1.12 Functional brain imaging and cognition

The functional neuroimaging approach depends on characterization of hemodynamic responses to relatively transient processes. Neuroimaging allows us to observe the subset of memory processes described as active memory processes (encoding and retrieval) that can be isolated in time (R. L. Buckner & Koutstaal, 1998). On the other hand, neuroimaging is unlikely to demonstrate areas involved in temporal distribution of processes related to storage and consolidation (R. L. Buckner & Koutstaal, 1998).

PET (Positron Emission Tomography) imaging has been used to map areas of the brain involved in processing deep encoding versus shallow encoding. PET imaging studies have shown that word generation activated a pathway of brain regions including left prefrontal areas, the anterior cingulate, and the right-lateral cerebellum. (E. Tulving, Kapur, Markowitsch, et al., 1994). Thus researchers concluded that active encoding of verbal information was tied to activation of a brain pathway including the left prefrontal cortex and functionally related structures. Kapur et al (Kapur, et al., 1994) tested deep encoding by asking subjects to decide whether visually presented words represented entities that were either living or non-living. They also tested “shallow encoding” by asking subjects to decide whether the word contained the letter “a”. Imaging data during performance of these tasks demonstrated robust left pre-frontal activation overlapping with the regions activated by the word generation tasks. Gabriel and colleagues have explored the functional anatomical correlates of another deep encoding task, in which participants view words and then decided whether they fell into the category of abstract (e.g. hope) or concrete (e.g. tree) words (J. D. E. Gabrieli, Desmond, John E., Demb, J.B. , Wagner, A.D., Stone M.V., Vaidya,

C. J., Glover, G.H., 1996). They found significantly greater left prefrontal activation during this deep encoding task than during a shallow encoding task in which subjects simply decided whether words were presented in uppercase or lowercase letters (Demb, et al., 1995). Fletcher et al (P. C. Fletcher, et al., 1995). They compared later recall performance when participants engaged in word generation concurrently with either an easy distractor task (high levels of recall, 83%) or a difficult distractor task (moderate levels of recall 69%). Word generation paired with easy distraction, which presumably allowed for more elaborate encoding, showed significantly greater left pre-frontal activation than was observed during word generation in conjunction with the difficult distractor task.

The hippocampus is strongly associated with declarative memory (Nyberg, McLntosh, Houle, Nilsson, & Tulving, 1996), which involves conscious recall and recognition for verbal, spatial and numeric material. Face recognition, an example of declarative or explicit long-term memory, involves small regions of the left and right fusiform and inferior temporal gyri (Allison, et al., 1994). Fletcher et al have demonstrated activation of the hippocampal system during memory tasks such as encoding of faces, words, scenes or objects (Bernard, et al., 2004). Fletcher et al have also previously demonstrated differences in brain activation seen at different levels of encoding, with deep encoding during learning trails resulting in left prefrontal cortex and medial temporal lobe activation (Paul C. Fletcher, et al., 2003). Kapur et al have suggested that when subjects process verbal stimuli in a semantic manner, either under experimental or real life conditions, it involved increased neuronal activity in the left inferior pre-frontal cortex.

Thus from these studies, the left prefrontal cortex, at or near Broadmann areas 44 and/ or 45 and sometimes extending anteriorly and dorsally, is activated when subjects are engaging in tasks that lead to long term storage as assessed by later explicit retrieval tasks (R. L. Buckner & Koutstaal, 1998). Tulving et al have suggested a model whereby the left and right prefrontal lobes are a part of an extensive neuronal network that sub serves episodic remembering, but with the two prefrontal hemispheres playing different roles. Left prefrontal cortical regions are differentially more involved in retrieval of information from semantic memory and in simultaneously encoding novel aspects of the retrieved information into episodic memory. Right prefrontal cortical regions, on the other hand, are differentially more involved in episodic retrieval (E. Tulving, Kapur, Craik, Moscovitch, & Houle, 1994). Increased activity in the hippocampal region, irrespective of the individual's intention to remember, leads to a more readily retrievable memory trace (Kapur, et al., 1994).

Para moved up in the same section.

In contrast to the consistent findings regarding left prefrontal involvement in memory encoding, there have been sporadic findings of medial temporal lobe involvement in encoding processes. Deep vs. shallow and other verbal encoding manipulations often have failed to detect differential activation in these regions (Desmond, 1996; P. C. Fletcher, et al., 1995; Kapur, et al., 1994). A number of studies have reported medial temporal lobe involvement in encoding when novel, complex visual scenes or faces are presented (J. D. E. Gabrieli, Brewer, Desmond, & Glover, 1997; Grady, et al., 1995; Haxby, et al., 1996; Stern, et al., 1996; E. Tulving, Markowitsch, Craik, Habib, & Houle, 1996). However hippocampal activation in relation to verbal encoding has been inconsistent. It

seems possible that critical memory-related processes sub served by medial temporal lobe structures may not include acute and differential activation during the initial encoding of information or may involve relatively sparse neural changes in relation to encoding (R. L. Buckner & Koutstaal, 1998).

Working memory is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and problem solving (E. E. Smith & Jonides, 1998). Previous fMRI studies have consistently demonstrated involvement of prefrontal and parietal regions in verbal working memory in humans (G. D. Honey, Bullmore, & Sharma, 2000).

Although frontal lobe activation is often bilateral, the left ventrolateral frontal cortex appears to be primarily concerned with the maintenance of verbal information whereas the right ventrolateral frontal cortex is more involved with maintenance of spatial information (P. C. Fletcher & Henson, 2001). There may also be anatomical divisions within the frontal cortex that subserve different processes with the ventrolateral frontal cortex being activated during tasks requiring maintenance of information, and the dorsolateral frontal cortex being more involved during tasks requiring manipulation of information (P. C. Fletcher & Henson, 2001). On the other hand, typical spatial working memory tasks activate right-hemisphere prefrontal, occipital, parietal and premotor cortices (Jonides, et al., 1993).

Buckner and Tulving (R. Buckner, & Tulving, E. , 1995) proposed that these functional neuroimaging studies demonstrate how multiple kinds of information processing might interact to promote long-term memory, as is evidenced by overlapping areas of encoding and working memory processes in the brain.

Effortful word generation tasks, verbal working memory tasks, and long term memory encoding tasks all activate similar brain pathways including left prefrontal regions and related structures. Processing that requires verbal elaboration (deep processing) appears to activate left prefrontal cortex selectively whereas well automated tasks involving verbal information (shallow tasks) do not (Demb, et al., 1995). Shallow encoding tasks thus may lead less often to the formation of explicit long term memories because they do not initially require representation of the information in prefrontal cortex, the anatomical substrate that supports higher level representations necessary for conscious retrieval.

1.13 Conclusions

The literature so far has shown that oral glucose can increase cognitive performance as evidenced by rodent experiments and human studies. Though there are wide variations in the study designs amongst the human studies (dose of glucose and type of cognitive task applied, for example), once can draw some useful conclusions.

Firstly, the dose of glucose shown to affect cognition favourably depends on the age group studied. Smaller doses (25 g) have been shown to be effective in young college-going adults whereas higher doses of 50-75 g of glucose have been shown to be effective in older individuals (Claude Messier, 2004). Secondly, the chances of finding a facilitatory effect of glucose depend not only on the choice of the cognitive task administered but also on the level of task difficulty (Sunram-Lea, et al., 2002). Also tasks which are cognitively more demanding or involve a second interfering task are more sensitive to glucose

facilitation (Foster, 1998). In general, hippocampally based tasks, such as long-term declarative memory (CVLT II) has been shown to be enhanced with oral glucose (Sunram-Lea, et al., 2002). Thus for this study 25 g glucose was used to study performance and brain activation patterns to encoding tasks in young healthy university graduates.

Brain activation patterns have enhanced our understanding of the processing of some of these cognitive processes. Bilateral frontal and medial temporal lobes are the main players in the formation of memory processes (Kapur, et al., 1994; E. Tulving, Kapur, Craik, et al., 1994). The intensity of activity on the left and the right halves of these regions tend to vary, for example with the type of information being handled (verbal vs. nonverbal) (Jonides, et al., 1993). Encoding is associated with activation in both the right and left prefrontal lobes and the medial temporal lobe (J. D. E. Gabrieli, Desmond, John E., Demb, J.B. , Wagner, A.D., Stone M.V., Vaidya, C. J., Glover, G.H., 1996; Nyberg, McIntosh, et al., 1996). Greater hippocampal activity during performance of an encoding task predicts how well the item is subsequently remembered (P. C. Fletcher, et al., 1995). Deeper encoding results in greater activation in the regions of left prefrontal cortex and medial temporal lobe as compared to shallow encoded tasks (Paul C. Fletcher, et al., 2003). Intensity of activity in the hippocampal region correlates with subsequent successful retrieval (Kapur, et al., 1994). The left prefrontal cortex has been shown to be responsible for retrieval of semantic memory whilst the right with episodic retrieval (Kapur, et al., 1994)

Working memory tasks are manipulated by the frontal lobes. Left ventrolateral frontal lobe and parietal regions are concerned with performance of verbal working memory tasks whereas the right prefrontal cortex is associated with the

performance of spatial working memory task (P. C. Fletcher & Henson, 2001; G. D. Honey, et al., 2000).

Thus there is an overlap of the brain regions involved in processing encoding and working memory with the left prefrontal lobe primarily handling tasks with verbal information (R. Buckner, & Tulving, E. , 1995).

1.14 Regulation of appetite

Rationale: In addition to studying the cognitive affects of a small dose of oral glucose and simultaneous brain activation patterns; this study also looked at the effects of oral glucose on brain activation in response to food images. This aspect of the study was subsequently added on to our study design.

The topical work done by Farooqi et al. (Farooqi, et al., 2007) locally showing the manipulative effects of leptin on appetite control prompted us to perform this study. Leptin, a key hormone involved in regulation of energy balance, was shown to manipulate the limbic (hedonic) regions of the brain, mainly the nucleus accumbens by changing feeding behaviour and hunger scores in congenitally leptin deficient children. Thus, the hypothesis was that glucose, a nutritive substrate and primary fuel of the brain could also potentially influence energy balance by regulating the appetite pathways in the brain.

In particular, in overnight fasted (hungry) healthy adults (given a sweetened drink), visualization of food pictures would elicit activation in the limbic regions of the brain. Further, a drink of 25 g of glucose would dampen the activation in these regions, as individuals would no longer be perceived to be hungry. If this was the case, differences in activation patterns to various types of food (appetising vs. bland) would be studied.

The following paragraphs give a brief overview of our current knowledge of appetite control and the various modalities used to study this subject.

1.14.1 The glucostatic theory

The role of glucose as a regulator of energy balance has been a subject of debate for more than half a century. Glucose as a modulator of energy balance was first proposed by Jean Mayer more than 50 years ago and was called the 'glucostatic theory'. This theory proposes a central role of glucose in the regulation of food intake (J Mayer, 1953, 1955; J. Mayer & Bates, 1952). The glucostatic theory postulated that reduced glucose utilization in critical brain regions leads to perception and expression of hunger, and increased glucose utilization in these same glucosensitive sites leads to decreased hunger and cessation of eating. Mayer (J Mayer, 1953, 1955; Mayer.J & Bates, 1952) proposed that decreased glucose utilization or metabolic hypoglycaemia, the point at which the peripheral arterio-venous difference in blood glucose becomes negligible and glucose was no longer entering 'metabolising cells' was the signal for meal initiation. He suggested that the glucostatic theory would account for the short term control of hunger and fluid intake, whereas he invoked a 'lipostatic' mechanism to account for the long term regulation of body weight and energy balance. Following this there have been numerous studies supporting and refuting the glucostatic hypothesis. These researchers and further studies emphasized the role of decreased glucose utilization or decreased intracellular glucose rather than the absolute level of blood glucose as the stimulus for meal initiation. Moreover, it was suggested that this theory should be renamed as the glucodynamic theory rather than the glucostatic theory, as it is the pattern of dynamic changes in glucose which was sensed rather than its absolute level (nadir (low) glucose

initiating food intake, whilst rising blood glucose terminating ingestion) (J Mayer, 1953, 1955; J. Mayer & Bates, 1952) . On the basis of observations that changes in glucose use affected ingestion, Mayer (J Mayer, 1955) proposed the glucostatic hypothesis whereby glucose-sensing neurons participated in the short-term regulation of energy intake.

1.14.1.1 Rodent Studies

Several studies have been conducted in rodents to study the pattern of blood glucose changes surrounding meals. In support of Mayer's hypothesis, it was found that 6-8% dips in plasma glucose were associated with meal initiation (Louis-Sylvestre & Le Magnen, 1980). Certainly large reductions in plasma glucose levels or glucose availability can stimulate food intake (Ritter, Murnane, & Ladenheim, 1982). Investigation of the effects of modest changes in blood glucose levels within the physiological range, in rodents, has found differences in appetite responses between blood glucose levels of 5-8 mmol/; with fullness greater at 8 mmol/l (Abizaid, et al., 2006). Moved up from below. When the level of glucose in blood is continuously monitored in freely feeding rats, by means of an indwelling intravenous catheter, Campfield et al. (L. A. Campfield & Smith, 1990; L. S. F. Campfield, 1986) observed that beginning a few minutes prior to when a "spontaneous" meal is initiated, blood glucose decreased. In rats, every observed spontaneous meal was preceded by a small (approximately 12%) but reliable decline in blood glucose (L. Arthur Campfield, Brandon, & Smith, 1985).

However it remains uncertain whether brain can actually detect small changes, within the physiological range, in plasma glucose or if this might be a primary

stimulant of meal initiation (Levin, Dunn-Meynell, & Routh, 1999). Alterations in plasma glucose of 2mM alter brain glucose levels by 0.2-0.3 mM within 10 min, and this is associated with changes in firing of glucose-sensing neurons (Silver & Erecinska, 1998). But these levels are still far greater than the <0.5 mM changes in plasma glucose postulated to trigger meal initiation (Louis-Sylvestre & Le Magnen, 1980). However, central neurons do respond to larger changes in peripheral glucose levels transmitted from portal vein glucosensors by direct neural inputs (Hevener, Bergman, & Donovan, 1997) and by direct action of glucose that enters the brain by a transport-mediated process (Vannucci, Maher, & Simpson, 1997). On the other hand, some researchers have postulated that eating is initiated even when energy supplies are ample (Woods, Schwartz, Baskin, & Seeley, 2000), suggesting that eating is an inefficient way to get calories in blood rapidly, due to digestive and absorptive lags. Others have suggested that initiation of eating secondary to a dip in glucose is functional only in extreme metabolic emergencies, as a protective mechanism (Epstein AN, 1975).

1.14.1.2 Human Studies

When blood glucose was measured over a 2-6 h period using continuous glucose monitoring, in participants who were isolated from food and hunger cues, changes in hunger and meal requests were preceded by, and correlated with, brief transient declines in blood glucose in 83% of participants (L. S. F. Campfield, 1986). The same association between hunger ratings and transient declines in blood glucose was seen when acute insulin infusions (5mU/kg body weight

administered over 2 min) were used to mimic spontaneous transient declines in blood glucose. No changes in hunger ratings were observed while blood glucose was stable; however, hunger ratings increased after insulin-induced decreases in blood glucose (L. Arthur Campfield, Smith, Rosenbaum, & Hirsch, 1996). These findings suggest that hunger and meal initiation are associated with rapid declines in blood glucose in human subjects although the mechanisms underlying this association have not been elucidated (L. Arthur Campfield, et al., 1996).

Hunger early in the test meal increases once the palatable meal is tasted, this response has been termed the appetiser effect (Castiglione, 2002). Preloading test meal reduces intake to a greater extent when subjects ingest a bland test meal compared with a palatable meal (Robinson, 2001; Yeomans, Lee, Gray, & French, 2001) suggesting that palatable meals may override partially normal satiety process.

Though some of the rodent studies tend to favour the Mayer's hypothesis about a dip in plasma glucose initiating ingestion of a meal and thus controlling appetite, human studies are less convincing. In spite of human studies showing small decreases in plasma glucose prior to meal initiation, the underlying physiological mechanisms remain unclear and the clinical relevance of this phenomenon is not unknown.

In this study the role of glucose in the control of appetite by was investigated by studying the brain activation patterns to different blood glucose levels (with and without 25 g of oral glucose). The expectation was to see different activation patterns on visualization of food pictures in the fasted versus the post glucose ingestion state. This could provide indirect evidence that variations in glucose levels in the body influenced appetitive behaviour.

Studies directed towards energy balance and regulation has traditionally focussed on metabolic controls and mainly focussed on the hypothalamus in the brain, which is undoubtedly a significant player in appetite control. Compelling evidence over the last decade has now highlighted the importance of the other brain regions, like the cortico-limbic brain systems known to be involved in cognition, reward and emotion, to regulate the neural circuitry of appetite control. Functional neuroimaging techniques have been an instrumental tool in expanding our knowledge of central regulation of appetite. I have used functional magnetic imaging for my studies. I have briefly explained the principle underlying this technique in the next paragraph.

1.14.2 Neuroimaging of the brain

The field of cognitive neuroscience, particularly related to studies involving functional imaging techniques, has experienced explosive growth over the past two decades. Functional imaging of the brain has been a valuable resource to study the complexity of appetite control and food intake in humans. Coupled with this is the rapid development of non-invasive brain imaging techniques. Together, brain imaging techniques and measures of local neuronal activity have permitted researchers to investigate regions of the brain that are functionally related to a variety of sensory and cognitive tasks (Raichle, 1998). Functional neuroimaging techniques include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), magnetoencephalogram (MEG), electroencephalogram (EEG), and other methods. The great strength of functional brain imaging is that it can contribute uniquely to a task by providing a broad and detailed view of the processing architecture of cognitively engaged

networks. Underpinning all the modalities of investigations is the common thread that increases in neuronal activity is preceded by increase in regional blood flow.

Principle of fMRI: Functional brain imaging with fMRI is based on a remarkably consistent relationship between regional changes in the cellular activity of the brain and changes in circulation and metabolism of that region. Though brain represents 2% of the body weight, it accounts for ~20% of the body's oxygen, and hence glucose consumption (Clark DD, 1999). fMRI, like other neuroimaging techniques (PET, Single-emission computed tomography (SPECT)) provides information about state dependent changes in local neuronal activity. Increases in local neuronal activity are associated with a greater increase in regional cerebral blood flow than needed to supply cells with oxygen; for this reason, increases in local neuronal activity result in a lower concentration of deoxyhaemoglobin, which can be detected using MRI (Tataranni & DelParigi, 2003). fMRI detects changes in the magnetic properties of haemoglobin, resulting from neural demands for oxygenated blood. The fMRI scanner detects changes in this hemodynamic response and records the blood oxygen level-dependent (BOLD) signal. The higher the proportion of oxyhaemoglobin (diamagnetic) relative to deoxyhaemoglobin (paramagnetic), the less interference to the radio frequency pulse generated by the scanner, and the stronger the BOLD signal and brighter the image (Gibson, Carnell, Ochner, & Geliebter). In other words, fMRI measures the different magnetic spin between oxygenated and deoxygenated haemoglobin, the levels of which change with neuronal activity. Though BOLD imaging is the dominant technique for fMRI activation, arterial spin labelling (ASL) technique is also being increasingly used. The ASL

technique gives better resolution for areas which involve air-tissue interfaces like the orbito-frontal cortices (Cacioppo, 2007).

Compared with PET, fMRI has superior temporal (seconds versus minutes) and spatial (~1mm versus ~4 mm) resolution, which allows more rapid changes in neuronal activity that occur in smaller areas of the brain to be visualised (Lingford-Hughes, 2005). Moreover, the relative safety, absence of radioactivity, and high spatial and temporal resolution of fMRI, has resulted in it eclipsing PET as the dominant approach in neuroimaging research (Gibson, Carnell, Ochner, & Geliebter, 2010). Indeed (Tataranni & DelParigi, 2003), fMRI is more accessible and less expensive than PET. Functional neuroimaging provides a fundamental advantage by allowing investigation of the whole brain, making it possible to study the system rather than restricting the investigation to pre-selected regions of interest.

Limitations of fMRI include difficulty in studying certain areas of the brain such as the orbitofrontal cortex due to its proximity to air-filled sinuses, which causes in-plane distortion of echoplanar images (W. D. Killgore & Yurgelun-Todd, 2006). Another region of the brain susceptible to this effect is the hypothalamus. The hypothalamus is surrounded by a vascular network and in close proximity to a sinus cavity, potentially limiting spatial resolution in imaging studies (Tataranni & DelParigi, 2003). Despite these limitations, functional imaging of the hypothalamic response to a meal has been pursued in a number of studies (J. F. Gautier, et al., 2000; Liu, Gao, Liu, & Fox, 2000; Tataranni, et al., 1999). Furthermore, although there are more established atlases and parcellation protocol (e.g. Talairach space) for human compared to animal brains, there is still no widely accepted standard (Talairach, 1988, 1993). Imprecise labelling can be

a limitation when imaging micro-anatomical structures, such as the hypothalamus and brainstem (Bohland, Bokil, Allen, & Mitra, 2009). Certain artefacts associated with fMRI are difficult to adjust especially when there is a significant head movement between scans (because of long duration between scans, or movements of the jaw, both of which may be present in eating and drinking studies (Frahm, Merboldt, & Hänicke, 1988). In addition, on/off design of most study paradigms limit capture of functional data. Nevertheless, there is no doubt that fMRI has the greatest potential for improving our ability to both describe the time course of the brain events that control eating and provide the most precise map of where these events occur (Logothetis, 2000; Thompson, Peterson, & Freeman, 2003).

Critical to the interpretation of the functional neuroimaging is the knowledge of the cellular events associated with the local changes in blood flow, metabolism (i.e. relative increase in glycolysis) and tissue oxygenation as evidenced by functional imaging. The most parsimonious explanation for this observation is that the glycolysis is related to metabolic changes in astrocytes associated with increased clearance of glutamate from the synapse (Magistretti, Pellerin, Rothman, & Shulman, 1999; Mintun, et al., 2001; Shulman, Hyder, & Rothman, 2001). It has been suggested recently that the astrocytes are also a critical link between neurons and blood vessels in orchestrating the changes in blood flow associated with changes in neuronal activity (Zonta, et al., 2003). Spiking activity of the neurons has been used as the gold standard in assessing the ability of functional imaging signals to track events of interest within the brain (Hyder, Rothman, & Shulman, 2002; A. J. Smith, et al., 2002). On the other hand, others researchers propose synaptic events, as reflected in local field potentials, as being

most influential in determining the signals obtained with functional imaging (Schwartz, et al., 1979; Frank R. Sharp, 1976; F. R. Sharp, Kauer, & Shepherd, 1977).

1.14.3 Control of energy balance

Food procurement has its history as an evolutionary conserved survival mechanism. For example, the honey bees engage in a dance to communicate the location and abundance of a food source to other worker bees in the hive. (von Frisch, 1967). Thus at a given time in evolution, large portions of the nervous system have been dedicated to procuring food. Memorial representations of foods and food cues are available to the foraging human long before food is actually seen, smelled, or tasted. Paramount to the functioning of this system is the control of appetite.

Appetite has variably been defined as the desire to eat food, felt as hunger and serves to regulate adequate energy intake to maintain metabolic needs. Regulation of appetite the (appestat) has been a subject of much research in the last decade. Appestat has been loosely defined as the brain centre (probably the hypothalamus) concerned in controlling of appetite. Human food intake relies on a complex hierarchy of cortical processing which include obtaining stable sensory information, evaluation of desirability, and choosing the appropriate behaviour. Part of this processing is linked to basic homeostatic regulation, which has been elucidated in great details in animal models with mammals with humans sharing many subcortical circuits and molecules, such as leptin and ghrelin.

However, human food intake is not only regulated by homeostatic processes as illustrated by our easy overindulgence on sweet foods beyond homeostatic needs

and epidemic proportions of obesity which has become a major health problem (Kohn & Booth, 2003). The regulation of human food intake relies on the interaction between homeostatic regulation and hedonic pleasure. This complex subcortical and cortical processing involves higher order processing, including learning memory, planning and prediction and gives rise to conscious experience of not only the sensory properties of food (such as identity, intensity, temperature, fat content, and viscosity) but also the valence elicited by the food (including, most importantly, the pleasure experienced) (Kringelbach, 2005).

Food intake is a precisely controlled act that can potentially be fatal if the wrong decision is taken to swallow toxins, micro-organisms, or non-food objects on the basis of erroneously determining the sensory aspects of the food. Humans have therefore developed elaborate food behaviours which are aimed at balancing, conservative risk-minimising and life preserving strategies, with occasional novelty seeking in the hope of discovering new sources of nutrients (Rozin, 2001).

It is thus becoming obvious that behind the ‘simple’ decision to eat or not eat a particular food, the brain is actually functioning at its highest order. Indeed, central to all the models of food regulation, is the fact that the various signals and messages converge at a ‘central headquarters’ to execute the final outcome. This then translates to the final decision to either eat or reject the particular food substance in question.

Current teaching dictates that appetite control is managed by two distinct systems, namely the ‘homeostatic system’ and the ‘hedonic system’. However recent explosion of knowledge of central regulation of appetite has blurred the distinction of these two systems, and has indeed highlighted the extensive

overlap of functions of the components each of these systems. Furthermore, there is a growing body of evidence detailing the common characteristics between the central centres controlling energy balance and pathways of addiction. Also areas of the brain traditionally associated with mood and addictive behaviour, the limbic system, have now been identified in addition, to be key players in the central control of appetite. The pancreatic hormone, insulin, which enters the brain from the circulation and acts there to reduce energy intake, was the first hormonal signal to be implicated in the control of body weight by the central nervous system (CNS). Newer players into the field of appetite control range from previously unrecognised roles of well known peptides on the brain such as protein peptide Y (PYY) (Batterham, et al., 2003) to the discovery of newer peptides like leptin (Farooqi, et al., 2007).

The homeostatic system is predominantly comprised of the hypothalamus and brain stem, and appears to drive food intake based on caloric need or energy balance (H.-R. Berthoud & Morrison, 2008; Nisbett, 1972). The role of the hypothalamus in the non-conscious regulation of energy homeostasis is well established. Key molecules governing energy homeostasis are highly conserved across species and it is therefore assumed that similar mechanisms may be operating in the human hypothalamus (Tataranni & DelParigi, 2003). To ensure adequate nutrition, it is necessary for the brain to have intrinsic circuitry that regulates the levels of various nutrients in the blood and in the body stores.

The increase of food intake (hyperphagia) triggered by a period of fasting is a simple but compelling example of food intake. The consequent recovery of lost body weight to baseline values, accompanied by the gradual return to normal levels of energy (Harris, Kasser, & Martin, 1986), is testimony to a regulatory

process that is both precise and robust. Aiding in this decision making are various cortical and sub-cortical brain regions, which have been described below (Fig.1.1).

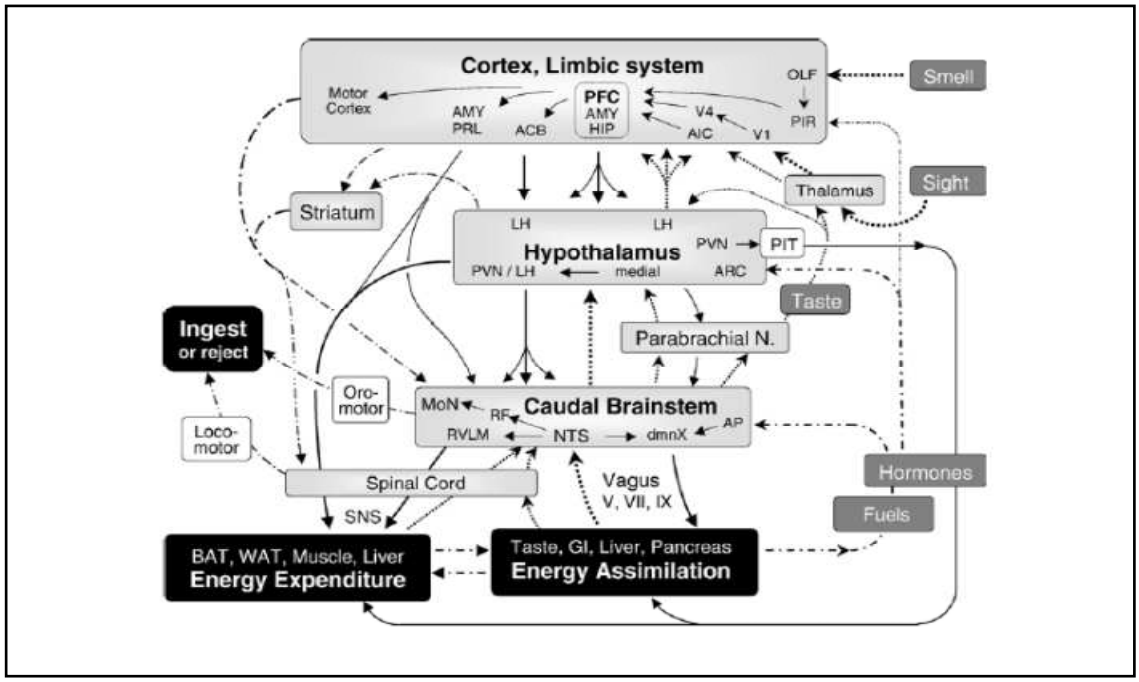

Figure 1.1: Neural network controlling food intake and energy balance (H.-R. Berthoud, 2007)

Schematic diagram of information flow involved in both internal-homeostatic and external control of food intake and energy balance. Signalling from the internal milieu or external environment to the brain is either mediated by primary and higher order sensory neurons or hormones and substrates. Centrifugal signalling from the brain to the effector organs is either mediated by premotor and motor neurons or by hormones. Abbreviations: ACB, nucleus accumbens; AIC, agranular insular cortex; AMY, amygdala; AP, area postrema; ARC, arcuate nucleus; dmnX, dorsal motor nucleus of vagus; LH, lateral hypothalamus; HIP, hippocampus; MoN, motor nuclei for oro-motor control; NTS, nucleus tractus solitarius; OLF, olfactory bulb; PFC, prefrontal cortex; PIR, piriform cortex; PIT, pituitary gland; PRL, prelimbic cortex; PVN, paraventricular nucleus of the hypothalamus; RF, medullary reticular formation; RVLM, rostroventrolateral medulla; SNS, sympathetic nervous system; V1/V4, visual processing areas 1,4; V, facial nerve; VII, trigeminal nerve; IX, glossopharyngeal nerve.

As is illustrated in the above diagram, the network controlling food intake and energy balance is extremely intricate and a dynamic process. This diagram (H.-R. Berthoud, 2007) illustrates the schematic flow of information in both internal-

homeostatic and external control of food intake and energy. Signalling from the internal milieu or external environment to the brain is either mediated by primary or higher order sensory neurones or hormones and substrates.

All this has generated renewed interest in understanding and unravelling of the biology and physiology of energy balance and appetite control. In addition, cutting edge technology has now made it possible to study brain behaviour in multitude of ways which was not possible before. This has not only enhanced the discovery of previously unknown metabolic processes and substrates but has also enhanced our understanding and knowledge of well established metabolic pathways and processes. These results also open up potential new avenues in the management of disorders associated with dysregulated appetite control and energy balance states such as obesity.

1.14.4 Central systems involved in appetite control and food consumption

The cortical and subcortical regions of the brain constitute a highly evolved system and incorporate some of the basic and rudimentary instincts related to feeding. This system takes into account not only real time ongoing inputs to the brain regions involved in appetite control in the form of taste or visual stimuli for example, but also background substrate status (peptide, glucose levels for example) and also memory of past events related to feeding.

For ease of discussion the central control of appetite has been anatomically divided into cortical and subcortical regions.

1.14.4.1 Cortical regions involved in control of appetite

Consuming sufficient food to maintain adequate energy stores is the sine qua non for survival for all species in the animal kingdom. For mammals, which must maintain a stable body temperature in even the most hostile climates to survive,

this problem is even more acute. Maintaining a high metabolic rate requires constant availability of large amounts of energy stores. For small mammals that do not retain heat well, life consists mainly of obtaining and consuming food (Saper, Chou, & Elmquist, 2002). To insure that this activity takes a high priority in brain function, mammalian brains have evolved several potent and interrelated neuronal systems that drive feeding behaviour.

All of the classic five senses (vision, hearing, smell, taste and touch) are involved in the regulation of food intake. Foremost, of course the sensing of food occurs when food is grasped and delivered to the mouth. This includes taste, smell, and somatosensory (this includes temperature, viscosity, pungency and irritation) input primarily from our oral and nasal cavity. This sensory input is vital in deciding whether to swallow or reject a potentially poisonous food. Taste is sensed by taste receptor cells arranged in taste buds which are primarily found on the tongue but also on other areas in the oral cavity such as the soft palate, the pharynx, the larynx, and the epiglottis (Thomas R. Scott & Plata-Salamán, 1999). Thus input through the visual, tactile and auditory sensations are able to serve as conditioned stimuli. The exact mechanisms by which representations of experience with specific foods are formed, stored and recalled are not well understood, but likely involve processing steps through each sensory channel and the generation of polymodal representations in specialized cortical areas. (H.-R. Berthoud, 2007). Further research has gone into localising the cortical centres representing the central control of these senses.

The following illustration (Fig. 1.2) taken from Moll et al (Moll, Zahn, de Oliveira-Souza, Krueger, & Grafman, 2005), shows the chief cortical regions involved in the regulation of appetite and energy balance in the body. These

chiefly include the various lobes of the frontal and temporal cortices.

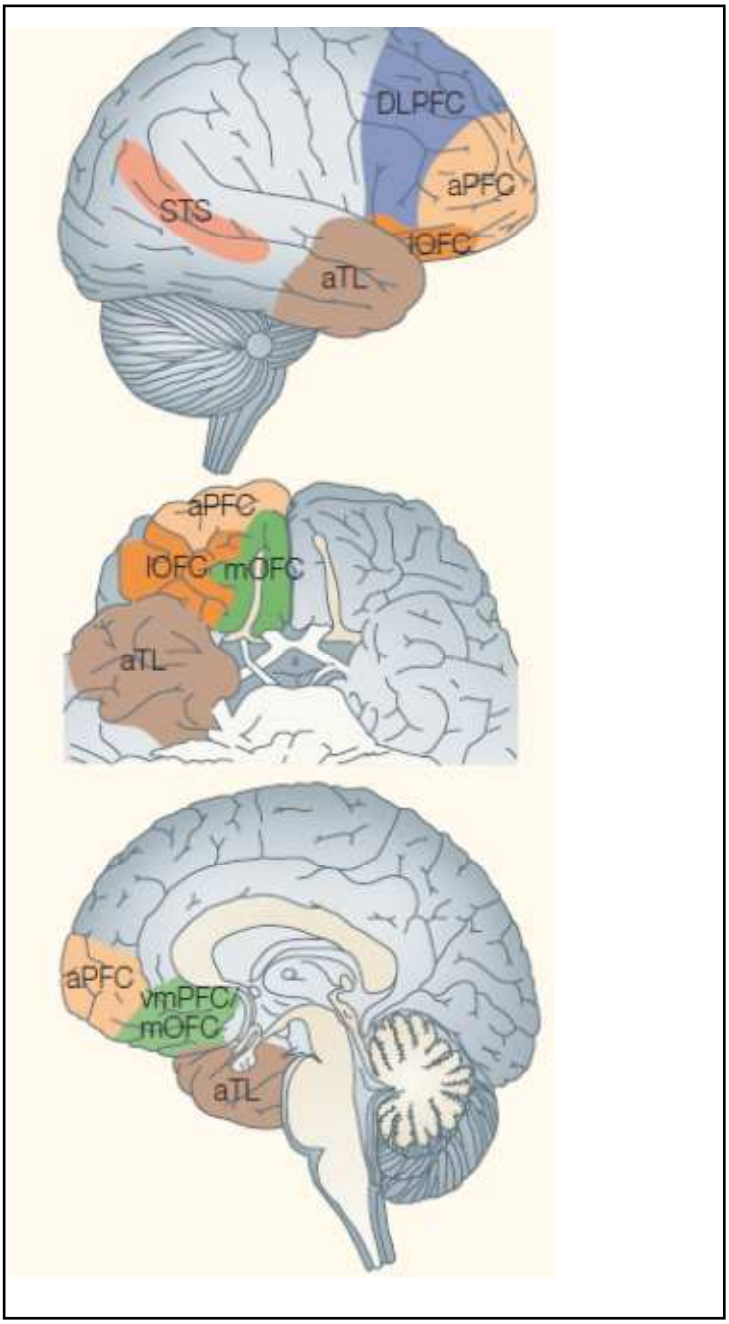

Figure 1.2 Cortical regions of the brain involved in control of appetite

Cortical regions include the anterior prefrontal cortex (aPFC), the medial and lateral orbitofrontal cortex (mOFC and lOFC), the dorsolateral PFC (DLPFC; mostly the right hemisphere) and additional ventromedial sectors of the PFC (vmPFC), the anterior temporal lobes (aTL) and the superior temporal sulcus (STS) region (Moll, et al., 2005)

The insular cortex and associated areas

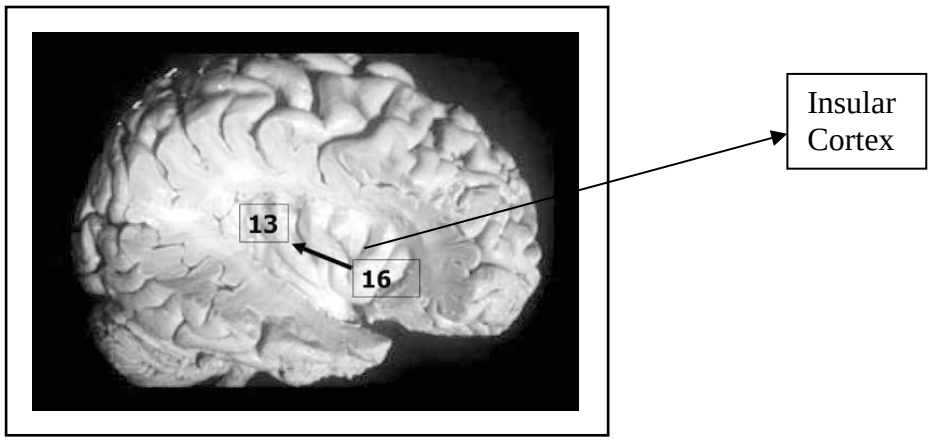

Figure 1.3: Macroscopic view of the insula

The insular lobe corresponds to the fifth lobe of the brain including Brodmann areas 13 through 16. The insula is buried in the lateral sulcus, covered by the operculum (consisting of the opercular parts of the frontal, parietal and temporal lobes) that is opened here (Dupont, Bouilleret, Hasboun, Semah, & Baulac, 2003)

Non-invasive imaging techniques have clearly demonstrated that simply thinking about food can modulate neural activity in specific brain areas known to be involved in the cognitive controls of appetitive behaviours. (Arana, et al., 2003). The largest functional magnetic neuroimaging study of taste processing from 38 right handed subjects (13 women and 25 men) described by Kringelbach et al revealed 3 cortical activation foci to the main effects of taste in the human brain. These were bilateral activation of the anterior insular/ frontal opercular cortex (Fig.1.3) with a slightly stronger response on the right side, the medial caudal orbitofrontal cortex and the left dorsolateral prefrontal cortex.

The locations of the anterior insular/frontal opercular cortex in the standard brain space in Montreal Neurological Institute (MNI) co-ordinates: [x,y,z: 38,20-4] and [x,y,z:-32, 22,0] are the likely bilateral sites of primary taste cortices. The insular cortex, in particular its most anterior portion, is considered a limbic-related

cortex. The insula has increasingly become the focus of attention for its role in body representation and subjective emotional experience. Functionally speaking, the insula is believed to process convergent information to produce an emotionally relevant context for sensory experience. More specifically, the anterior insula is related more to olfactory, gustatory, viscera-autonomic, and limbic function, while the posterior insula is related more to auditory-somesthetic-skeletomotor function (Kringelbach, de Araujo, & Rolls, 2004c). fMRI experiments have revealed that the insula has an important role in pain experience and the experience of a number of basic emotions, including anger, fear, disgust, happiness and sadness, functional imaging have also implicated the insula in conscious desires, such as food carving and drug craving (Kringelbach, et al., 2004c). The right anterior insula has been shown to support a representation of visceral responses accessible to awareness, providing a substrate for subjective feeling states (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004).

The insula is well situated for the integration of information relating to bodily states into higher- order cognitive and emotional processes. The insula receives information from ‘homeostatic afferent’ sensory pathways via the thalamus and sends output to a number of other limbic related structures such as the amygdala, the ventral striatum and the orbitofrontal cortex (OFC). It also encodes the motivational value of food, with its activity decreasing with eating and subjects become les motivated to further food intake (Dakin, et al., 2001).

The second region identified was the medial caudal OFC. The location in the MNI coordinates: [x,y,z:6,22,-16] is likely to coincide with the secondary taste

cortex, which fits well with the subsequent neurophysiological recordings in medial parts of the macaque OFC (Pritchard et al 2005).

Lastly the third region in which activity was found was the left dorsolateral pre-frontal cortex (DPFC) in the posterior part of the middle frontal gyrus (Brodmann Area 46): [x,y,z: -42, 26, 36]. This could aid higher cognitive processes in guiding complex motivational and emotional behaviour. Using functional magnetic resonance imaging, researchers have found that individual variation in trait reward sensitivity (as measured by the Behavioral Activation Scale) highly correlated with activation to images of appetizing foods (e.g., chocolate cake, pizza) in a fronto–striatal–amygdala–midbrain network. These findings suggested that there was considerable personality-linked variability in the neural response to food cues in healthy participants (Beaver, et al., 2006).

Furthermore, at a lower statistical threshold, taste related activity was found in the anterior cingulate cortex (I. E. de Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003a; I. E. T. de Araujo, Kringelbach, Rolls, & Hobden, 2003b; Ivan E. T. de Araujo, Kringelbach, Rolls, & McGlone, 2003c; Kringelbach, O'Doherty, Rolls, & Andrews, 2003; J. O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001b; John P. O'Doherty, Deichmann, Critchley, & Dolan, 2002; Small, et al., 2003; David H. Zald, Hagen, & Pardo, 2002; D. H. Zald, Lee, Fluegel, & Pardo, 1998). Regions of the anterior cingulate cortex thus may contain taste-related activity which can help the role of this region in executive control.

The decision about food intake is multi-modal incorporating information gleaned from taste, smell, somatosensory receptors in the nose and oral cavity, temperature of the food, viscosity, fat content and pungency of the food. Studies

using multimodal combinations of taste and smell using neuroimaging have shown that a region of the left OFC is responsible for the hedonic representations of the food (Kringelbach, et al., 2003). The insula is also involved in the brain's hedonic response to taste stimuli. It possibly sets the tone for how strong of a signal is transmitted to areas that further process hedonic taste experience (A. Wagner, et al., 2007). This suggests a dual role of the insula in sensory processing and affective processing of taste. Thus the frontal opercular/insular region (primary gustatory area) seems to be sensitive not only to the intensity but also to the palatability of a tastant (J. O'Doherty, et al., 2001b). In a PET study by Tataranni et al, there was increased activity in the middle insula in response to taste stimulation in hungry (after 36 h of last fast and shortly before the administration of a satiating amount of liquid meal) obese individuals. In contrast, there was no significant change of activity in hungry normal-weight individuals. Post-obese individuals, who are at high risk for the recurrence of weight gain, exhibited an obese-like increase of insular activity, suggesting that the insular response to sensory-stimulated anticipation of food reward may represent a neural marker of increased propensity to weight gain (A. C. K. Del Parigi, Salbe AD, Hill JO, Wing RR, Reiman EM, Tataranni PA, 2002).The insular cortex has been implicated in a variety of functions related to the integration of the autonomic response with ongoing behaviour and emotion (Allen, Saper, Hurley, & Cechetto, 1991), and gastrointestinal motor and sensory phenomenon (Penfield, 1955), including swallowing (Hamdy, et al., 1999), distension of the oesophagus (Aziz, et al., 2000) and stomach (Stephan, et al., 2003), as well as in response to flavour (Dalton, Doolittle, Nagata, & Breslin, 2000; I. E. de Araujo, et al., 2003a; Kringelbach, et al., 2003; Small, Jones-

Gotman, Zatorre, Petrides, & Evans, 1997), taste (Small, et al., 2003; Small, et al., 1999), food texture (Ivan E. de Araujo & Rolls, 2004) and tongue somatosensation (Pardo, Wood, Costello, Pardo, & Lee, 1997), smell (Cerf-Ducastel & Murphy, 2001; I. E. de Araujo, et al., 2003a; Savic & Gulyas, 2000; Small, et al., 1997; Zatorre, Jones-Gotman, Evans, & Meyer, 1992), hunger for food (A. Del Parigi, et al., 2002b; J.-F. Gautier, et al., 2001; J. F. Gautier, et al., 2000; Tataranni, et al., 1999), thirst (Denton, et al., 1999), hunger for air (Banzett, et al., 2000), and drug craving (Kilts, et al., 2001; G.-J. Wang, et al., 1999). The insular cortex has also been implicated in food reward and flavour-guided behaviour in rats (Cubero & Puerto, 2000; Ragozzino & Kesner, 1998) and humans (LaBar, et al., 2001; J. O'Doherty, et al., 2000; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001). Taken together, these reports indicate that the insular cortex participates in many aspects of eating behaviour, serving perhaps as the “ingestive” cortex of the brain (T. R. Scott & Verhagen, 2000; Small, et al., 2001). Greater insular activity is associated with higher motivation to eat (Small, et al., 2001). It has also been suggested that in conditions like anorexia nervosa, rate-limiting dysfunction of neural circuitry integrated by the insula could be the responsible offender (Nunn, Frampton, Gordon, & Lask, 2008).

Brain imaging in non-human primates has shown that there are sets of neurones in the caudolateral orbitofrontal cortex that responds to tastes of food only when the animal is hungry (E. T. Rolls, 1997). The response of these neurones decreases to almost zero when the animal has eaten to satiety and is specific for the food eaten. This observed event is referred to as sensory-specific satiety (SSS), a decreased desire to consume more of a food just eaten while still

desiring more food. It is related to human eating behaviour and contributes to satiation, that is, the termination of a meal (De Graaf, De Jong, & Lambers, 1999; Raynor & Epstein, 2000).

The Orbitofrontal cortex (OFC)

The OFC is one of the most important nodes linking sensory and hedonic systems involved in appetite and food consumption in the human brain. Indeed, primary taste cortex (i.e., agranular insular cortex) has efferent connections to the (OFC) and the other major limbic areas including NAc, IHA , and amygdala. The OFC receives multimodal inputs including gustatory, olfactory, visual, and somatosensory information. For example, some OFC neurons respond to the oral texture of fat (B. Rolls & Moran, 1999). Outputs from this region of the cortex project to the striatum, the ventral midbrain, and the sympathetic nervous system (H. R. Berthoud, 2002). In rats, electrical stimulation of the OFC initiates feeding (Bielajew & Trzcinska, 1994), and infusion of various neuropeptides or neurotransmitters into the OFC can alter respiratory quotient and energy expenditure as thermogenesis (Iain S. McGregor, Menéndez, & Atrens, 1990a; I. S. McGregor, Menendez, & Atrens, 1990b; Westerhaus & Loewy, 2001). Functionally, neuroimaging studies have shown that both pleasant and unpleasant stimuli activate the orbitofrontal cortex (J. O'Doherty, et al., 2000). Furthermore, the OFC is regarded as the brain region integrating different modalities- smell, taste and texture (Edmund T. Rolls, 2001). The human OFC also plays an important role in representing the reward value of liquid food stimuli (Kringelbach, et al., 2003) which correlates with its subjective pleasantness; as studied using fMRI. This effect is also consistent with an important role for the

orbitofrontal cortex in human emotion and motivation, and associated subjective states (Kringelbach, et al., 2003).

A study investigating the nonspecific satiation effects of chocolate where subjects were allowed to eat chocolate beyond satiety, found modulation in cortical chemosensory areas including insula, caudomedial and caudolateral OFC, suggesting reward value of food is represented here. They further noted differences in activity in the medial and lateral caudal part of the OFC suggesting differential representation of reward and punishment in this region (Small, et al., 2001). Activity in OFC has in fact been implicated with negative dissonance (pleasantness) of musical chords and intensely pleasurable responses to music have been associated with activity in OFC, ventral striatum, cingulate and insular cortex in studies investigating the effects of auditory stimulation (Blood & Zatorre, 2001). Further compelling evidence from drug studies have found responses to cocaine in the PFC, ventral striatum, and other reward related brain structures (Breiter, et al., 1997). Thus the OFC has been implicated in a wide variety of tasks.

A large meta-analysis has demonstrated medio-lateral and antero-posterior functional distinctions in the OFC, (Kringelbach, 2002 ; Kringelbach & Rolls, 2004a). Activity in the medial orbitofrontal cortex has been shown to relate to the monitoring of the reward value of many different reinforcers (involving mechanisms for learning and memory), whereas the lateral OFC activity has been proposed to be related to the evaluation of reinforcers which leads to change in ongoing behaviour. Furthermore, it has been shown that more complex and abstract reinforcers (such as monetary gain and loss) being represented is

represented more anteriorly than simpler reinforcers such as taste and pain (Kringelbach, 2002 ; Kringelbach & Rolls, 2004a).

The OFC has been extensively implicated in representing and monitoring the changing reward value of primary (including gustatory and somatosensory stimuli) and learned (including olfactory and visual stimuli) (Kringelbach & Rolls, 2004a). A caudal and lateral area of the orbitofrontal cortex has been involved in sensory-specific satiety, both in response to the odour of a solid food eaten to satiety (J. O'Doherty, et al., 2000) or to the orosensory experience of a liquid food consumed to satiety (Kringelbach, et al., 2003). This is the same region noted to be activated by O'Doherty et al (J. O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001) in response to a symbolic monetary gain and in proportion to the magnitude of this reward, as well as deactivated in response to a symbolic loss of money (punishment). Wang et al showed marked increase (24%) in brain metabolism, using FDG-PET, by presentation of food (G.-J. Wang, et al., 2004), providing evidence of the high sensitivity of the human brain to food stimuli. These changes were largest in the superior temporal, anterior insula and orbitofrontal cortices. Further, the increased activation in the right orbitofrontal cortex correlated significantly with increases in self-report of hunger and desire for food.

Animal studies implicating the limbic regions of the brain have shown that lesion experiments in rats suggest different but complimentary roles for the orbitofrontal cortex and basomedial amygdala in learning about the representations of specific experiences with food and using them to guide appetitive behaviour. It had long been demonstrated that food intake can be conditioned over time by repeatedly pairing the presentation with tone or light in

hungry rats. After learning this task even sated rats will approach and consume food upon exposure to the conditioned stimulus (Weingarten, 1983). Thus the authors demonstrated an increase in free feeding following presentation of the pavlonian cue, a phenomenon called “conditioned potentiation”. Through selective lesion techniques involving the basal ganglia and the OFC in rats, another group showed that the basolateral amygdala seemed critical to learning representations that link cues to the incentive properties of outcomes, but not for maintaining such representations. In contrast, the OFC seems to be critical for maintaining memorial representations that link cues to the incentive properties of outcomes, for updating them with new information, and for using them to guide appetitive behaviour. (Pickens, et al., 2003). Furthermore, neurons in the basolateral and basomedial amygdala, and orbitofrontal prefrontal cortex were selectively activated by conditioned potentiation and projected to the lateral hypothalamus, a key area for energy control. Thus this evidence indicates that these pathways are functionally involved in this cue-potentiated feeding during satiation. It also provided a key functional anatomical link between the reward-motivational systems in the amygdala and pre-frontal cortex with the hypothalamus (primary homeostatic centre). (Petrovich, Holland, & Gallagher, 2005). The OFC is richly interconnected with the limbic system, particularly the amygdala and the perirhinal cortex; this network may serve to integrate affective, visceral, and primary sensory information (Price, 1999).

1.14.4.2 The subcortical control of appetite: Mesolimbic dopamine reward system

Certainly one of the most potent drives for feeding is its rewarding nature. Few experiences in life are more satisfying than consuming a well-prepared meal, and

the complex flavours and textures of food are best appreciated when one starts in a hungry (rather than satiated) state. Because of these rewarding properties, humans and animals will work for food (the quintessential definition of a reward, i.e., the willingness to engage in otherwise unrelated behaviours that quickly extinguish in the absence of the reward) (Saper, et al., 2002).

The subcortical control of appetite is regulated by the limbic system which is also called the hedonic region of the brain or the ‘addiction centre’ Fig. 1.4. Areas of the brain involved in processes of anticipation and reward include the projection areas in the ventral striatum (especially the nucleus accumbens (NAcc)) (Knutson, Adams, Fong, & Hommer, 2001), dorsal striatum (Delgado, Stenger, & Fiez, 2004; David H. Zald, et al., 2004), OFC (E.T Rolls, 2004), and other areas of mesial prefrontal cortex (Knutson, Fong, Bennett, Adams, & Hommer, 2003; Ramnani, Elliott, Athwal, & Passingham, 2004). This network has been collectively described as the ‘reward pathway’ that responds to subjectively positive stimuli. Thus the reward pathways involve several entities of the brain, predominantly involving the limbic systems. There is overwhelming work delineating the role of these regions in appetite control using various modalities like studies in animal models (dopamine deficient mice), behavioural and psychological studies and functional imaging studies. Understanding the pathways involved in reward perception paves way to the understanding of the hedonic controls of appetite.

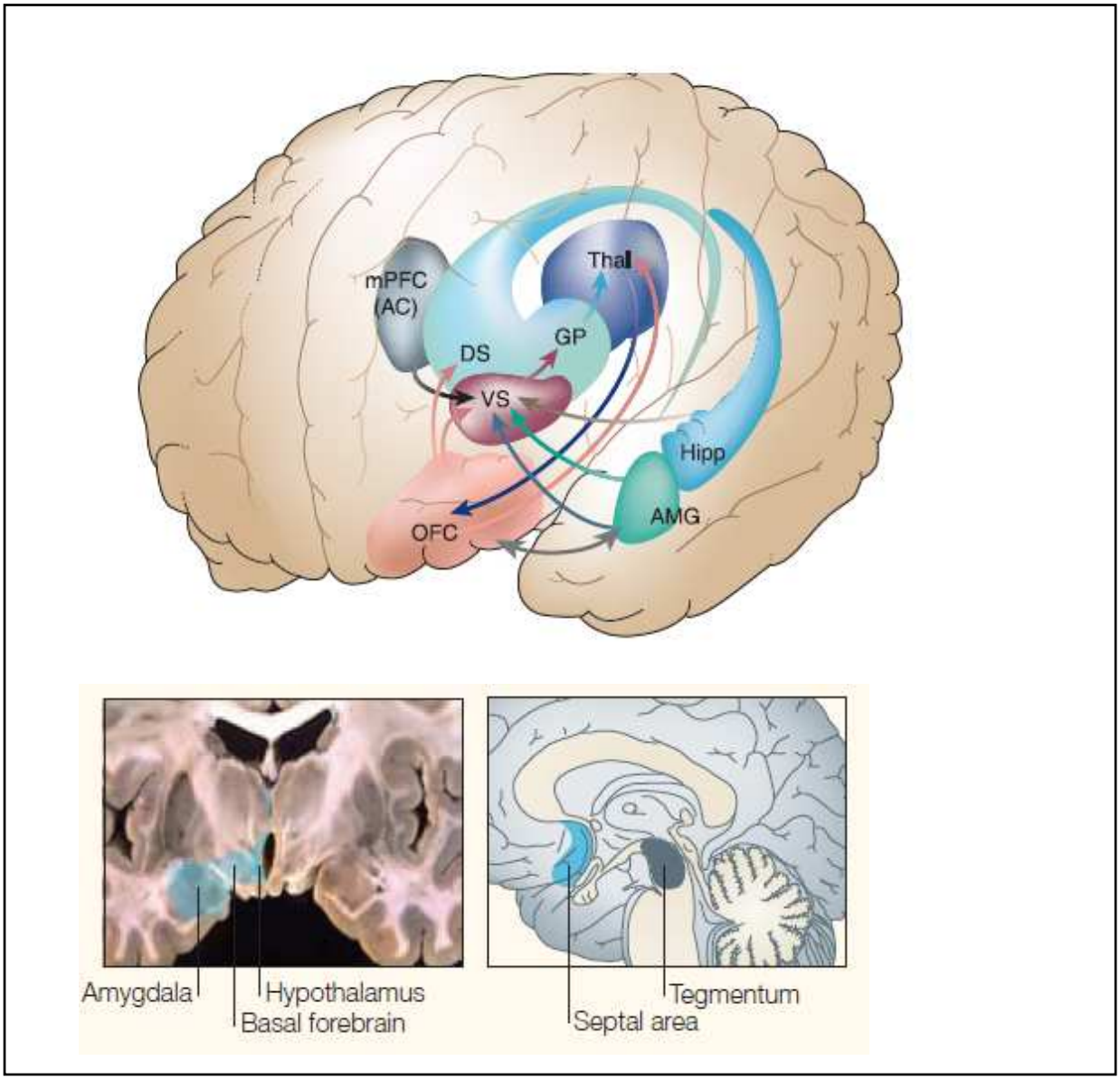

Figure 1.4 Interactions between chief limbic regions involved in appetite control (top panel) and chief subcortical regions involved in hedonic control of appetite (lower panel)

In this diagram, the green and blue arrows depict glutaminergic projections; the orange arrows, dopaminergic projections and the pink arrows GABAergic projections (Everitt & Robbins, 2005; Moll, et al., 2005). Acb, nucleus accumbens; AMG, amygdala;. GP, globus pallidus ; Hipp, hippocampus; mPFC, medial prefrontal cortex; AC, anterior cingulate cortex; OFC, orbitofrontal cortex; VS, ventral striatum; DS, dorsal striatum; Thal, thalamus. Subcortical structures include the amygdala, ventromedial hypothalamus, septal area and nuclei, basal forebrain (especially the ventral striatum/pallidum and extended amygdala), the walls of the third ventricle and rostral brainstem tegmentum (Moll, et al., 2005).

1 Introduction

The mesolimbic dopamine system including the ventral tegmental area (VTA) of the midbrain and extending to the nucleus accumbens in the striatum, is considered the major reward pathway (Haber & Knutson, 2009). The mesolimbic dopamine reward system is a higher brain centre that is important in neurobiological control of food intake (Palmiter, 2007). This is clearly demonstrated in dopamine deficient mice, as they are hypoactive and hypophagic and die of starvation within 3 weeks of age (Szczypka, et al., 1999). Activation of mesolimbic dopamine neurons in the ventral tegmental area (VTA) leads to dopamine outflow from the nucleus accumbens (NAc). This mesolimbic dopamine-accumbal projection is critical to reward related behaviour and has been well studied in models of drug addiction (Kalivas & Volkow, 2005). Food palatability and hedonic value are critical to the overall regulation of food intake and significantly contribute to obesity by overriding long-term homeostatic control in today's highly palatable, energy-rich environment (Andrews & Horvath, 2008) . Highly palatable foods increase dopamine concentrations in the NAc (Hernandez & Hoebel, 1988), and the hedonic value of sucrose can be attenuated by dopamine antagonists (Bailey, Hsiao, & King, 1986).

Recent evidence suggests that neurons in the hypothalamus can sense and respond to the changes in metabolic value of ingested nutrients. In a set of behavioural experiments, de Araujo et al used behavioural, neurochemical and electrophysiological modalities to study whether the mesolimbic dopaminergic system, critical for reinforcing food palatability and hedonic value, could also sense metabolic value of ingested nutrients independent of taste (Ivan E. de Araujo, et al., 2008). The authors showed that KO (knock-out) mice (mice lacking a functional transient receptor potential channel M5, TRPM5, highly

expressed in taste receptor cells), were acutely insensitive to the orosensory or "sweet" rewarding properties of sucrose. As expected, water deprived WT (wild-type) mice were more strongly attracted to sucrose solutions compared to water (as measured by number of licks for water), whereas KO mice exhibited no preference for sucrose over water. Additional preference tests confirmed that the KO mice were insensitive to the orosensory "sweet" rewarding properties of sucrose. These sweet insensitive mice then allowed the authors to test the critical question of whether animals could detect the caloric value of ingested substances. WT and KO mice were exposed to a "conditioning protocol" that allowed KO mice to associate sipper side with post-ingestive caloric load (I.E. water versus the highly calorific sucrose solution). Strikingly their results indicated that both WT and KO mice consumed more sucrose. These results argued that KO mice were making a choice preference purely based on the detection of the postingestive reinforcing properties of the sucrose solution (increased caloric load). When the experiment was repeated with sucralose (noncaloric, but highly palatable sucrose derived sweetener), the WT mice consumed more sucralose (avid for sweet taste) than water during the conditioning period but the KO mice did not. Thus the WT mice were reinforced by sweet taste regardless of whether the drink was the highly caloric sucrose or the noncaloric sucralose. Conversely, the KO mice showed a specific preference for calorific content and were not influenced by sweet taste, in the absence of any calorific advantage. In this experiment the authors excluded the possibility that differences in plasma glucose underlay the observed effect. Furthermore, dopamine levels in nucleus accumbens were significantly higher in WT mice post sucrose and sucralose, confirming that dopamine release in the NAc

reinforces the hedonic value “taste” of sugars, even if no calories are present. On the other hand, KO mice exhibited no increase in NAc dopamine upon ingestion of sucralose, although they showed significant increases in NAc dopamine after sucrose consumption, indicating that caloric load activates the brain dopamine reward independent of “sweet” taste sensation (Andrews & Horvath, 2008; Ivan E. de Araujo, et al., 2008).

The brain response to the ingestion of a meal in normal-weight individuals suggested the presence of an orexigenic domain, mainly represented by limbic and paralimbic areas, including the orbitofrontal and insular cortices, anterior cingulate, and hypothalamic region, and of a satiation domain, almost exclusively represented by prefrontal areas (Tataranni, et al., 1999). Furthermore Tataranni et al proposed a model to explain the differences in the brain response to a meal in obese and lean individuals. This model predicts that the prefrontal cortex signals satiation by sending inhibitory inputs to the limbic/paralimbic areas, thus suppressing hunger. In obese individuals, the prefrontal cortex may be working harder to suppress chronically hyperactive orexigenic areas. Alternatively, the hypothalamus in the obese individuals may be resistant to the inhibitory effects of the prefrontal cortex (A. Del Parigi, et al., 2002).

1.14.5 Lessons from addiction studies

Further insight into the study of appetite regulation by the limbic system is offered by studies focussing on the central mechanisms of drug addiction. Functional imaging of areas involved in substance abuse has been intensively studied and has contributed to our knowledge of neuroimaging in addiction. This

is relevant and interesting as there are several common characteristics between the neural circuits of the addiction systems and the appetitive systems.

Drug abuse and addiction, and certain types of obesity can be understood as resulting from habits that strengthen with repetition of the behaviour and that become increasingly harder for the individual to control despite their potentially catastrophic consequences (Nora D. Volkow, Wang, Fowler, & Telang, 2008). In general, irrespective of the patient populations, substances abused, and use of different neuroimaging studies, several key areas involved in addiction have been identified. Areas such as the amygdala, anterior cingulate, dorsolateral prefrontal cortex and orbitofrontal cortex, which are involved in attention, emotional processing, goal-directed behaviour, associative learning, decision making, integration of information and response suppression, are commonly activated. In particular, the role of the orbitofrontal cortex has gained prominence because of its crucial role in impulse control and decision making. (London, Ernst, Grant, Bonson, & Weinstein, 2000; Nora D. Volkow & Fowler, 2000).

Consumption of food, other than eating from hunger, and some drug use are initially driven by their rewarding properties, which in both instances involves mesolimbic dopamine pathways. Food activates brain reward circuitry both through palatability (involves endogenous opioids and cannabinoids) and through increase in glucose and insulin concentrations (involves dopamine increases), where as drugs activate this same circuitry via their pharmacological effects (via direct effects on dopamine cells or indirectly through neurotransmitters that modulate dopamine cells such as opiates, nicotine, γ-aminobutyric acid or cannabinoids (Nora D. Volkow & Wise, 2005).

1 Introduction

Functionally the limbic reward system mediates the rewarding, reinforcing, and emotional aspects of stimuli. From the anatomical perspective, there is a general consensus that the lateral hypothalamic area (LHA), amygdala, select regions of the cerebral cortex, the ventral tegmental area (VTA), and the ventral striatum or the nucleus accumbens (NAc) are components of the circuitry (De Olmos & Heimer, 1999; Everitt, et al., 1999). Activation of VTA dopamine neurons, and release of dopamine within the NAc, has long been viewed as indicative of reward enhancement. Food craving (defined as an intense desire to eat a specific food) has been suggested to be the evolutionary source for cravings of all kinds including cravings for drugs and alcohol (Pelchat, Johnson, Chan, Valdez, & Ragland, 2004). Pelchat et al used a two part technique to study fMRI brain activation to food cravings. Threshold for craving was reduced through diet manipulation (monotonous diet) and cravings were triggered during BOLD fMRI sessions by having subjects imagine the sensory properties of favourite foods (a cue-induction technique). They had two groups of 10 healthy volunteers, one who received monotonous diet and another who were maintained on their normal diet. Both groups were shown food names and were allowed to think of the most desirable version of their liked food, in a block fashion. The monotonous diet group showed greater activation in the left hippocampus, left insula, and right caudate nucleus (Pelchat, et al., 2004). These areas are also activated during drug craving supporting a common circuitry for desire for natural and pathological rewards (Nora D. Volkow, Fowler, Wang, & Goldstein, 2002).

The opioid and the serotonin pathways can modulate the mesolimbic dopaminergic activity (Spanagel & Weiss, 1999), suggesting a link between ‘liking’ and ‘wanting’ food and a possible basis for ‘food craving’(Mercer &

Holder, 1997). The association of taste with ‘wanting’ food and food reward (positive reinforcement) is related to the release of dopamine in the meso-limbic circuitry of the brain (Martel & Fantino, 1996). Moreover reduced D2 receptors could contribute to loss of self control in detoxified drug-addicted subjects and with decreased metabolic activity (regional brain glucose metabolism) in the orbitofrontal cortex, anterior cigulate gyrus and dorsolateral pre-frontal cortex (N. D. Volkow, Fowler, J.S., Wang, G. J, Hitzmann, R., Logan, J., Schlyer, D. J., Dewey, S.l. & wolf, A. P. , 1993; G.-J. Wang, et al., 2001). These areas are traditionally involved with inhibitory control (Goldstein & Volkow, 2002) and with emotional processing (Phan, Wager, Taylor, & Liberzon, 2002) and dopamine impairments in these regions could underlie the compulsive drug intake that characterizes addiction. (Nora D. Volkow & Wise, 2005). Thus Volkow et al postulate that high levels of D2 receptors could protect against addiction by modulating prefrontal regions involved in inhibitory control and emotional regulation. Likewise, reductions in D2 receptor activity in morbidly obese subjects was associated with reduced glucose metabolism in the prefrontal regions of the brain, namely the dorsolateral prefrontal cortex, medial prefrontal cortex and the cingulate gyrus. This suggests that decreases in D2 receptors in obese subjects contribute to overeating in part through dysregulation of prefrontal regions implicated in inhibitory control and emotional regulation (Nora D. Volkow, et al., 2008).

On the other hand, during active cocaine addiction, the prefrontal regions are hypermetabolic, suggesting drug induce dopamine increases in the striatum activates OFC and cingulate gyrus which result in craving and compulsive drug intake (N. D. Volkow, et al., 1991; Nora D. Volkow, et al., 2008). Imaging in

obese subjects have shown increased activation of prefrontal regions upon exposure to a meal, which is greater in obese than in lean subjects(J. F. Gautier, et al., 2000). When food- related stimuli are given to obese subjects, medial prefrontal cortex is activated and cravings are reported (J. F. Gautier, et al., 2000; Miller, et al., 2007; G. J. Wang, Volkow, Thanos, & Fowler, 2004). These prefrontal regions could reflect a neurobiological substrate common to the drive to eat or the drive to take drugs. Abnormalities of these regions could enhance either drug- or food-oriented behaviour, depending on the sensitivity to the reward and/or established habits of the subject (Nora D. Volkow, et al., 2008).

Food deprived subjects were studied while stimulated with a neutral or food-related stimulus (conditioned cues). Food stimulation significantly increased dopamine in striatum and these increases correlated with the increases in self-reports of hunger and desire for food (N. D. Volkow, et al., 2002). This suggest the involvement of striatal dopamine signalling in conditioned responses to food and the participation of this pathway in food motivation in humans (Nora D. Volkow, et al., 2008). Thus Volkow et al proposed a model illustrating the overlap of the neurocircuitry in addiction and obesity, as illustrated in Fig 1.5.

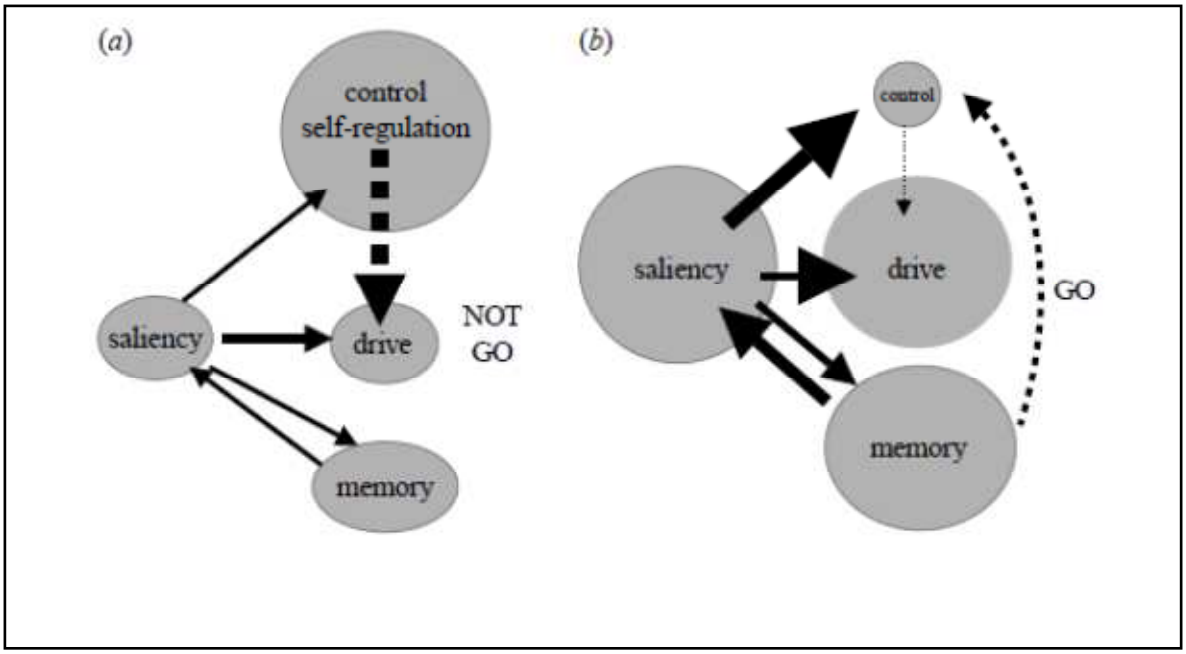

Figure 1.5 Overlap of neural circuitry in addiction and obesity

Model of brain circuits involved with addiction and obesity: reward/saliency motivation/drive, memory/conditioning and inhibitory control/emotional regulations. Disrupted activity in brain regions involved with inhibitory control/emotional regulation when coupled with enhanced activation of reward/saliency and memory/conditioning leads to enhanced activation of the motivational/drive circuit and the resultant compulsive behaviour (drug taking or food ingestion) when the individual is exposed to the reinforcer (drug or food), conditioned cues or a stressor. Note that circuits that regulate mood as well as internal awareness (interoception) are also likely to modulate the ability to exert control over incentive drives. (a) Healthy brain, (b) dysregulated brain (Nora D. Volkow, Fowler, & Wang, 2003b).

According to this model, reward/saliency, motivation/drive, memory/conditioning and inhibitory control/emotional regulations are the key players of the neurocircuitry. Disrupted activity in the brain regions involved in inhibitory control/emotional regulation when coupled with enhanced activation of reward/saliency and memory/conditioning leads to enhanced activation of the motivational/drive circuit and the resultant compulsive behaviour (drug taking or food ingestion) when the individual is exposed to the reinforcer (drug or food), conditioned cues or a stressor. (Nora D. Volkow, et al., 2003b). Nasser et al suggest reduced sensitivity to the “sensory-specific satiety” as an alternative way in which altered taste responses promote excessive energy intake and contribute to the development or maintenance of obesity in humans (Nasser, 2001).

This model has therapeutic implications for it suggests a multi-prong approach that targets strategies to: decrease the rewarding properties of the problem reinforcer (drug or food); enhance the rewarding properties of alternative reinforcers (i.e. social interactions, physical activity); interfere with conditioned-learned associations (i.e. promoting new habits to substitute for old ones); and strengthen inhibitory control (i.e. biofeedback), in the treatment of drug abuse/addiction and obesity (Nora D. Volkow, et al., 2003b).

In addition to the dopaminergic system, the endocannabioid system has also been shown to be involved in control of food intake via both central and peripheral mechanism (Di Marzo & Matias, 2005). Brain endocannabinoids system controls food intake at two levels, First, it tonically reinforces the motivation to find and consume foods with a high incentive value, possibly by interacting with the mesolimbic pathways involved in the food reward mechanisms. Second, it is activated 'on demand' in the hypothalamus after short-term food deprivation and then transiently regulates the levels and/or action of other orexegenic and anorectic mediators to induce appetite (Di Marzo & Matias, 2005). The endocannabinoids system interacts with the mesolimbic system by enhancing dopamine release in the nucleus accumbens shell (Duarte, et al., 2003; A. N. A. Verty, McGregor, & Mallet, 2004) or by synergising with opioids through as yet undefined mechanisms (R. Z. Chen, Huang, Shen, MacNeil, & Fong, 2004; Kirkham & Williams, 2001; Rowland, Mukherjee, & Robertson, 2001; A. Verty, Singh, McGregor, & Mallet, 2003; C. M. Williams & Kirkham, 2002). In addition to its central effects, the endocannabinoid system also has peripheral actions which contribute to its role of appetite control. The endocannabinoid system may also reduce satiety by acting on the vagus nerve, as suggested by the

anorectic action of peripherally administered rimonabant and by the reversal of this action following destruction of the vagal capsaicin-sensitive nerves that also mediate cholecystokinin (CCK)-induced satiety (Gomez, et al., 2002).

1.14.6 Role of neuropeptides in the limbic region

Neuroimaging methods have been used to examine brain activation in response to food stimuli following manipulation of appetite related hormones. For example, in a study combining i.v infusions of ghrelin with fMRI, Malik et al (Malik, McGlone, Bedrossian, & Dagher, 2008) measured brain activation in response to pictures of highly palatable foods (e.g. pizza, hamburger) versus nonfood stimuli (e.g. scenery pictures) following a 3-h fast in lean healthy males (n= 12) before and after a 20 min ghrelin infusion. They compared the results with those from similarly timed scans conducted in a control group (n= 8) receiving no ghrelin. Post-infusion increases in response to food (versus non-food) pictures were observed in the amygdala, orbitofrontal cortex, insula and striatum, comprising areas that are associated with encoding the reward value of stimuli (Edmund T. Rolls, 2000) and have shown activation to appetising food images (Stice, Spoor, Ng, & Zald, 2009; Stoeckel, et al., 2008). Thus ghrelin activates dopamine neurones in the VTA and increases dopamine turnover in the nucleus accumbens of the ventral striatum (Balleine, 2005; Jerlhag, et al., 2007). They suggested that the orexigenic effect of ghrelin is associated with an up-regulation of mesolimbic dopaminergic activity accompanied by an increase in motivational salience of high-energy foods (Lenard & Berthoud, 2008; Malik, et al., 2008)

In addition to signalling in the periphery, a number of hormones produced in the brain also regulate appetite. Neuropeptide Y (NPY) and agouti-related protein-

producing neurones in the arcuate nucleus are activated by ghrelin to stimulate food intake, where as serotonin from the raphe nucleus inhibits feeding (Arora & Anubhuti, 2006; Leibowitz, Weiss, Walsh, & Viswanath, 1989). Similar to the peripheral effects, centrally released endocannabinoids (Wenger & Moldrich, 2002) increase food intake, whereas GLP-1 (Tang-Christensen, et al., 1996), and oxyntomodulin (Dakin, et al., 2001) decrease it. By contrast to ghrelin, PYY appears to have different actions according to site of administration and release. Suppression of food intake has been found by peripheral administration (Batterham, et al., 2003), whereas increases have been found on central administration (Corp, Melville, Greenberg, Gibbs, & Smith, 1990; Hagan, 2002), highlighting the complexity of neural and hormonal signals relating to appetite control. Studies have also combined direct hormone infusions with fMRI. In a double-blind placebo-controlled crossover study by Batterham et al (Batterham, et al., 2003), eight lean healthy males were infused with physiologically relevant doses of PYY $_{3\text{-}36}$ to mimic the hormone profile after satiation, whereas, on another day, they received saline infusions designed to stimulate the fasted state. All participants were scanned throughout both infusions, and blood draws were taken every 10 min throughout the 100-min procedure to measure hormone levels, with visual analogue scale ratings made every minute to assess subjective appetite. Thirty minutes following the scan, participants consumed a mixed buffet meal and caloric intake was measured. Correlational analyses revealed increased activation with PYY 3-36 infusion and corresponding decrease with saline infusion in the left orbito-frontal cortex, parabrachial nucleus, ventral tegmental area, insula, anterior cingulate cortex, ventral stratum (globus pallidus, putamen), regions of the frontal, parietal, temporal and cerebellar cortices and

posterior hypothalamus (including VMH), providing evidence for tight yoking between gut hormones and brain activity. Furthermore, caloric intake in the buffet meal was predicted by activation in the hypothalamus in the saline condition, and by deactivation in higher-order reward areas (i.e. orbitofrontal cortex) in the PYY condition. This was interpreted as indicating a switch from homeostatic determination of feeding in the fasted state to hedonic determination of feeding in the satiated state (Neary & Batterham, 2009).

In a study of leptin replacement in genetically leptin-deficient adults, Baicy et al (Baicy, et al., 2007) reported reduced fMRI activation in the leptin supplemented group in areas involved with hunger (insula, parietal and temporal cortex) and greater activation in regions linked to inhibition and satiety (PFC) in response to visual pictures of food (e.g. fried chicken, cheeseburgers) compared to neutral stimuli (e.g. brick walls). Another group extended this to leptin deficient adolescents, demonstrating marked fMRI activation in the ventral striatum in response to food images presented in both the fed and fasted states, which was markedly reduced following 1 week of leptin administration. (Farooqi, et al., 2007). Rosenbaum et al (Rosenbaum, Sy, Pavlovich, Leibel, & Hirsch, 2008) have applied this methodology to individuals with common polygenic obesity. In their study, six obese inpatients that had lost 10% of their initial weight on a liquid diet were given twice-daily subcutaneous injections of leptin or saline for 5 weeks. Response to visual presentation of actual foods was assessed at baseline, after weight loss, and after leptin and saline administration. Post-administration, the saline group showed significantly greater activation to food cues compared to the leptin group in areas including the insula, parahippocampal gyrus, and middle and superior frontal gyri, consistent with a relatively greater

appetitive responsivity and drive to eat (Rosenbaum, et al., 2008). Thus these studies suggest that leptin down-regulates 'hedonic' activation in reward areas in response to food stimulation, and simultaneously up-regulates homeostatic control by enhancing the central response to peripheral satiety signals (Farooqi, et al., 2007; Hukshorn, et al., 2002).

Other studies have focussed on specific effects of sweet taste on brain activation patterns. Study of effects of glucose ingestion on the hypothalamus by Smeets et al revealed prolonged signal decrease in the upper hypothalamus 2-5 minutes following ingestion of 75 glucose solution and lasted for ~30 minutes. Water, aspartame and maltodextrin had no such effect on the hypothalamus. In addition insulin measurements revealed an early rise in concentrations following ingestion of glucose only and with glucose and maltodextrin at 5-10 minutes post ingestion. However, as maltodextrin causes insulin changes but no hypothalamic activation, they suggested that the early (<5 minute) decrease in hypothalamic signal was attributable to glucose per se rather than insulin. Moreover, a combination of taste (sweetness) and energy content was thought to be important in triggering a hypothalamic response and a adaptive response to sweetened beverages (Smeets, de Graaf, Stafleu, van Osch, & van der Grond, 2005a).

Chen et al have studied the hypothalamic response after glucose ingestion in 24 lean and overweight rats after fMRI. The hypothalamic fMRI signal was transiently lowered in all rats within 19.5 - 25.5 minutes of oral glucose consumption, although the decrease was greater in the lean than the overweight rats, with no change observed in control animals (M. Chen, et al., 2007).

Imaging studies investigating neuronal responses to oral administration in humans have provided data on the relationship between plasma glucose and

insulin and appetite regulation. Matsuda et al (Matsuda, et al., 1999) administered a 65 g oral glucose load to ten obese versus ten lean male and female adults after a 12-h fast. Oral glucose ingestion started 10 minutes after subjects were placed in the scanner. Following ingestion, lean participants showed deactivation in the lower posterior quadrant of the hypothalamus including the VMH, and obese participants showed a slower and smaller response. In the upper anterior hypothalamic region including the PVN, there was slight deactivation and a relative delay in hypothalamic inhibition in obese versus lean participants. The decrease (4-8%) in BOLD signal in the lower posterior hypothalamus started 4 min after ingestion and lasted approximately 10 min in all subjects, providing information about the lag time of homeostatic neural responses. There was a positive correlation between the time to reach maximum response in the lower posterior hypothalamus and upper anterior hypothalamus and fasting glucose and insulin concentrations in both obese and lean subjects. The findings of Matsuda et al suggest that delayed activation of satiety centres (e.g. VMH) following glucose consumption may contribute to excessive intake in obese individuals (Matsuda, et al., 1999). Thus fMRI and PET studies have shown that in obese individuals the decrease in hypothalamic activity following a meal is significantly reduced compared with lean individuals (Matsuda, et al., 1999)

In a similar study done by Liu et al (Liu, et al., 2000), 21 healthy adults were given a 75 g oral glucose load after a 12-h fast. A reduction in hypothalamic activity (up to 4%) was observed initially at 1-2 min and then again at 7-12 min following ingestion. Smeets et al recently extended these findings by varying glucose load and adding water condition to rule out the possibility that

hypothalamic signal decreases independently over time. In this study of 15 lean healthy males, 25 g or 75 g of glucose was administered in 300 ml of orange-flavoured water, and the hypothalamic signal (mostly in the upper anterior hypothalamus) was shown to be significantly lower (1-2.5%) than in water condition (300 ml) for up to 30 min post ingestion. This decrease was significantly greater for 75 g than for 25 g glucose load, supporting a dose-response pattern (Smeets, de Graaf, Stafleu, van Osch, & van der Grond, 2005b).

The exact neurophysiological mechanisms underlying these finding in the hypothalamus are unclear. It is possible that the meal directly inhibits hypothalamic neuronal activity, which may be elevated in a state of hunger (Tataranni & DelParigi, 2003). Alternatively, the meal may activate inhibitory pathways (prefrontocortical hypothalamic pathways) (A. Del Parigi, et al., 2002), which in turn suppress the neuronal activity of the hypothalamus. It is also unclear what components of the meal (glucose) or of the physiological response to it (insulin, other gut hormones, autonomic nervous system afferents signals) may mediate the observed hypothalamic response. Thus it is difficult to resolve if these responses relate primarily to the role of the hypothalamus in the regulation of glucose metabolism or of energy homeostasis (Tataranni & DelParigi, 2003).

Thus in essence, appetite control is a highly evolved and intricate pathway incorporating several central and peripheral hunger and satiety signals and is modulated to various external appetitive stimuli and ingested nutrients and indeed the internal central appetite control mechanisms can adapt to changing external appetitive environment over time, suggesting a dynamic model of appetite control.

These data illustrate that neuropeptides, originating both centrally (e.g. Neuropeptide Y) and peripherally (e.g. leptin) influence the hypothalamo-limbic regions of the brain to regulate appetite. In addition, presence of glucose manipulates hypothalamic activity as observed with brain imaging techniques, suggestive of either a primary glucose effect or a secondary effect of hormonal and peptide secretion in response to glucose. This study decided to test the effects of oral glucose, a vital brain substrate, in hypothalamo-limbic brain areas in response to viewing food images, to see if presence of glucose manipulated activity in these regions under different physiological conditions (post 25 g glucose vs. sweetener) and thus influence energy balance in the body.

1.14.7 Factors influencing brain responses to foods (pictures)

Brain responses to food (pictures) has been shown to vary with the physiological state of the body (hungry/satiated), calorific content of food and individual's nutritive status (lean vs. obese), to state a few.

1.14.7.1 Nutritional state

It is now well recognized that nutritional state impacts on food reward. Acute fasting or more chronic negative energy balance and weight loss increases the appeal and pleasantness for food (Cabanac, 1971; Cameron, Goldfield, Cyr, & Doucet, 2008; Stoeckel, Cox, Cook Iii, & Weller, 2007; Uher, Treasure, Heining, Brammer, & Campbell, 2006). Goldstone et al investigated whether acute fasting increased the degree to which the brain's reward circuitry is engaged by high-calorie compared with low-calorie foods, in *a priori* regions of interest including ventral striatum, amygdala, anterior insula and orbito-frontal cortex. Their

results demonstrated that fasting (skipping breakfast) enhanced the engagement of brain reward system by high calorie foods, with activation bias for high-calorie foods over low-calories foods seen bilaterally within the ventral striatum, amygdala, anterior insula and the medial and lateral OFC when fasted but not after eating breakfast. Acute fasting also increased activity in the ventral striatum, amygdala, insula and medial OFC in response to food vs. non-food stimuli compared when fed (Farooqi, et al., 2007; Fuhrer, Zysset, & Stumvoll, 2008; Hinton, et al., 2004; Holsen, et al., 2005; LaBar, et al., 2001; Uher, et al., 2006). The subjective rating of hunger when fasted for at least 5 h has been shown to correlate with activation in the insula to food pictures (Porubská, Veit, Preissl, Fritsche, & Birbaumer, 2006). Activity in the amygdala and OFC has also been associated with enhanced memory of food stimuli when fasted (Morris & Dolan, 2001). Furthermore, the behavioural data showed that subjective food appeal was only biased towards high-calorie foods when fasted (A. P. Goldstone, et al., 2009). Acute fasting also increases activity in the ventral stratum, amygdala, insula and medial OFC in response to food vs. non-food stimuli compared when fed (Farooqi, et al., 2007; Fuhrer, et al., 2008; Hinton, et al., 2004; Holsen, et al., 2005; LaBar, et al., 2001; Uher, et al., 2006). The subjective rating of hunger when fasted for at least 5 h has been shown to correlate with activation in the insula to food pictures (Porubská, et al., 2006). Activity in the amygdala and OFC has also been associated with enhanced memory of food stimuli when fasted (Morris & Dolan, 2001). Parigi et al have also studied the neuroanatomical correlates of hunger and satiation. Administration of a meal to hungry individuals was associated with increased neuronal activity in the prefrontal cortex (generally involved in the inhibition of inappropriate response

tendencies) and decreased neuronal activity in the hypothalamus, thalamus, several limbic/paralimbic areas (generally involved in affect and motivation), basal ganglia, temporal cortex and cerebellum. Among the limbic/paralimbic areas, they observed decreased activity in response to meal in the insular cortex, the anterior cingulate (selectively involved in response to noxious stimuli) and the orbito-frontal cortex (known to respond to hunger in non-human primates) (A. Del Parigi, et al., 2002b).

In normal weight individuals, in a fasted hungry state, visual food vs. non-food stimuli is reported to produce greater activation in regions including the amygdala, insula and orbitofrontal cortex (Fuhrer, et al., 2008; Gordon, et al., 2000; Hinton, et al., 2004; Holsen, et al., 2005; LaBar, et al., 2001; Porubská, et al., 2006; Simmons, Martin, & Barsalou, 2005; St-Onge, Sy, Heymsfield, & Hirsch, 2005; G.-J. Wang, et al., 2004). These areas are also implicated in neural response to smell and taste (Angelo Del Parigi, et al., 2002; Jay A. Gottfried, O'Doherty, & Dolan, 2002; J. P. O'Doherty, 2007; Small, et al., 2007).

Central processing of food pictures using a block design of food and non-food pictures in healthy normal weighted individuals has been studied by Frank et al (Frank, et al., 2010). Areas of the brain active on viewing food pictures compared to non-food pictures showed greater activation in the insula and orbitofrontal cortex suggesting a temporo-insulo-opercular and orbitofrontal network involved in food processing. Stronger activation was found in food-specific areas like orbitofrontal cortex and insula for high-calorie food, whereas there was no significantly different BOLD activation in the amygdala, indicating that this may be an area specific for explicit evaluation of food pictures. In addition there was more occipital activation to high calorie foods as compared to

low calorie foods (Frank, et al., 2010). On analysing the fMRI images for effects of satiation (hungry and not hungry states were successfully manipulated as measured on hunger scores), no significant differences in the imaging data were observed. On analysis of gender effects on the brain activation, the superior medial frontal lobe (involved in reward – seeking behaviour and social cognition) showed higher activation in women when showed the high-calorie pictures when hungry compared to when not hungry. This suggested that women had a lower ability to suppress hunger (Frank, et al., 2010). Another study looking at fMRI brain activation in lean healthy individuals to food versus non-food pictures (Porubská, et al., 2006), demonstrated greater activity in the left orbitofrontal cortex and the insular/opercular cortex bilaterally with a stronger focus on the left side, on viewing food pictures. Further, the activity in the insular cortex on sides, the left operculum, and the right putamen were modulated by the subjective feeling of appetite concerning the food stimuli presented.

fMRI studies examining the modulating effects of calorie content, hunger and attention focus of food reward processes in the human brain reveals activation of a large network of left-sided brain regions, including the fusiform gyrus, ventral stratum, amygdala, bilateral insula/ frontal operculum, anterior cingulate cortex, premotor area, dorsolateral prefrontal cortex and medial orbitofrontal cortex (Siep, et al., 2009). Satiated healthy females show stronger activation in these reward processing areas on presentation of low-calorie foods whilst hungry healthy females show a stronger BOLD response in these areas when presented with high-calorie foods. Strong BOLD activity was observed in the amygdala and medial orbitofrontal cortex when participants evaluated the food pictures

compared to when they did not (Siep, et al., 2009). Del Parigi et al demonstrated that obese individuals responded to satiation with greater activation of the prefrontal cortex and greater deactivation of the limbic/paralimbic areas compared to lean individuals, suggesting obesity may be associated with abnormal neuronal activity in certain regions of the brain, some of which may have a role in the pathophysiology of the disease (A. Del Parigi, et al., 2002b). Another study designed to investigate differences between obese and lean subjects in their response to the sensory experience of a liquid meal in a state of extreme hunger and expectation of feeding, using PET and ^{15}O-water , showed significant activation in the sensory areas (middle-dorsal insular cortex), associative areas (temporal cortex), paralimbic areas (orbitofrontal and posterior cingulate cortices), and midbrain. Interestingly the insular response was proportional to the percentage of body fat and the degree of adiposity was the main determinant of this neuronal response (DelParigi, Chen, Salbe, Reiman, & Tataranni, 2005). This may be due to greater central sensitivity to the fat content and/or texture of the liquid formula meal. Or a greater anticipatory effect of the sensory stimulation in obese as compared to lean individuals (DelParigi, et al., 2005). Saelens et al. (Saelens & Epstein, 1996) report that food is more reinforcing in the obese than the lean, and this is reflected in the differences in functioning of the dopaminergic pathway between the lean and the obese (G.-J. Wang, et al., 2001). Cornier et al (Cornier, Von Kaenel, Bessesen, & Tregellas, 2007) studied the neuronal responses to visual food-related stimuli in thin individuals (BMI < 24Kg/m^2) 2 days post eucaloric intake and 2 days post 30% overfeeding, using fMRI. They demonstrated robust neuronal activation in the inferior temporal visual cortex, posterior parietal cortex, premotor cortex, and

hippocampus (consistent with visual processing and attention) and hypothalamus to foods of high hedonic value than to neutrally rated foods. Two days of overfeeding led to significant attenuation of these responses (Cornier, et al., 2007), suggesting interactions between the hedonic and the homeostatic systems of regulation of energy.

Thus these studies indicate that the brain activation patterns to food pictures vary relative to baseline metabolic state (fasting vs. fed) (Porubská, et al., 2006) and nutritional status (lean vs. obese) (A. Del Parigi, et al., 2002b). Feeding was associated with increased activity in the prefrontal cortices, mainly involved in inhibitory responses (A. Del Parigi, et al., 2002b) following visual food stimuli. On the other hand fasting was associated with activation in hedonic brain regions including ventral striatum, amygdala, insula and OFC in response to visual food stimuli (LaBar, et al., 2001).

1.14.7.2 Appetising vs. bland

Brain activation is greater in ventral stratum, OFC and amygdala when viewing appetizing compared with bland or disgusting foods which are correlated to individual differences in reward sensitivity as assessed by Behavioural Activation Scale. This implicates the fronto-striatal-amygdala-midbrain network in the neural responses to food cues in healthy participants (Beaver, et al., 2006). Other studies have shown greater activity in the insula, medial and dorsal prefrontal cortex when viewing high versus low calorie foods (Gordon, et al., 2000; W. D. S. Killgore, et al., 2003). Hinton et al showed greater activation in the amygdala and OFC when choosing highly preferred foods from a menu (Hinton, et al., 2004). On the other hand, low calorie foods (e.g. salads and

cereals) caused activation in the primary gustatory cortex, including bilateral superior and transverse temporal gyri, and inferior left hemisphere somatosensory cortex, as compared to non-food stimuli (W. D. S. Killgore, et al., 2003). Furthermore obese subjects are reported to have greater activation in the ventral striatum, amygdala, insula, and medial and lateral OFC when viewing high-calorie vs. low calorie foods, as compared with non-obese subjects (Stoeckel, et al., 2008). High calorie foods yield significant activation within the medial and dorsolateral prefrontal cortex, thalamus, hypothalamus, corpus callosum, and cerebellum. Low-calorie foods yield smaller regions of focal activation within medial orbitofrontal cortex; primary gustatory/somatosensory cortex; and superior, middle and medial temporal regions (W. D. S. Killgore, et al., 2003).

One such study looking at brain mechanisms involved in energy homeostasis and appetite regulation in humans using food cues to individuals attempting weight loss, showed significantly greater activation on viewing ‘fattening’ foods (as compared to non-fattening foods) in the brain regions involved in many different aspects of food intake regulation. These included the regions of the hypothalamus, caudate, putamen, nucleus accumbens, thalamus, midbrain, right insula, left amygdala, prefrontal cortex, and occipital lobe (Schur, et al., 2009a). Thus the authors concluded that neural circuitry primarily engaged in energy homeostasis (hypothalamus), satiety perception (hindbrain), reward processing (midbrain ventral tegmental area, ventral striatum) and cognitive control of behaviour (orbitofrontal cortex, prefrontal cortex) is selectively attuned to representations of foods perceived as fattening. Thus it is plausible that attention, motivation, and cognitive areas have developed to selectively attend to

environmental food cues in conjunction with a reward system that reinforces their ingestion (Schur, et al., 2009a). Neuroimaging has also enhanced our understanding of peoples' choices of the types of food consumed. fMRI studies on healthy normal-weight adult women as they viewed coloured food photographs has been studied by Killigore et al. The images presented were from three categories: high calorie foods, low calorie foods, and non-edible dining-related utensils. Both food categories were associated with bilateral activation of the amygdala and ventromedial prefrontal cortex. High calorie foods yielded significant activation within the medial and dorsolateral prefrontal cortex, thalamus, hypothalamus, corpus callosum, and cerebellum. Low calories foods yielded smaller regions of focal activation within medial orbitofrontal cortex, primary gustatory/somatosensory cortex; and superior, middle and medial temporal regions. They also suggested that the amygdala may be responsive to a general category of biologically relevant stimuli such as food, whereas separate ventromedial prefrontal systems may be activated depending on the perceived reward value or motivational salience of food stimuli (W. D. S. Killgore, et al., 2003).

These studies demonstrate that brain activation patterns differ in response to caloric value of food, with increased activation in hedonic regions of the brain in presence of calorie – rich food images (Schur, et al., 2009b).

1.14.7.3 Gender

Effects of fasting and gender on cerebral processing of visual and gustatory food-related stimuli have been studied using fMRI (Uher, et al., 2006). Uher et al reported significant gender differences in the processing of visual food- related

stimuli in modality-specific cortices and in the inferior occipito-temporal cortex, with stronger responses in women than men. Food vs. non- food showed greater activation in the right angular gyrus and in the left anterior insula (Uher, et al., 2006). Another FDG-PET study looking at gender differences in brain activation with food stimulation and voluntary control of hunger (visual, smell, taste)(G.-J. Wang, et al., 2009), revealed that cognitive inhibition of hunger, suppressed activation of various limbic areas (left amygdala, left hypothalamus, left OFC, left uncus, right stratum, right insula, anterior cingulate gyrus, parahippocampus, and cerebellum) in men. In women, there was no such decrease in activation of the reward areas of the brain even though they reported decrease in subjective feeling of hunger. Study of the responses of the brains of women to ingestion of a meal has revealed areas of the brain responsible for satiation (J. F. Gautier, et al., 2000). They showed that satiation, consisting of a liquid meal after 36 hours of fasting was associated with increased rCBF in prefrontal and occipital regions and decreased rCBF in limbic/paralimbic areas, basal ganglia, temporal cortex, and cerebellum. Moreover, obese individuals responded to satiation with greater activation of the prefrontal cortex and greater deactivation of some of the limbic/ paralimbic areas compared to lean subjects. In addition, there was satiation induced deactivation in the vicinity of the amygdala and nucleus accumbens in obese women (J. F. Gautier, et al., 2000). This suggests differential brain responses to satiation in lean and obese women.

Effects of gender on fMRI brain activation to hunger and satiation has also been studied by Del Parigi et al (Angelo Del Parigi, et al., 2002). They found extensive similarities in the brain responses to hunger and satiation between men and women with fMRI. In particular, their study indicated that hunger elicited

greater activation of brain regions mainly involved in processing of emotion in men (temporal lobe, posterior cingulate, parahippocampal gyrus, and dorsolateral prefrontal cortex) than women. On the other hand, satiation elicited more extensive activation of neocortical areas involved in sensorial association (occipital cortex) and behavioural planning (venterolateral prefrontal areas) in women than in men, suggesting retrieval of visual information to process the sensation of satiation (Angelo Del Parigi, et al., 2002).

These studies seem to suggest that brain responses to food stimuli tend to vary between the genders with greater activity in the reward areas of the brain following a meal in women than in men (G. J. Wang, et al., 2009), though this finding has not been consistent across other studies.

1.15 The feed-forward theory

Studies have shown that a rat that is satiated for glucose in solution, and will drink no more of it when access is prolonged, will promptly return to ingestion when offered laboratory chow, milk, or even glucose itself in powdered form. The resulting bout of ingestion, called ‘a second meal’ may lead to higher caloric intake as high as or higher than the initial ‘first meal’ of glucose solution, which leads to satiety for that solution (D. G. Mook, Dreifuss, & Keats, 1986). The authors concluded that the satiety for glucose solution reflects one post-ingestive mechanism or set of mechanisms. Then, access to chow or powder must feed forward to reset the necessary conditions for satiety, recruiting a new satiety mechanism with different properties. The feed-forward component appears necessary because this happens only when, and if, the chow or powder is actually encountered. If the second commodity is withheld (as in an 80-minutes session of

access to solution), intake remains inhibited over the second half of the session. Therefore, the authors suggested that the stimulus properties of the powder must feed forward as a “metabolic expectancy” or an “efference copy” (D. G. Mook, J.A.Brane and J. A. Whitt, 1983) to cancel or relax the original postingestive inhibition (D. G. Mook, et al., 1986).

Thus studies from the 1980’s were already showing that the homeostatic system was not a singular player in the field of energy balance but in fact seemed to be under a more powerful influence of a stronger controlling system that could supersede its signals and engage the animal to consume more food that necessary, i.e. the hedonic system.

1.16 Psychological basis for appetite control and feeding

Alongside physiologists, psychologists and neuroscientists have also contributed to the knowledge of the elaborate feeding systems and the drivers of appetite. Indeed, the psychological theories explaining feeding behaviour seem to be of paramount importance and relevant in today’s world and could potentially be a focus of therapeutic intervention in tackling the epidemic of obesity. Various theories have been proposed using different models to explain the brain mechanisms associated with energy regulation and appetite.

It is known that the relative abundance of food and its reward value often over-ride the physiological signals of hunger and satiety (Nestle, et al., 1998). On the other hand, the rewarding effects of food are modulated by the internal state such that a food that is pleasurable when one is hungry may be unpleasant after satiation (Tataranni & DelParigi, 2003). Thus there has been a progressive dysregulation of the reward networks on repeated exposure to palatable foods

over several years. For human and even rodents, metabolic need (negative energy balance) is not the only motivation to consume food. Other motivations to feed can be traced to the rewarding features of simultaneously available attractive foods, temporal factors (time of day, season), emotion and cognition (learning, memory and social cues). Collectively, these factors influence “non-homeostatic” food intake (Grill, Skibicka, & Hayes, 2007).

The psychopharmacology of food reward comprises at least two phases: anticipation and consumption (K. C. Berridge, 1996). Anticipation is usually elicited by the presentation of a sensory cue, which reliably signals the forthcoming delivery of a rewarding stimulus. Using fMRI, O’Doherty et al measured the brain activity in subjects exposed to one of three arbitrary visual stimuli, each of which reliably predicted the subsequent delivery of a moderately pleasant sweet taste, a moderately unpleasant salty taste, or a neutral control solution. In keeping with the central role of reward, dopaminergic areas, such as the midbrain, amygdala, and ventral stratum (nucleus accumbens), showed higher activity in anticipation vs. receipt of actual reward (John P. O'Doherty, et al., 2002). Hunger has been shown to increase the response to the sight of food in some of these limbic/paralimbic areas (LaBar, et al., 2001) and the sight of food increased regional blood flow in the right parietal and temporal cortex in obese but not normal weight women (Karhunen, Lappalainen, Vanninen, Kuikka, & Uusitupa, 1997).

1.16.1 Liking vs. wanting

It is widely acknowledged that an important stimulus for eating is not hunger but the anticipated pleasure of eating determined by the sensory qualities of palatable foods (Pinel, Assanand, & Lehman, 2000; Woods, et al., 2000). It has been said that humans have evolved to eat more than is required to met their immediate nutritional needs (Pinel, et al., 2000). Whereas this behaviour was once adaptive in an environment characterized by food scarcity, it became maladaptive in the modern environment where a variety of foods with highly rewarding value are almost always available. Indeed, the human eating system did not evolve to cope with the continuous exposure to highly tempting foods such as French fries, hamburgers or chocolate cookies (Pinel, et al., 2000). The maladaptive response of overeating under conditions of abundance is often explained in terms of difficulties to resist the temptation of the immediately rewarding value of palatable foods. However, from an evolutionary perspective it seems more likely that humans (and other animals) would be able to attribute proper values to delayed rewards when it enhances the chance of maximizing their long-term gain. That is, they would be able to exert self-control and deny immediate rewards in the interest of delayed by bigger rewards. The view that self-control may have played an important role in human evolution of (eating) behaviour seems at odds with the dominant evolutionary explanation that immediate pleasure from food drives decisions about food intake (Ruud van den Bos & de Ridder, 2006).

Two essential components of the reward system that regulates eating behaviour are the hedonic experience of eating and appetitive behaviour involved in attempts to obtain food (A. E. Kelley, Baldo, Pratt, & Will, 2005). The hedonic

experience or sensory pleasure of eating is determined by the palatability of foods as been labelled as 'liking' (K. C. Berridge, 1996; K. C. Berridge & Robinson, 2003). 'Liking', under control of opioids, deals with the immediate appraisal of food items and has been shown to be active when subjects (rats) are more or less sated (M. F. Barbano & Cador, 2005). When hungry or (not sated) it appears that the specific qualities of food items are of less relevance than the mere consummation of food items per se, leading to the ingestion of large quantities of food regardless of their hedonic quality (A. E. Kelley, et al., 2005). Opioid induced overeating in sated subjects, especially of highly palatable and caloric food, serves to increase fat stores that promote survival under conditions of future famine. It may thus be hypothesized that the role of opioid-mediated 'liking' and consummation of foods becomes stronger when basic nutritional needs are met (M. F. Barbano & Cador, 2005; H.-R. Berthoud, 2004; A. E. Kelley, et al., 2005).

The second component of the reward system relates to the degree to which anticipated pleasure is translated into action (Salamone & Correa, 2002; Spruijt, van den Bos, & Pijlman, 2001; R. van den Bos & Cools, 2003; R. van den Bos, Houx, & Spruijt, 2002). Animal studies of 'wanting' behaviour have shown that rats showed higher levels of anticipatory activity prior to the arrival of food when hungry than when they are sated- thus demonstrating the opposite pattern from 'liking' behaviour, which proved to be higher in sated subjects (M. Barbano & Cador, 2007). Until now, evolutionary accounts of failure to regulate eating in the midst of plenty have focussed on the 'liking' part of the reward-system, emphasizing the pleasure of eating as a powerful mechanism to protect from future famine (Mela, 2006; Pinel, et al., 2000). Yet the 'wanting' part of the

reward system seems more sensitive to environmental variability as it directly relates to the amount of effort individuals are willing to (or must) spend to obtain food and is therefore a more promising candidate to explain maladaptive overconsumption from an evolutionary perspective - especially as this relates to an imbalance between the reward system and self-control (Ruud van den Bos & de Ridder, 2006).The rewarding properties of modern food-items may be so powerful that 'wanting' is extremely activated and thus overrides self-control. Exposing humans to an environment with a large variety of foods which differ in rewarding value makes them extremely vulnerable to the strongly rewarding food items that are immediately available (Ruud van den Bos & de Ridder, 2006).

It has also been argued that self-control plays an important role in the decisions involving food intake. Self-control is often referred to as the choice of a more-delayed outcome that is ultimately of more value over a less-delayed outcome that is ultimately of less value (Ainslie, 1974; Logue, 1988). The somatic marker hypothesis proposes that decision making is a process that is influenced by marker signals that arise in bioregulatory processes, including those that express themselves in emotions and feelings (Bechara, Damasio, & Damasio, 2000; Damasio, Everitt, & Bishop, 1996). This influence can occur at multiple levels of operation, some of which occur consciously and some of which occur non-consciously. These processes involve various cortical (e.g. OFC) and subcortical components (e.g. amygdala). Using the Iowa Gambling Task (IGT), Van den Bos et al have shown that enhancing the rewarding properties of some items relative to others increases the chance that individuals cannot suppress responding to them, even though they know that this choice will turn out disastrous in the long run. Thus they argue that exposing healthy humans to an environment with a

large variety of foods that differ in rewarding value make them extremely vulnerable to the strongly rewarding food items that are immediately available (Ruud van den Bos & de Ridder, 2006). Sensory- specific satiety is thought to modulate food consumption in rodents (Kent C. Berridge, 1991). In humans, the only study aimed at assessing the change in brain activity associated with transition from a state of high motivation to eat food (chocolate) to a state of aversion revealed decreases in neuronal activity in the primary gustatory cortex, striatum, midbrain, subcallosal region and caudomedial orbitofrontal cortex and prefrontal cortex, thus providing circumstantial evidence for an additional role of brain dopaminergic pathways in the regulation of food intake (Tataranni & DelParigi, 2003).

Thus a dynamic interaction between the reward system and the self-control systems seems a more plausible explanation of the way decisions are made about food intake under natural, thus uncertain conditions. As abundance of food in our society is a reality, it may be the omnipresence of highly rewarding palatable foods that is an important factor in compromising a balanced decision about the trade-off between short-term gains (acquisition of highly palatable foods) and long-term gains (health benefits) (Ruud van den Bos & de Ridder, 2006). Lowe et al have studied the fMRI responses to food images in dieting versus non-dieting people. The dieters were labelled as ‘restrained eaters’ and the non-dieters were labelled as ‘Unrestrained Eaters (UREs)’. They demonstrated that ‘Restrained Eaters’ engage in counter-regulatory eating stemming from eating less than wanted rather than less than needed. They studied brain activation of normal weight restrained eaters (REs) and unrestrained eaters (UREs) in fasted and fed state and when viewing pictures of highly and moderately palatable

foods. 'Unrestrained Eaters' showed bilateral widespread activation to high palatability food stimuli when fasted, in areas associated with hunger, memory and motivation. In the same state restricted eaters showed virtually no activation. On the other hand, while viewing high palatability foods in fed state, REs showed activation in areas associated with goal-oriented planning, expectation of reward and goal-defined behaviour (left orbito-frontal cortex and dorsolateral prefrontal cortex), and food craving and desire (left insular cortex). Whereas activation for UREs under the fed state was found in areas related to satiation and memory. Thus they concluded that, when food deprived, REs ate relatively little, not because they were dieting, but because they were less motivated to eat and consumption of a filling meal by REs paradoxically increased the reward value of palatable food, thus creating a state of hedonic hunger (M R Lowe, 2008).

It has been suggested that in disorder of eating or appetite, such as in anorexia nervosa and in obesity, subjects are unable to recognise internal eating cues (such as epigastric empty hollow sensations, EHS) and that subjective cues are significant in homeostatic eating. Thus recognition of these internal cues along with low mental arousal (feeling of pleasure) should result in better regulation of energy intake (Lovell-Smith, Ciampolini, & Borselli, 2008).

1.16.2 Valence vs. Salience

Previous experiences influence eating behaviour/pattern. Emotional states differ in many ways, but one of the most fundamental is valence or how positive or negative an emotion feels (J. A. Russell, 1980). Distinguishing between positive and negative has fundamental implications for both subjective experience and

behaviour. Positive emotions are linked to approach and negative emotions are linked to avoidance (Cooper & Knutson, 2008). Increased activation of nucleus accumbens has been demonstrated with increasing magnitude of anticipated rewards, (Breiter, Aharon, Kahneman, Dale, & Shizgal, 2001; Knutson, et al., 2001), and increasing self-reported positive arousal (Drevets, et al., 2001), and decreasing NAcc activation with painful stimulation or increasing potential losses (Becerra, Iadarola, & Borsook, 2004; Tom, Fox, Trepel, & Poldrack, 2007). This valence account is thought to make the key prediction that anticipatory NAcc activation will correlate with positive emotional experience and so will predict approach behaviour. On the other hand, other researchers have shown that the salience of an incentive cue and not its valence drives NAcc activation and thus NAcc activation does not necessarily distinguish positive from negative emotional experience.

Salience has been defined behaviourally as one that increases the chance an organism will need to make an important behavioural response in near future. Crucially this response might involve either an approach or withdrawal, with cues posing danger or a need to escape will hold as much (if not more) salience as cues predicting reward (Cooper & Knutson, 2008). Thus according to this definition, the NAcc promotes attention towards important or unexpected events rather than promotes approach behaviour (K. C. Berridge & Robinson, 1998; Redgrave, Prescott, & Gurney, 1999). Cue salience might increase with absolute incentive magnitude (good or bad), incentive uncertainty or the contingency of the response (i.e. how important an organism's response is to the outcome.). NAcc activation increases with behavioural demands or interference (Tricomi, Delgado, & Fiez, 2004; Zink, Pagnoni, Chappelow, Martin-Skurski, & Berns,

2006; Zink, Pagnoni, Martin-Skurski, Chappelow, & Berns, 2004) in response to novel non-rewarded events (Zink, Pagnoni, Martin, Dhamala, & Berns, 2003) or in anticipation of painful stimulus (K. C. Berridge & Robinson, 1998; Jensen, et al., 2003). Existing studies have yielded conflicting evidence as to whether all salient outcomes increase NAcc activation (John P. O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003; Pagnoni, Zink, Montague, & Berns, 2002). Valence can be negative or positive and salience can vary between high to low. Further studies have suggested a two-component account to describe NAcc activation. Cooper et al (Cooper & Knutson, 2008) have shown that valence and salience each partially account for NAcc activation during incentive processing, but neither provides a complete account.

1.17 Model for interaction between sensory and hedonic systems

A possible model has been proposed for the interaction between sensory and hedonic systems in the human brain using as an example one hemisphere of the orbitofrontal cortex. This is illustrated in the following figure (Figure 1.6) Information is shown as flowing from the bottom to the top of the figure.

Sensory information about primary (e.g., taste and smell) and secondary (e.g., visual) reinforcers is carried from the periphery to the primary sensory cortices (e.g., anterior insula/frontal operculum for taste and pyriform cortex for smell), where stimulus identity is decoded into stable representations (Small, et al., 1999; Zatorre, et al., 1992). This information is then conveyed for further multimodal integration in brain structures such as posterior parts of the orbito-frontal cortex (I. E. de Araujo, et al., 2003a; Small, et al., 1997). The reward value of the reinforcer is assigned (e.g., in more anterior parts of the orbito-

frontal cortex) (J. A. Gottfried, O'Doherty, & Dolan, 2003; Small, et al., 2003) from where it can be used for influencing subsequent behaviour (e.g., lateral parts of the anterior orbito-frontal cortex, anterior cingulate cortex (Kringelbach, et al., 2003) and dorsolateral prefrontal cortex (Kringelbach, et al., 2004c; Wallis & Miller, 2003), monitored as a part of learning and memory mechanisms (e.g., in medial parts of the anterior orbitofrontal cortex) (I. E. T. de Araujo, et al., 2003b; J. A. Gottfried, et al., 2003) and made available for subjective hedonic experience (e.g., mid-anterior orbitofrontal cortex) (Kringelbach, et al., 2003). The reward value and thus also the subjective hedonic experience of a reinforcer, can be modulated by hunger and other internal states (J. A. Gottfried, et al., 2003; Kringelbach, et al., 2003; J. O'Doherty, et al., 2000), while identity representation is remarkably stable and not subject to modulation (I. E. T. de Araujo, et al., 2003b; Edmund T. Rolls, Kringelbach, & De Araujo, 2003).

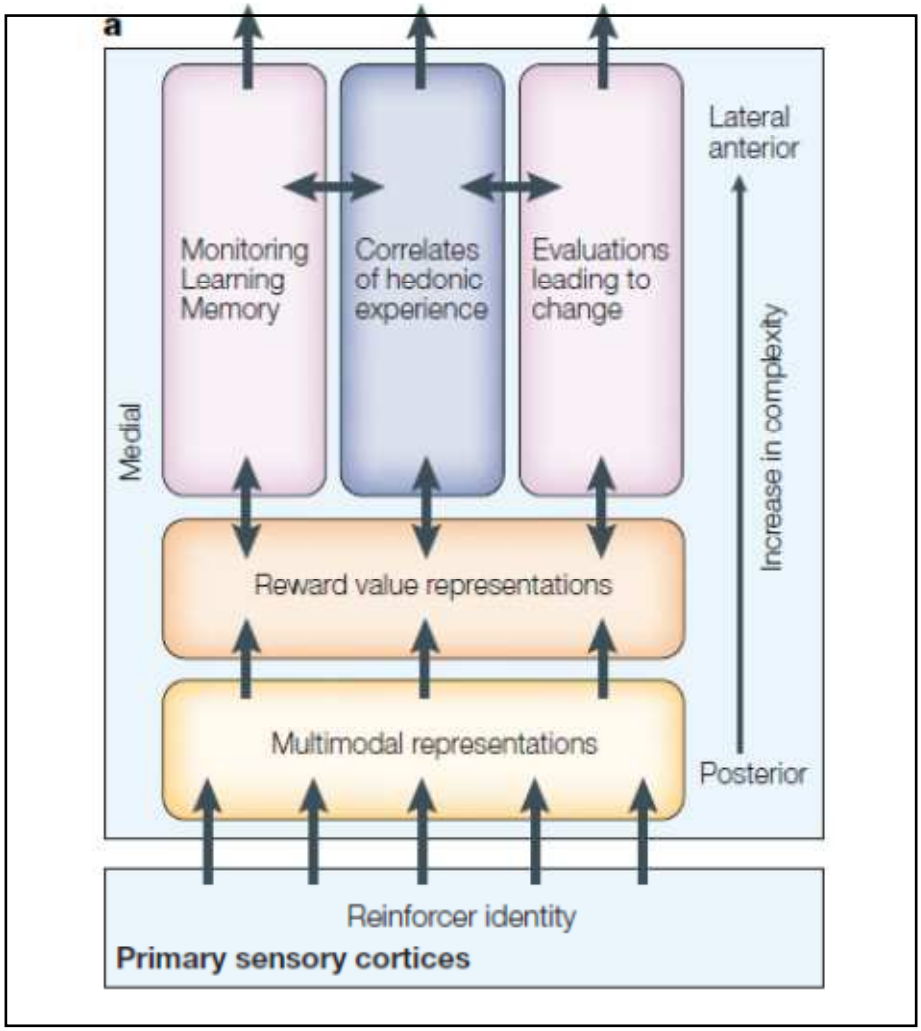

Figure 1.6 Model for interaction between hedonic & sensory systems (H.-R. Berthoud, 2007)

1.18 Conclusion

Food intake is essential to sustain life, and the sensory systems of taste and smell are amongst the most fundamental building blocks of the brain's natural reward systems (Ann E. Kelley & Berridge, 2002). It has been proposed that humans' higher cognitive functions may have evolved to support the required cognitive processing involved in the sophisticated foraging necessary for the sustained food intake needed for omnivores such as humans (Kringelbach & Rolls, 2004a). Thus the major players in the control of feeding and energy intake include

several areas of the neocortex (hippocampus and amygdala), striatum (nucleus accumbens, ventral pallidum), hypothalamus (lateral, perifornical) and ventral midbrain (ventral tegmental area). These are very different from the limited neural substrate comprised by the mediobasal, paraventricular and perhaps lateral hypothalamus and the caudal brainstem typically held responsible for the homeostatic regulation of energy balance (H.-R. Berthoud, 2007). Though homeostatic mechanisms are traditionally known to initiate and end feeding, hedonic eating can override homeostatic cues to eating. Various factors influence feeding behaviour, including nutritive state (LaBar, et al., 2001), nature of food (W. D. S. Killgore, et al., 2003) and presence of circulatory peptides such as leptin (Farooqi, et al., 2007). In addition, pleasure can guide the initiation and cessation of feeding (A. E. Kelley, et al., 2005).

Various neuropepetides (PYY $_{3\text{-}39}$, leptin) have been shown to influence activity in the various limbic structures (Batterham, ffytche, et al., 2007; Farooqi, et al., 2007) of the brain. This work entertained the hypothesis that glucose a chief source of energy to the brain, could also possibly affect the limbic structures in the brain to influence appetitive behaviour. The current study initially looked at brain activation patterns in relation to performance of cognitive tasks under influence of oral glucose. Thus the study design was well-placed to extend the area of interest of the experiment.

This aspect of the research was set out to look at differences in the brain activation patterns on fMRI to food images, under influence of oral glucose. The hypothesis was to observe greater activation in the limbic regions of the brain involved with hunger (insula, parietal and temporal cortices) in the fasted state whilst activation in these regions would be dampened following 25 g glucose on

viewing food images, similar to leptin (Farooqi, et al., 2007). Further, if this was true, differences in activation would be found on observing appetising (amygdala and OFC) as compared to bland foods, as demonstrated by Killgore at al. (W. D. S. Killgore, et al., 2003).

1.19 Main aims of the research project:

1. To examine the effects of changes in blood glucose on memory function and upon accompanying patterns of brain activity (Study 1).
2. To identify memory differences between non-diabetic subjects (Study 2), and patients with type 1 diabetes (T1DM) with and without exposure to severe hypoglycemia (Study 3) characterized in terms of performance deficits and changes in brain activation.
3. To examine whether differences occurring as a consequence of exposure to severe hypoglycemia in T1DM are reversible.
4. To examine also fMRI activation of glucose-sensing areas of brain at different glucose nadirs in T1DM patients with and without hypoglycemia unawareness

Prior to embarking on complex clamp studies in people with diabetes, the cognitive performances and brain activation patterns of a small dose of oral glucose were studied in non-diabetic healthy individuals (Study 1). This study would lead up to exploration of the effects of altered glucose levels on memory performance in diabetic subjects.

Specific aims and hypotheses for Study1:

1. Cognitive performance on long-term verbal memory tasks would be enhanced following 25 g of oral glucose (vs. sweetener).

2. This difference in cognitive performance under different drink conditions would be mirrored by differences in brain activation patterns.

In addition, contemporary work by Farooqi et al (Farooqi, et al., 2007) showing effects of leptin on the nucleus accumbens and appetite regulation, prompted the study of glucose load on a further cognitive domain, namely brain activation patterns in response to images of foods.

3. Additional hypothesis was to see increased brain activation in limbic regions of the brain on viewing food pictures following ingestion of the control drink, aspartame. This state would be perceived as the fasted state and would thus activate the hedonic circuitry on presentation of food (images).
4. Activation in the limbic regions would be dampened following 25 g of oral glucose.

The first part of the volume of this thesis is made up of the study of the cognitive performances and the brain activation patterns of a small dose of oral glucose in healthy volunteers. The second part describes the study of brain activation patterns on viewing food imagery under influence of glucose and sweetener.

Chapter 2: Methods and Materials

In this chapter, the general methods and the materials used in the study are described.

2.1 Recruitment of volunteers

Thirteen healthy, right handed, volunteers, aged 18 to 60 years were recruited by advertising in the local 'Cambridge Graduate Union' newsletter and by posting flyers at place of work (in accordance with the local ethics). Assuming a coefficient of variation (CV) of 25% and glucose mediated difference in recall of 25% (Sunram-Lea, et al., 2002) then n=12 gave 90% power to detect similar difference with alpha of 0.05.

Those with previous history of diabetes, major neurological illness (epilepsy, previous seizures) or psychiatric illness (including alcohol and drug dependence, major depression) likely to affect the interpretation of the fMRI scans or performance of the cognitive tasks, were excluded from the study. Volunteers were screened for substance abuse, smoking and presence of any other contraindications for magnetic resonance imaging (MRI). Subjects were also screened for cardiovascular risk factors and for presence of any major organ dysfunction. Female subjects were screened for pregnancy (last menstrual period (LMP) and with commercially available urine pregnancy test kit (Axis-Shield Pregnancy Test ® UK, AC-FHC-U101, sensitivity 10 IU/ml). They were weighed, had their height measured and their BMI was calculated. Only volunteers with healthy BMI (</= 25 kg/m^2) were recruited. The protocol was approved by the Local Research and Ethics Committee (LREC) of Addenbrooke's Hospital, Cambridge, UK. Each volunteer gave written informed consent for the studies and further consent and screening for MRI safety was performed

independently by the Neuroimaging Department (Wolfson Brain Imaging Centre, WBIC). They arrived at the Imaging Centre, on the morning of the test day. Subjects requiring visual refractive error correction were offered MRI safe lenses.

2.2 Experimental procedure

Thirteen healthy non-obese right handed subjects (9 males 4 females, BMI 23.7+/-1.07, age 26.8 $\pm$ 1.1 years) were studied on two occasions at least 1 week apart. Studies were approved in advance by a local research ethics board and volunteers gave fully informed consent to participate. Subjects fasted from 10 PM the night prior to studying with studies being performed between 9 and 11 am the following morning. Studies were performed in random order with subjects blinded as to study order. For this study, volunteers had a single intravenous catheter inserted retrograde in the left antecubital fossa, following anaesthetisation of the skin by injection of 1% lignocaine (local anaesthetic) intradermally. The left arm was chosen, so that the right arm could be free for using the button box inside the scanner for recording responses to cognitive functions. 0.9% normal saline solution (0.9%) was infused to keep the vein patent throughout the experiment. During scanning blood samples were collected every 5 minutes, passed out from the scanning room through a dividing wall and analysed in the adjacent room.

Following cannulation and during the 45 minute acclimatisation period, subjects practiced the cognitive tasks once, on a laptop, in the preparation room. Immediately prior to entering the scanner, subjects consumed over the period of a minute a drink containing 200 millilitres of water along with 25 g of glucose on one study day and on the control day 425mg (5 tablets) of aspartame (“Canderel”, Merisant, High Wycombe, UK). The dose of aspartame was selected to match for sweetness and

both drinks were further disguised with lemon juice (Jif lemon juice, Unilever Foodsolutions, UK, 3 squirts per drink). Oral glucose was preferred to intravenous glucose to keep the overall study design simple when glucose clamp studies were performed (Appendix 1).

During scan acquisition, subjects viewed images projected on the computer screen (housed in the ante room) via a mirror positioned over the head. Subjects underwent a battery of cognitive tests lasting for 1 hour designed to examine memory performance. During scanning, samples were collected every 5 minutes after ingestion of drink up until the end of the scanning period (60 minutes). Blood samples were drawn from the cannula by an investigator inside the scanning room and passed outside through a hole in the dividing wall, for analysis of plasma glucose. Additional plasma samples were collected at 15 minute intervals for measurement of insulin and glucagon. Importantly, subjects were not disturbed or re-cannulated if there were problems with the sampling catheter, so a complete sampling profile was possible only in eight out of thirteen volunteers on the glucose day and seven out of thirteen volunteers on the sweetener day. Subjects were blind to their glucose levels at all times.

At the end of the experiment, the cannula was withdrawn, the subjects were given a meal and they then left the Imaging Centre. The same volunteers then returned for the second part of the study after an interval of at least a week and underwent a similar protocol (with a different drink). Subjects received financial reimbursement for their participation.

2.3 Dose of glucose and sweetness testing

2.3.1 Dose of glucose

Studies done by Sunram-Lea et al (Sunram-Lea, et al., 2002) showed that ingestion of 25 g of oral glucose load (in comparison to a sweetener like Aspartame) in healthy subjects resulted in a rise in blood glucose (T0 baseline, t25 and t45 are 25 and 45 minutes respectively) and an improvement in recall performance up to 24 hours later (immediate recall averaged over 5 trails (IFR), delayed recall within 45 minutes of ingestion (DFR) and 24 hours later (DFR). In this the blood glucoses were significantly high at 25 minutes post ingestion of glucose drink. This is illustrated in the following figure (Fig. 2.1) adapted from the paper.

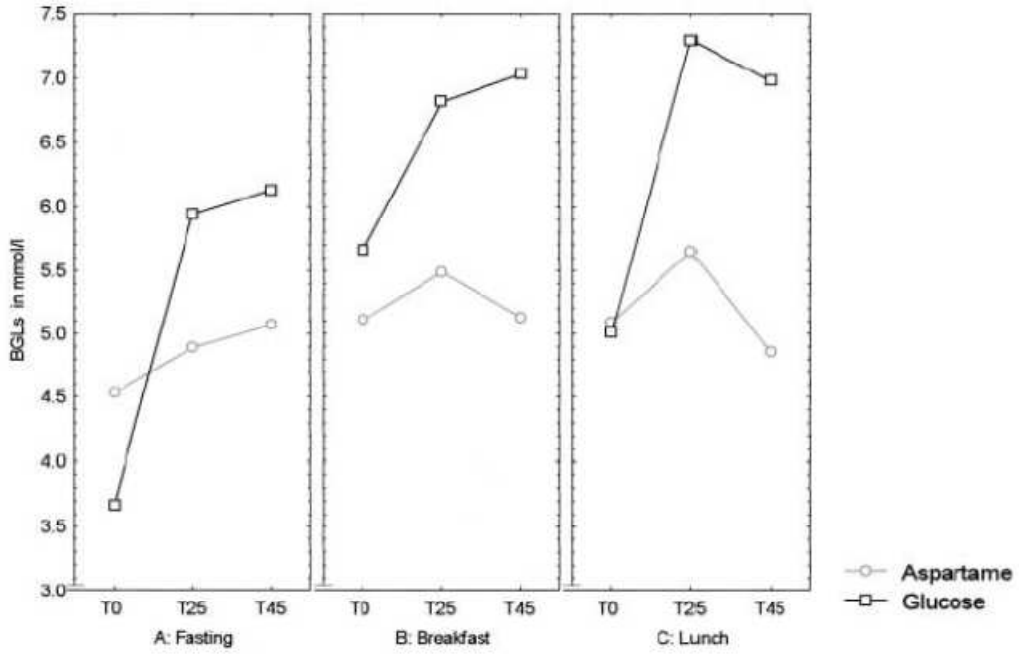

Figure 2.1 Graph illustrating glycemic response post 25 g glucose

(Sunram-Lea, et al., 2002)

Based on this preliminary data, cognitive performances and related anatomical brain activation in healthy non-diabetic volunteers was tested following 25 g of oral glucose load.

2.3.2 Sweetness Testing

Critical to the study was matching drinks for taste and sweetness. In advance of the fMRI studies, double-blinded sweetness tasting was performed by collaborators in Lancaster in a group of 7 healthy volunteers (3 females) using 2 tests (Sünram Lea et al., unpublished data). Given the critical importance of this information for this study, the experiment has been briefly described below.

Aspartame was favoured as a placebo as it has a sweet taste without the bitter chemical or metallic aftertaste reported in other artificial sweeteners. Aspartame is an artificial sweetener that is 180 times sweeter than sugar in typical concentrations, without the high energy value of sugar. It has a caloric value similar to sugar (4 kcal/g), but the amounts used are small enough to consider aspartame essentially free of calories. Analogous to previous research the drinks were also flavoured with lemon juice to improve palatability (Foster *et al.,* 1998, Sünram-Lea *et al.,* 2001; 2002a; 2002b; 2004 & Green et al., 2001)

Seven independent judges (4 males, 3 females) were provided with six 300ml drinks (labelled 1-6) containing five dosages of Canderel aspartame tablets and one drink containing 25g of dextrose glucose. Tasting was carried out with uniform containers (disposable plastic cups) to ensure that participants will not be influenced by colour or other characteristics of the receptacle. The ratings were carried out in a laboratory setting and drinks were refrigerated for 30 minutes and then taken out of the fridge 10 minutes before the experiment. The experimenter and those participating in the sweetness matching test were blind to the drink contents. All drinks were flavoured with lemon juice. Participants were first asked to taste and experimental liquid and rate all drinks for how sweet they were on a five point scale (1 being the least sweet and 5 being the most sweet) and then match it to another liquid of perceived equivalent sweetness The Canderel dosage which was rated as most similar in

sweetness to the solution containing 25g of glucose was 5 aspartame tablets (Table 1).

Table 1 Sweetness Rating of Glucose and Aspartame Flavoured Drinks.

Drink	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5	Judge 6	Judge 7	Total
Glucose 25g	3	4	3	5	3	3	3	24
Aspartame 3 tablets	2	1	3	2	1	2	3	14
Aspartame 5 tablets	3	4	3	5	4	3	3	25
Aspartame 7 tablets	5	3	5	4	4	5	5	31
Aspartame 9 tablets	5	5		4	5	5	4	33
Aspartame 11 tablets	4	4	5	5	5	5	4	32

Looking at the sweetness rating, the mean sweetness rating for the glucose drink was 3.4 $\pm$ 0.3 with aspartame sweetness scores for the doses detailed above being 2 $\pm$ 4 (3 tablets), 3.6 $\pm$ 0.3 (5 tablets), 4.4 $\pm$ 0.3 (7 tablets), 4.7 $\pm$ 0.2 (9 tablets) and 4.6 $\pm$ 0.2 (11 tablets). In summary, the aspartame dose which was rated as most similar in sweetness to 25g of glucose was 5 Canderel tablets, a result in keeping with manufacturers' report for relative sweetness.

Next, using a double-blind procedure subjects were asked to compare glucose and 5 Canderal tablets to test if they could identify the two drinks. Participants were asked to swill their mouths with water and were then presented with two new drinks (labelled A or B) one containing 25g glucose the other containing 5 aspartame tablets. The participants were told that one drink contained glucose and the other contained an artificial sweetener. Participants were then asked to taste both drinks and identify which drink contained glucose. They were given a sheet of paper on which to make their response by circling one of three responses; Drink A, Drink B or Don't Know. Three participants circled a 'Don't Know' response, of the remaining four participants two correctly identified the glucose drink and 2 incorrectly believed that the aspartame drink was glucose. Therefore 43% of the sample admitted that they could not tell the difference between the two drinks and the remaining participants identified the drink at chance levels.

Taken together, these data show that a drink containing 5 Canderal tablets was equivalent in sweetness to a drink with 25 g glucose. In the subsequent fMRI studies, we therefore used this Canderal dose as the appropriate control drink.

2.4 Sample handling

All blood samples were immediately spun with Eppendorf Microfuge® centrifuge at 1300 revolutions per minute for 30 seconds for glucose and 1 minute for hormone samples, at room temperature. Plasma was separated and either immediately analysed for glucose or stored on dry ice (for ~ 3 hours) for later hormone measurements. These samples were subsequently stored in -20°C freezer for insulin and glucagon measurements.

2.4.1 Biochemical analyses

Bedside plasma glucose was measured using the Analox Glucose Analyser (Analox Instruments® GM9, Glucose oxidase method). The quality control (QC) was carried out by using the quality control (QC) solution provided by the Analox® Company. Insulin was assayed in singleton on a 1235 AutoDELFIA® automatic immunoassay analyser using a two-step time resolved fluorometric assay (Kit No. B080-101). All reagents, standards and consumables were those recommended and supplied by the manufacturer. Glucagon was measured by Meso Scale Discovery kit® (Gaithersburg, MD, USA), a two-site microtitre plate-based immunoassay with electrochemical luminescence detection.

2.5 Cognitive tasks

A set of cognitive function tests, comprising encoding and retrieval, continuous performance tasks (CPT) and working memory tasks (WM) was administered on each study day. Encoding and retrieval have been shown to glucose sensitive by other researchers (Sunram-Lea, et al., 2002). The WM and CPT tasks are widely used tasks engaging critical cognitive functions (attention and working memory). They were included to ensure that any changes observed in the key tasks were not a simple general attentional or working memory effect. These tasks weren't included because of any anticipation that they would be sensitive to glucose.

All tasks were displayed on a computer screen and responses were recorded using a button box. During each set of tasks, performance was measured and brain activation was determined using fMRI. The subjects practiced the tasks only once on a laptop prior to entering the magnetic resonance (MR) scanner and before drinking a sweetened solution. Thus sequence of tasks were, initial practice, followed by the test

drink and move into the scanner, then performance of the encoding task, followed by continuous performance testing (CPT), working memory (WM) and finally the picture (food/non-food) task. Following this the subjects came out of the scanner and were then tested for retrieval on a laptop. The task order was kept constant on all study days. The instructions given to the subjects prior to administering the tasks can be viewed in *Appendix 3*.

2.5.1 Declarative long-term memory

The declarative long-term memory task (or verbal long term memory) consisting of encoding and retrieval (measurable and consolidation and storage (non-measureable) has been repeatedly shown to be sensitive to glucose potentiation effects in various studies (Foster, 1998; Sunram-Lea, et al., 2002).

Numerous studies have examined declarative LTM and fMRI activation using a number of different cognitive tests. Locally, Fletcher's laboratory examined LTM in healthy subject using a list of nouns with two levels of encoding (deep and shallow) combined with fMRI scanning. The cognitive task was associated with specific fMRI changes which was different at the two depths of coding of the test. There was also a correlation between performance on the subsequent recall test and brain activation (Paul C. Fletcher, et al., 2003).

For this study, the encoding task was designed to include a shallow encoding task and a deep encoding task to tease out any differences in performance and imaging characteristics on the two occasions. For this task, participants were visually presented with a list of words on the computer screen and performed the two encoding tasks as previously described (Paul C. Fletcher, et al., 2003). However, due to technical and computational setback, the encoding data could not be analysed and is hence not reported here.

2.5.1.1 Deep encoding task

For this task the word was accompanied by an instruction to record whether the word was thought as pleasant/ not pleasant on the basis of any previous associations the word might have had in the person's lifetime (any pleasant or unpleasant connotations to the word). Participants were instructed to press one of the two buttons on a conveniently located keypad to indicate their choice (yes or no).

2.5.1.2 Shallow encoding task

The word was accompanied by an instruction to pay attention to whether there were an odd or even number of syllables in the word (e.g. STREAM odd?). A keypad response indicated their yes/ no decision.

For both tasks the chosen button for yes or no was held constant within participants. A total of 144 nouns were presented in a pseudo- randomised order, 72 for each of these two tasks.

After scanning, (approximately 60 minutes after initial list presentation/ encoding) participants were presented with a recognition memory task (Gardiner, 1988) of 200 words (2 seconds appearance with interstimulus interval of 1.5 seconds) including the 144 words that they had seen during scanning and instructed to indicate, by pressing one of the 3 buttons, whether it was (i) remembered from the study list and asked whether the word had pleasant connotations or not (pressing 'B' button on keyboard with right hand), (ii) remembered from the list and asked if the word had even/odd syllables (pressing 'N' button on the keyboard with right hand) (iii) do not remember seeing the word at all (pressing 'Z' button on the keyboard with left hand). The participants were not informed of this task at the time of performance of the encoding task.

2.5.2 Continuous performance task (CPT)

The continuous performance task is a widely used neuropsychological task for assessing vigilance and sustained attention (Nestor, et al., 1991; Nuechterlein, 1991; Rosvold, 1956). It also enables us to evaluate subject's reaction times. Existing literature does not suggest this task to be glucose-sensitive. It was administered to ensure that any changes observed in the key tasks (encoding and retrieval) were not a simple attentional effect.

This task has been shown to produce robust fMRI activation (Weintraub, 1985). In our Centre, Fletcher's laboratory has tested this task widely in healthy controls, several patient groups and in pharmacological studies, finding reliable activation in prefrontal, cingulate and sub-cortical regions.

The task consisted of a combination of numbers with either clear or poor imagery. Subjects were required to monitor the computer screen upon which numbers flashed up at a rate of roughly one per second. They were instructed to respond with a key press, as rapidly as possible, every time they saw a '0' and to withhold responses to all other digits. The tasks was run at two levels of difficulty, one in which stimuli were clear and easily visible and one in which they were degraded and a greater level of visual attention was required to pick out targets. Performance was measured in terms of ability to discriminate targets from non-targets and the reaction time when responding to a target. Brain responses on fMRI, in terms of the main effects of performing the task compared to a resting baseline and in terms of the impact of stimulus degradation were measured. This task is illustrated in the figure below (Fig. 2.2).

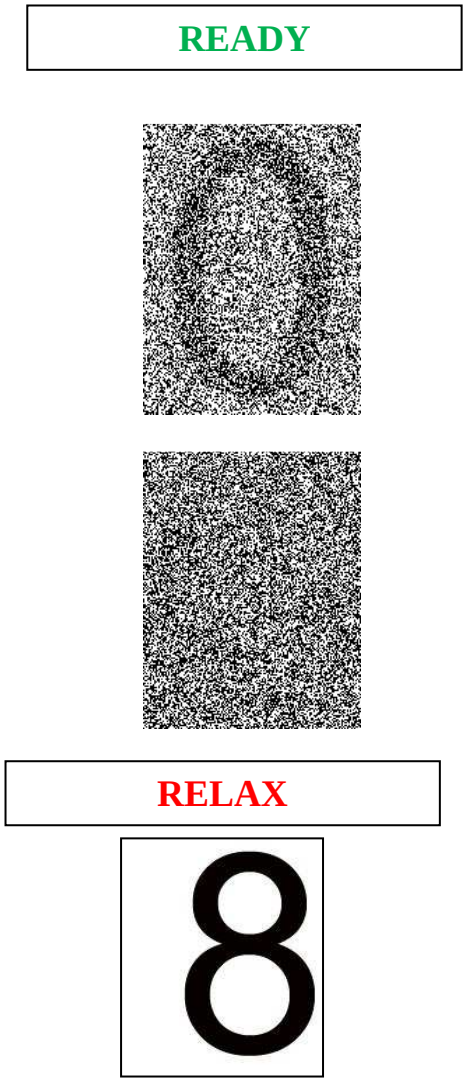

Figure 2.2: Continuous Performance Task

2.5.3 Working memory (WM) task

The working memory is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and problem solving (E. E. Smith & Jonides, 1998). This task has not been convincingly shown to be sensitive to glucose induced facilitation. It was administered to enable correct interpretation of data obtained during performance or other tasks. I was also interested in studying the brain activation patterns during performance of this task.

Fletcher and colleagues have recently used this working memory task to study brain activation patterns. They examined the effects of ketamine, confirming that the test produced robust fronto-parietal activation and that changes in performance on the cognitive test (with ketamine) were associated with changes in this pattern of fMRI activation (R. A. E. Honey, et al., 2004).

Participants were presented with a set of letters for 2.5 seconds, followed by an instruction cue (FORWARD or ALPHABET) for 1.5 seconds. Following a 7 second delay (during which a fixation cross was presented on the screen) a probe was displayed for 4 seconds during which participants were required to respond with a yes or no. If the cue was FORWARD, participants were required to remember the letters in the order they were presented and the response cue was a question about the position of the letter (e.g. was 'N' 2^{nd}?) and the participants were to give a yes response if that letter had been presented in that position in the initial display, and a no response if it had not. If the cue was ALPHABET, participants were required to mentally rearrange the letters presented into alphabetical order and to remember the letters in the new order. The response cue was again reading the position of the letter (e.g. was 'N' 2^{nd}) but in this condition participants had to give a yes response if that letter had that position alphabetically and a no response if it had not. For both the

FORWARD and the ALPAHABET conditions, participants were presented with three (low- load condition), four and five (high-load condition) letters. This is illustrated in the figure below (Fig. 2.3) which was projected on a computer screen.

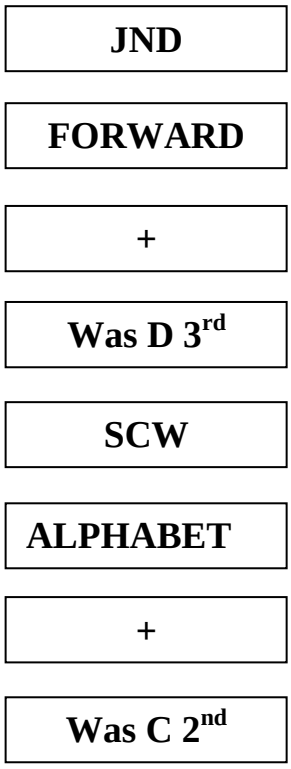

Figure 2.3: Working memory task

2.6 Picture tasks

Rationale: This part of the study was subsequent addition to our study design. The topical work done by Farooqi et al. (Farooqi, et al., 2007) showing the manipulative effects of leptin on appetite control prompted us to perform this study. In this study Farooqi et al. showed leptin treatment in two congenitally leptin- deficient adults resulted in changes in activation in the limbic regions of the brain, mainly the nucleus accumbens. This supported the notion that leptin acted on neural circuits governing food intake, providing key neuroanatomical insights into mechanisms of actions of leptin (Farooqi, et al., 2007). Thus, this seminal work encouraged the hypothesis that glucose, a nutritive substrate and primary fuel of the brain, could also influence energy balance and pathways regulating appetite in the brain.

The hypothesis was that in overnight fasted (hungry) healthy adults (given a sweetened drink), visualization of food pictures would elicit activation in the limbic regions of the brain. Further, a drink of 25 g of glucose would dampen the activation in these regions, as the individual would no longer be perceived to be hungry. If that was the case, one would further look for differences in activation patterns to various types of food (appetising vs. bland). A separate power calculation was not performed for this arm of the study.

Several studies have documented changes (decrease in signal) in the hypothalamic region on functional imaging following ingestion of glucose (M. Chen, et al., 2007; Liu, et al., 2000; Matsuda, et al., 1999; Smeets, et al., 2005a) with glucose doses ranging from of 25-75 g. Further studies have looked at the brain activation patterns on viewing food images under different metabolic conditions (fasting vs. fed). These studies have shown changes in activation in the regions of limbic/paralimbic areas,

insula and the orbitofrontal cortex (A. Del Parigi, et al., 2002b; Gordon, et al., 2000; Porubská, et al., 2006; G. J. Wang, et al., 2004).

The regions on imaging considered in the present study included the limbic/paralimbic areas, the insula, and the orbito-frontal cortex. The hypothalamus is difficult to map using the region of interest (ROI) analysis and was not studied. The 'picture task' used by Farooqi et al. (Farooqi, et al., 2007) was administered towards the end of our cognitive testing.

Between 45 and 60 minutes into the experiment, subjects were presented with a series of images (4 sec presentation) consisting of both foods and non-food items presented in a blocked format (each block made up of 5 successive food images or 5 non-food images so that each block lasted 20 seconds). Food and non-food images were matched for size, variety of colours and complexity. On occasion, images were repeated and subjects were asked to press a keypad if they recognised an image that had previously been viewed. Inherent to the design was a further sub-classification of the food items into appetising and bland foods. On exiting the scanner, subjects were shown a paper copy of the food images viewed and asked to rate using a visual analogue scale, their liking of the food images. An example of the images used is shown below (Fig. 2.4). See also *Appendices 4a and 4b*.

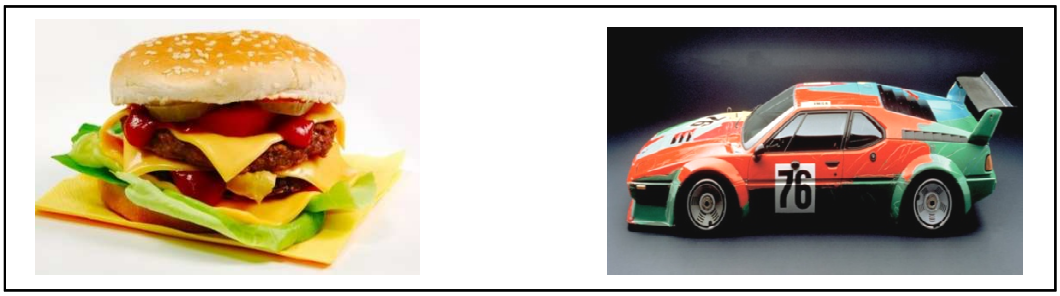

Figure 2.4: Food images task

The imaging results of this task are presented as an independent study in Chapter 4.

2.7 Brain Imaging and Analysis

Siemens Trio scanner operating at 3 Tesla in the Wolfson Brain Imaging Centre (WBIC) in Cambridge was used. A total of 502 Gradient echo T2*-weighted echo planar images (EPI) depicting blood oxygenation level dependent (BOLD) contrast were acquired for each subject. The first six images were treated as "dummy" scans and discarded to avoid T1 equilibration effects. Images were positioned at 30 degrees to the AC-PC plane and comprised 49 slices, each of 2mm with a 0.5 inter-slice gap. A TR of 2000ms was used with an echo time of 30ms and 90 degree Flip Angle. The scanner has a 192mm field of view with a 64x64 data matrix.

Data were analyzed using statistical parametric mapping in the SPM5 (SPM5: Wellcome Dept of Cognitive Neurology, London, UK). Statistical parametric mapping (www.fil.ion.ucl.ac.uk) (Friston, 1995) employs a mass univariate approach and culminates in a series of t-tests comparing signal (at each volume element (voxel) of the scan) across activation and control conditions. The design was counterbalanced in order to allow identification and exclusion of period effects. Essentially this involved a voxel by voxel implementation of the general linear model with individual subjects' responses to activation tasks identified and carried through to a group analysis identifying the common regional brain activations through a series of standard t tests. Group analyses were used to evaluate brain responses to the task and to identify the impact of the glucose drink upon these activations. The threshold was set at $p < 0.005$ uncorrected with minimum cluster size of 20 voxels.

Images were realigned then spatially normalized to a standard template and spatially smoothed with a Gaussian kernel (6mm at full width 3 half-maximum). The time series in each session were high-pass filtered (with cut-off frequency 1/120 Hz) and serial autocorrelations were estimated using an AR (1) model. Each of the three

blocks of stimuli (encoding, CPT and WM) events were modeled using a canonical hemodynamic response function (plus first derivative) convolved with a 20 second boxcar function placed at the onset of each block In addition, a parametric function was applied to each condition mean post-scan subjective liking. These functions were used as covariates in a General Linear Model and a parameter estimate was generated for each voxel for each block type. The parameter estimate, derived from the mean least squares fit of the model to the date, reflects the strength of the covariance between the data and the canonical response function for a given condition. The responses to each condition were modeled separately compared to baseline and parameter estimates taken forward to a group analysis treating inter-subject variability as a random effect. At the group level an ANOVA model was used reflecting the 2x2 factorial design with stimulus type (food and non food pictures) and pre-scan drink (glucose and aspartame) as independent factors.

The regions of brain activation were anatomically identified using the Pick atlas tool implemented in SPM5, with striatal and midbrain regions being identified using criteria described previously (Murray, 2008). For example, voxels within the regions of interest showing a significantly greater response for food compared to non-food pictures for the glucose and sweetener conditions combined were identified. Parameter estimates from each of the foci were extracted to identify and evaluate stimulus by drink interactions.

2.8 Statistical analyses

Assuming a CV of 25% and glucose mediated difference in recall of 25%, n=12 gave 90% power to detect similar difference with alpha of 0.05.

Data were analysed using ‘Windows Excel 2007 ®and GraphPad Prism®. Following the initial processing of the fMRI images using SPM5 (performed by Dr Swamy), the

statistical analyses for the images were performed by Dr Paul Fletcher. The cognitive tasks were statistically analysed by Dr Paul Fletcher.

The biochemical data were first log transformed and then analysed used paired t-tests. Empty cells in Excel 2007 were treated as missing. The contents of the cell were checked with the 'if' function with the 'isnumber' information function, e.g. if (isnumber (cell), log(cell),""). Biochemical data are presented as means (SEM) throughout, unless indicated otherwise.

Cognitive data were analysed using within-subjects analysis of variance (ANOVA).

SPM analyses results are reported using Z-scores mainly.

Chapter 3: Effects of Oral Glucose on Cognitive Performance and Brain Activation

3.1 Aims

To establish how and whether oral glucose alters memory tasks performance and associated brain activation responses as assessed by fMRI in healthy non diabetic volunteers.

3.2 Rationale

As set out earlier, the ultimate aim of the programme of work towards which this study contributed to was to examine the effects of changes in blood glucose during both short term and over a longer period on cognition and brain activation patterns in patients with type 1 diabetes.

As a logical first step in this overarching programme, the effect of oral glucose on these parameters in non-diabetic healthy individuals was examined.

3.3 Chapter abbreviations

GLU: Studies in which 25 g oral glucose ingested

CON: Control studies in which sweetener (5 tablets of Aspartame) ingested

WBIC: Wolfson Brain Imaging Centre, Cambridge, UK

3.4 Methods

The methods are described in detail in Chapter 2. In summary, 13 healthy subjects attended the WBIC on two separate occasions and underwent fMRI scans after consuming either glucose or sweetener (GLU or CON) in randomised single-blinded order. During the following 60 mins, subjects performed a series of cognitive tasks.

Presented below in order are data from blood sampling, cognitive performance and fMRI brain activation.

3.5 Results

3.5.1 Changes in Blood Chemistry

Due to challenges with sampling from venous catheters while subjects were lying inside the MRI scanner, blood sampling was only possible in eight of the thirteen subjects on CON day and 7 out of thirteen subjects in GLU days.

3.5.1.1 Plasma glucose results

As expected, baseline glucose values were similar on both study days (5.1 ± 0.00 vs. 5.2 ± 0.01 mM GLU *vs.* CON). On GLU days, intake of 25 g glucose orally resulted in a modest but significant rise in plasma glucose (6.9 ± 0.00 *vs.* 5.1 ± 0.01 mM GLU *vs.* CON at 30 mins, $p < 0.0001$, $t=6.775$, $df=12$). This significant difference persisted at 45 min and 60 min although plasma glucose had started to return towards baseline by the end of the study (Fig. 3.1).

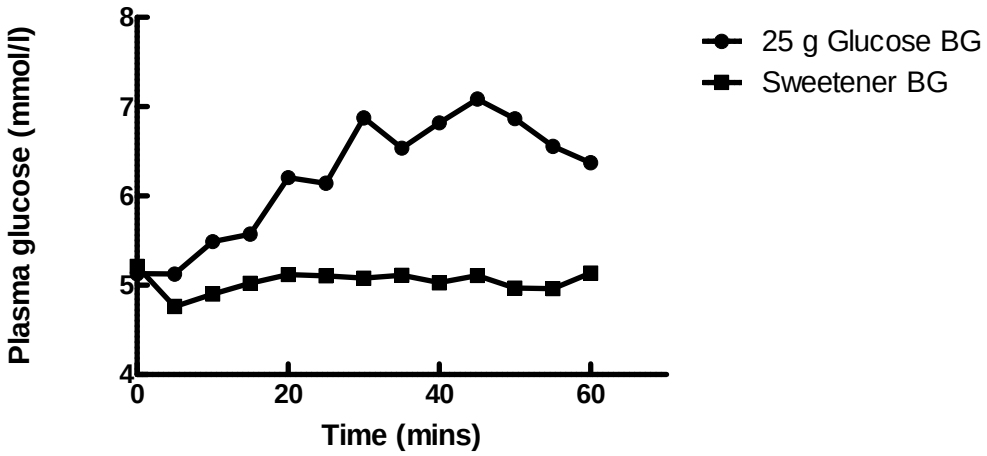

Figure 3.1 Plasma glucose on 25 g glucose days and sweetener day

3.5.1.2 Hormone results

In addition to glucose, insulin may have effects on cognition and memory. As relatively few samples were obtained at the 15 minute time point (3 samples). Hence insulin or glucagon data at the 15 minute mark was not analysed.

Baseline plasma insulin levels were not significantly different in both groups (33.33± 0.07 vs. 27.77 ± 0.08 pM/L, GLU vs. CON, p=0.37, NS).

As expected, in keeping with the rise in plasma glucose, insulin levels also rose in these healthy volunteers on GLU days as compared to CON days (fig 3.2). By 30 minutes, insulin levels had risen significantly in the GLU group (84.70 ± 0.08 vs. 22.85 ± 0.10 pmol/l). These changes persisted at 45 minutes (103.61 ± 0.07 vs. 19.00 ± 0.13, GLU vs. CON) and at 60 minutes (95.09 ± 0.10 vs. 19.31 ± 0.13, GLU vs., CON). p = 0.04, t= 3.44, df = 3. These results are illustrated below (Fig 3.2).

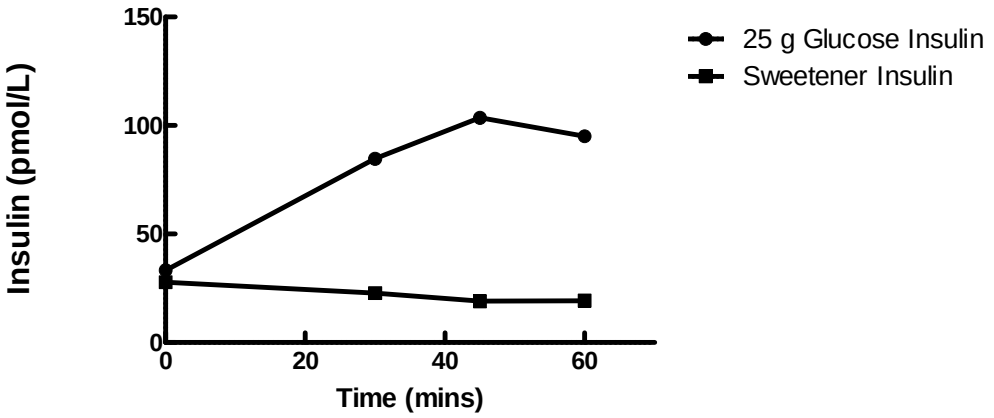

Figure 3.2 Plasma insulin levels on glucose and sweetener days

Plasma glucagon levels responds reciprocally with insulin, although, current literature does not suggest a cognitive effect of glucagon. Glucagon levels did not differ significantly between the GLU and CON days (fig 3.3). Mean glucagon levels (pg/ml GLU vs. CON) at 0 min= 75.94 ± 0.03 vs. 74.4 ± 0.02, 30

min= 57.33 ± 0.07 vs. 65.59 ± 0.02, 45 min = 72.24 ± 0.03 vs. 67.56 ± 0.02 and 60 min = 58.51 ± 0.05 vs. 62.14 ± 0.02. p = 0.58, t=0.6087, df= 3. These results are depicted in figure 3.3.

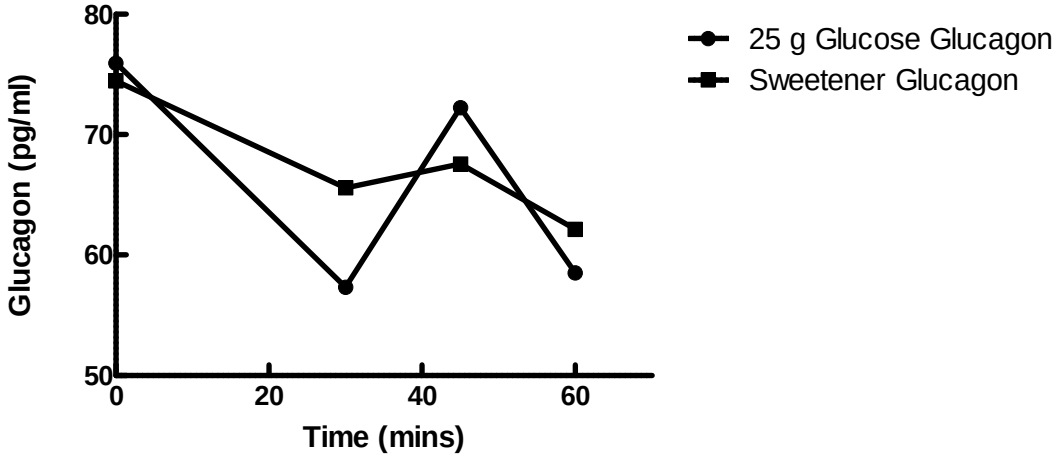

Figure 3.3 Plasma glucagon levels on GLU and CON days

3.5.2 Cognitive Performance Data

Cognitive data from 13 participants were analysed. Cognitive data were analysed using within-subjects analysis of variance (ANOVA).

3.5.2.1 Continuous Performance Testing:

The performance scores for this task are presented as a discrimination index (targets versus non-targets). The discrimination index represents the proportion of hits minus the proportion of false alarms (false alarm= wrongly saying 'yes' to a non-target, that is, inverse of correct rejections).

On GLU days the discrimination index was 0.98 (STD: 0.04) and on CON days 0.98 (STD: 0.03).

3.5.2.2 Working Memory

The behavioural results for the working memory task are presented as follows:

1. **Hits:** This indicates the correct identification of the target. The maximum score for this task was five. There was no effect of drink on performance. The correct hits GLU vs. CON were 4.17 vs. 4.08 for 3 digits, 4.33 vs. 4.33 for 4 digits, 4.08 vs. 3.83 for 5 digits. p= 0.69, F = 0.17, df = 1. Interaction between types of drink vs. task difficulty were non- significant with p = 0.93, F=0.07, df = 2. Bonferroni posttest was performed giving t= 0.18 for 3 digits, 0.0 for 4 digits and 0.53 for 5 digits. These results are illustrated in figure 3.4.

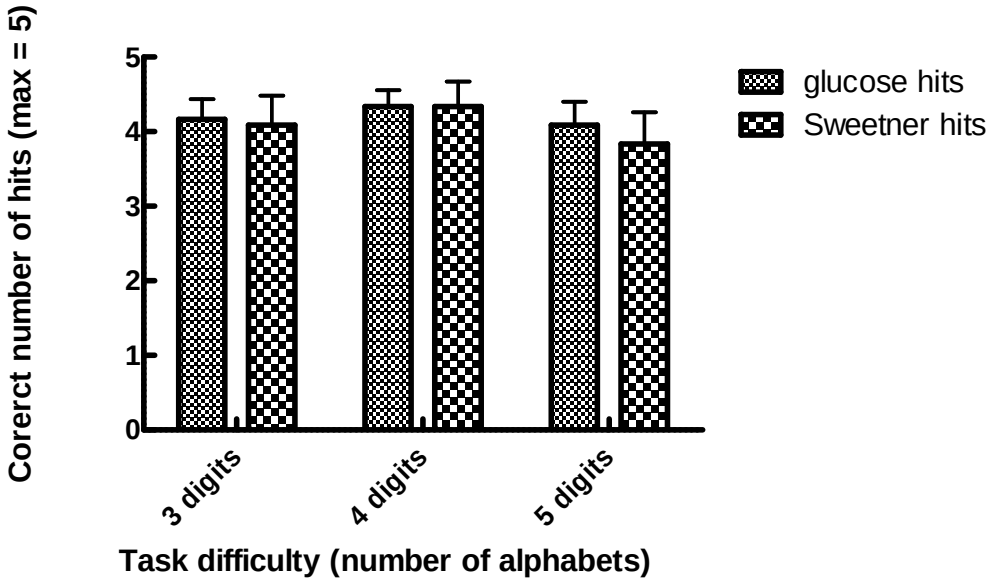

Figure 3.4 Working Memory (Frequency of correct hits)

2. **Correct Rejections:** This indicates the correct response to a 'No' answer. The maximum score for this task was five. Correct rejections GLU vs. CON were 4.67 vs. 4.58 for 3 digits, 4.25 vs. 4.41 for 4 digits and 4.25 vs. 4.33 for 5 digits. p = 0.76, F = 0.09, df = 1. Interaction between types of drink vs. task difficulty were non- significant with p= 0.85, F=0.16, df = 2. Bonferroni posttest was performed giving t= 0.26 for 3 digits, t= 0.52 for 4 digits and t= 0.26 for 5 digits. These results are shown in figure 3.5.

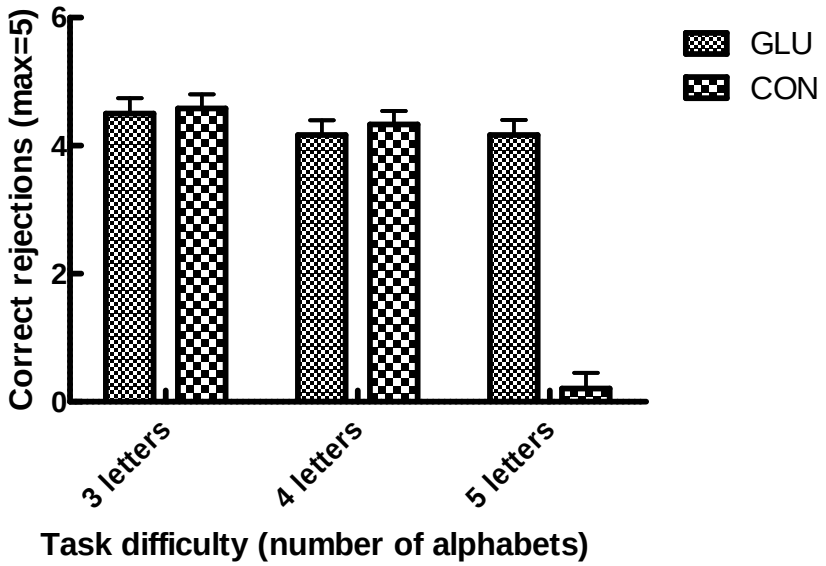

Figure 3.5 Working Memory (Frequency of correct rejections)

3. **Accuracy:** This is a measure of overall hits plus the correct rejections out of a possible maximum score of ten. Accuracy for GLU vs. CON was 0.88 vs. 0.86 for 3 digits, 0.86 vs. 0.88 for 4 digits, 0.83 vs. 0.81 for 5 digits. There was no significant difference in performance under the different drink conditions with p = 0.89, F = 0.02 and df = 1. Interaction between types of drink vs. task difficulty was not significant with p = 0.92, F= 0.08 and df = 2. Bonferroni posttest was performed giving t = 0.25 for 3 digits, t = 0.25 for 4 digits and t = 0.25 for 5 digits. These results are illustrated in figure 3.6.

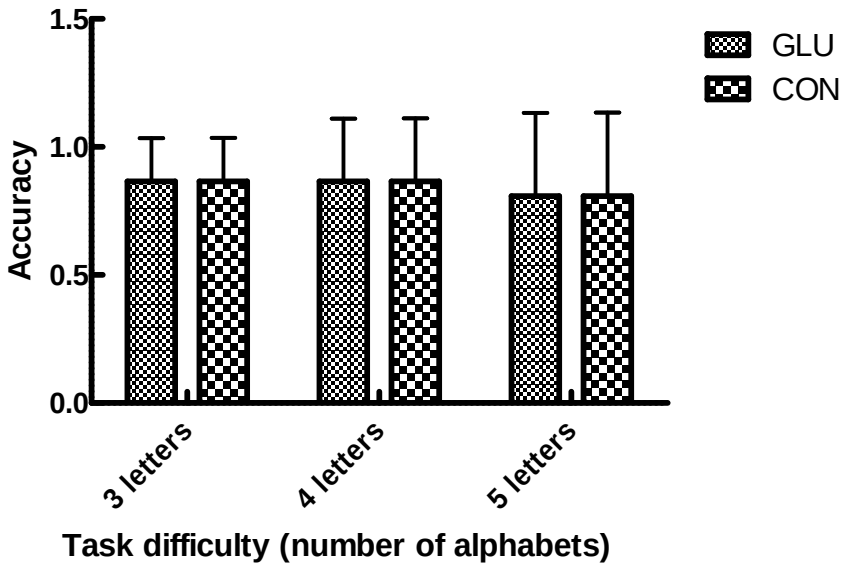

Figure 3.6 Working Memory (Accuracy)

4. **Performance or the 'Discrimination Index':** This represents the proportion of hits minus the proportion of false alarms (false alarm= wrongly saying 'yes' to a non-target, that is, inverse of correct rejections) (Fig. 3.7). The discrimination index for GLU vs. CON was 0.77 vs. 0.78 for 3 digits, 0.78 vs. 0.71 for 4 digits, 0.70 vs. 0.65 for 5 digits. There was no difference in performance on the two study days with p = 0.64, F = 0.21 and df = 1. Interaction between types of drink vs. task difficulty was not significant with p = 0.88, F = 0.13, df = 2. Bonferroni posttest was performed giving t = 0.13 for 3 digits, 0.53 for 4 digits and 0.40 for 5 digits. This is shown in figure 3.7.

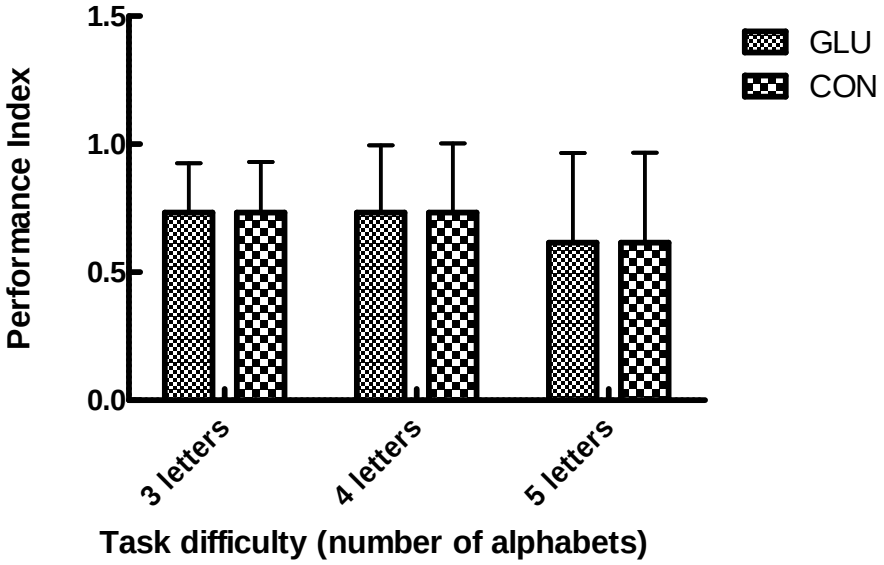

Figure 3.7 Working Memory (Performance Index)

3.5.2.3 Encoding

Due to loss of retrieval task performance data, direct comparison between cognitive performance and neural activation was not possible for this task. Lack of retrieval data made interpretation of encoding attempts difficult to analyse.

3.5.3 Functional Magnetic Resonance Imaging (fMRI) Results

Data from 13 participants were analysed.

3.5.3.1 Continuous Performance Testing (CPT):

For this task, the baseline condition "Relax" was used as a control. During performance of the CPT task, robust activation was seen in the regions of the inferior frontal gyrus, superior temporal/inferior parietal cortex on the left and the right sides on GLU days. Activation was also noted in the anterior cingulate/medial prefrontal cortex on GLU days. On CON days, performance of the CPT task was associated with activation in the regions of the inferior frontal gyrus, superior temporal/inferior parietal cortex on the left and the right hemispheres. In addition, activation as also noted in the anterior cingulate/medial prefrontal cortex, midbrain, cerebellum, right and left thalamus and the left putamen. These regions of activation survived a threshold of $p < 0.005$. The x y z co-ordinates of these regions and the Z scores indicating the statistical magnitude of the effect have been described in Table 2. The surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ uncorrected for multiple comparisons (Figures 3.8 and 3.9).

Regions surviving an uncorrected threshold of p<0.005 and cluster size of 20 voxels

	Region	**Coordinates (x,y,z)**	**Z score (t)**
CPT main effect (compared to control task) - Glucose			
Df (1,20)	**Inferior Frontal Gyrus**		
	Right	42, 10, 24	2.7 (3.1)
		32, 24, 8	3.5 (4.2)
	Left	-34, 18, 6	4 (5)
	Superior temporal/inferior parietal cortex		
	Right	30, -60, 44	3.6 (4.3)
		66, -34, 26	2.8 (3.1)
	Left	-56, -36, 20	3.9 (4.9)
	Anterior cingulate/medial prefrontal cortex	4, 14, 46	3.8 (4.6)
CPT main effect (compared to control task) - Sweetener	**Region**	**Coordinates (x,y,z)**	**Z score (t)**
Df (1,20)	**Inferior Frontal Gyrus**		
	Right	42, 24, -6	3.8 (4.7)
		42, 12, 24	3.6 (4.4)
	Left	-46, 20, -2	3 (3.4)
	Superior temporal/inferior parietal cortex		
	Right	32, -52, 52	3.7 (4.5)
		66, -42, 20	3.6 (4.4)
	Left	-30, -52, 52	3.8 (4.7)
		-54, -44, 28	3.4 (4)
	Anterior cingulate/medial prefrontal cortex	2, 16, 54	3.8 (4.7)
	Midbrain	6, -16, -8	3.6 (4.3)
	Cerebellum	38, -40, -26	3.5 (4.2)
	Thalamus		
	Right	10, -20, 18	3.4 (4)
	Left	-16, -14, 18	3.2 (3.7)
	Left putamen	-20, 0, 14	3.1 (3.5)

Table 2 Regional brain activation during performance of Continuous Performance Task (CPT)

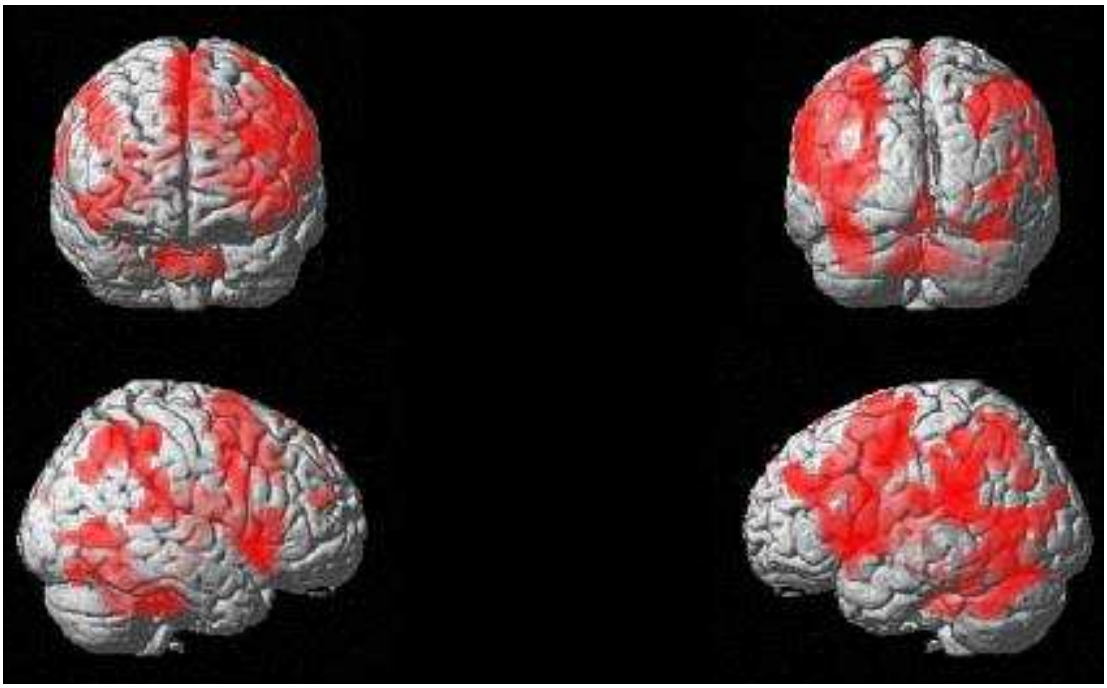

Figure 3.8 fMRI activation during CPT (vs. Control Task) for GLU

These surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ uncorrected for multiple comparisons

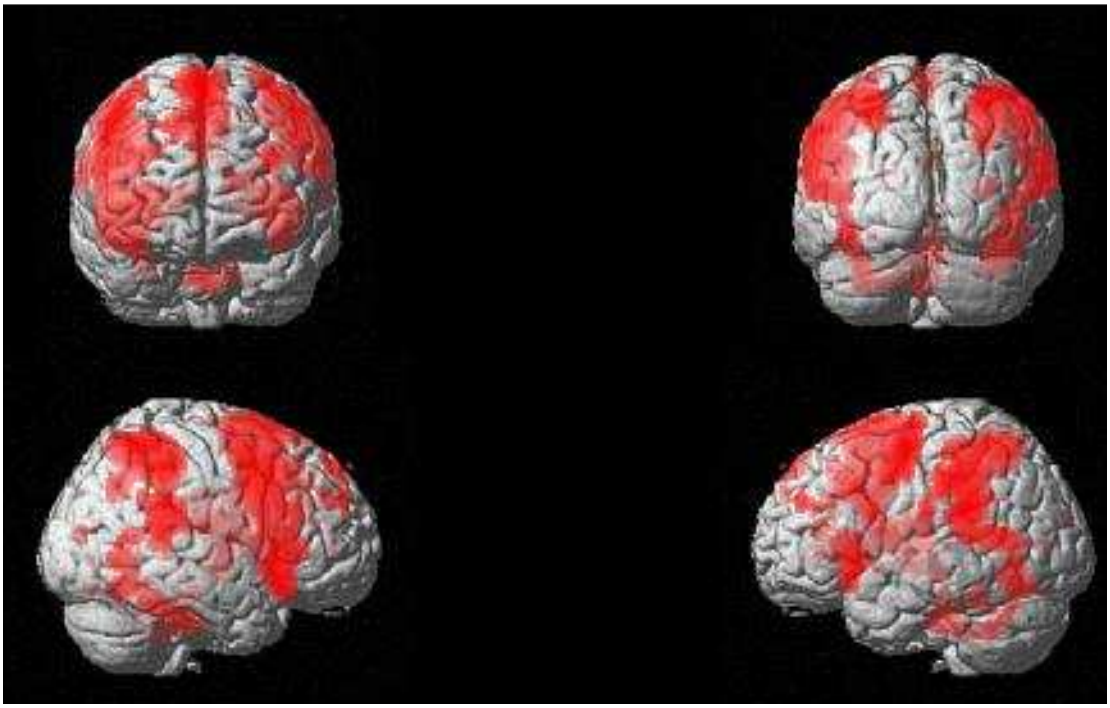

Figure 3.9 fMRI activation during CPT (vs. Control Task) for CON

These surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ uncorrected for multiple comparisons

3.5.3.2 Working Memory

For the WM task, activation was compared to simple fixation cross (taken as baseline). Activation was found in the regions of middle/inferior frontal gyrus, inferior parietal cortices on both hemispheres with GLU. Activation was also observed in both the right and the left cerebellar hemispheres and the midbrain on the GLU days. On CON days, activation was observed in the middle/inferior frontal gyrus and superior temporal/inferior parietal cortex bilaterally. These regions showed increase intensity of activation with increase in the difficulty of the working memory task, however, there was no difference in patterns of activation with the type of drink.

These regions of activation survived a threshold of $p < 0.005$. The x y z co-ordinates pertaining to the regions of brain activation have been detailed in table 3. The surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ (Figures 3.10 and 3.11).

fMRI and Working Memory

Regions surviving an uncorrected threshold of p<0.005 and cluster size of 20 voxels

	Region	**Coordinates (x,y,z)**	**Z score**
Working memory load effect Glucose			
Df (1, 18)	**Middle/Inferior Frontal Gyrus**		
	Right	30, 32, 24	3.2 (3.8)
		38, 30, 18	2.9 (3.4)
		26, 14, 28	3.2 (3.7)
	Left	-38, 38, 30	
	Inferior parietal cortex		
	Right	50, -20, 28	2.6 (2.9)
	Left	-46, -34, 44	3 (3.5)
	Cerebellum		
	Right	38, -66, -28	3.1 (3.6)
	Left	-28, -64, -32	3.8 (4.8)
	Midbrain	4, -28, -18	2.7 (3.1)
Working memory load effect Sweetener	**Region**	**Coordinates (x,y,z)**	**Z score**
Df (1, 18)	**Middle/Inferior Frontal Gyrus**		
	Right	50, 12, 46	3.3 (4)
		30, 30, 32	2.6 (3)
		48, 38, 20	2.6 (2.9)
		40, 50, 10	2.3 (2.6)
	Left	-46, 20, -4	2.5 (2.7)
	Superior temporal/inferior parietal cortex		
	Right	42, -34, 48	3.4 (4.1)
		32, -66, 60	2.8 (3.2)
	Left	-56, -46, 26	2.4* (2.7)

Table 3 Regional brain activation during performance of Working Memory Task

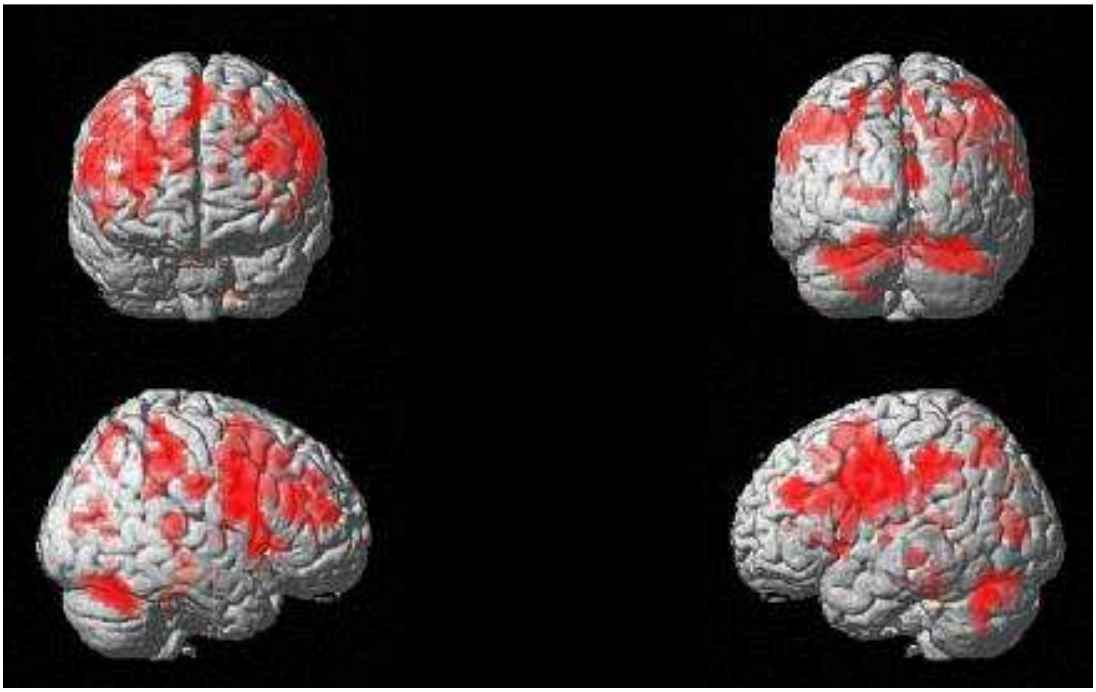

Figure 3.10 fMRI activation during WM (increased intensity with increasing work load) for GLU

These surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ uncorrected for multiple comparisons.

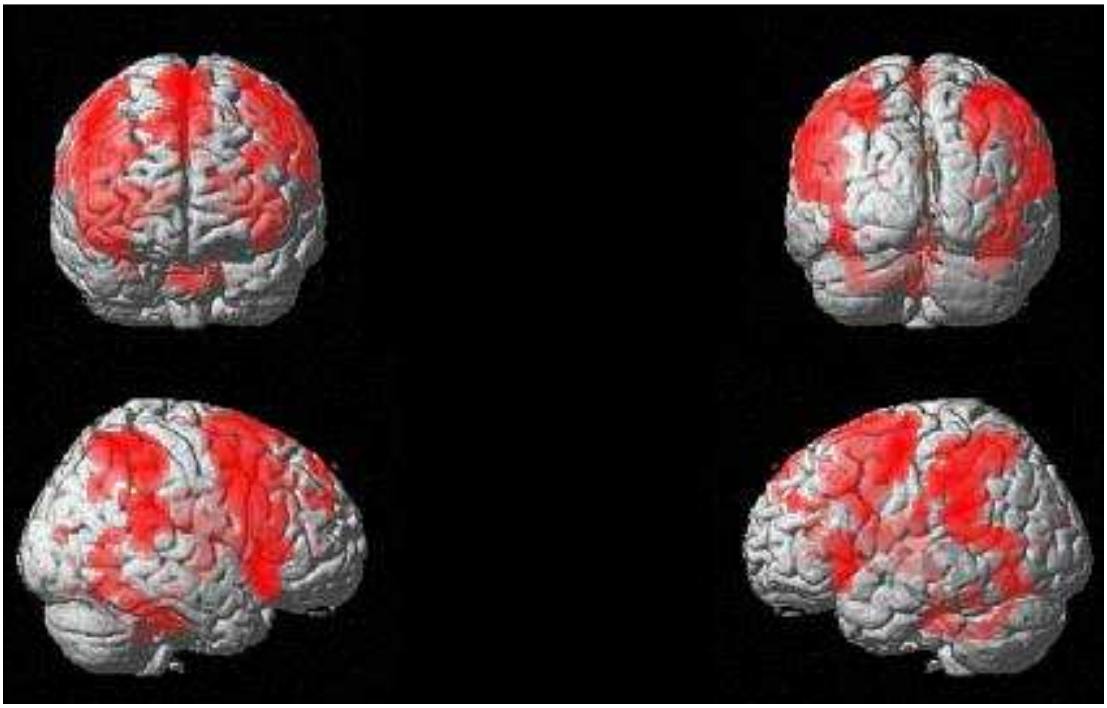

Figure 3.11 fMRI activation during WM (increased intensity with increasing work load) for CON

These surface regions in the figure represent activation observed with threshold set at a modest $p<0.05$ uncorrected for multiple comparisons.

3.5.3.3 Encoding

The fMRI images during encoding task was not analysed because of the lost retrieval data, as without the latter data, there was no way of knowing which trials involved successful encoding and which did not.

3.6 Discussion:

Facilitation of cognitive abilities has been an alluring and elusive subject for decades. Ingestion of glucose improves declarative long term memory (Sunram-Lea, et al., 2002), but the neuroanatomical basis of this phenomenon remains unexplored.

This study aimed to answer this question by studying the cognitive effects of a small dose of oral glucose on memory tasks whilst simultaneously studying the regions of brain activation during performance of these tasks, using fMRI.

Overall the study was well designed with sufficient power to unmask facilitative effects of glucose on cognitive performance. However, the study was rendered less powerful due to loss of critical data (retrieval data) making interpretation of the encoding task (shown to be glucose-sensitive in literature) difficult.

Thus my study contributes to the existing literature on effects of oral glucose load on cognitive performance. It additionally delineates the brain regions recruited during performance of these tasks under different metabolic conditions (glucose vs. sweetener).

3.6.1 Glucose dose

As was expected and as has been reported in various studies (Brandt, et al., 2006; Claude Messier, 2004; Sunram-Lea, et al., 2010a), 25 g of oral glucose increased plasma glucose levels significantly. For this study, 25 g oral glucose dose was used, rather than a higher dose, as collaborators of the study; Sunram-Lea et al have previously demonstrated facilitative effects on cognition at this dose. Moreover, according to the existing literature, glucose facilitative effects in young individuals (average age of our participants was 24 years) are observed at lower blood glucose values than that seen in healthy, older individuals (Claude Messier, 2004).

In this study, an oral load of 25 g of glucose gave a perceptible and a significant rise of blood glucose from the baseline, as in Sunram-Lea's study, associated with a corresponding rise in plasma insulin. In contrast, glucagon levels were unchanged by the oral glucose load. Plasma glucose at baseline was comparable

in both the groups, making any further observations in performances in the two groups comparable.

In humans, doses ranging from 25g to 75 g have been shown to be effective in demonstrating memory facilitation which corresponds to 300mg/kg to 1g/kg for a 75 kg human (Claude Messier, 2004). There is further thought that higher doses of glucose, show beneficial effects in healthy elderly individuals and in people with Alzheimer's disease, where as lowers doses of glucose have been found have facilitative cognitive effects on healthy younger individuals (Claude Messier, 2004). Human studies comparing glucose effects on cognition using different doses, such as 25 g and 60 g have found comparable blood glucose levels (Azari, 1991; Owen, et al., 2010b; Sunram-Lea, et al., 2010a) over similar time periods.

Azari et al gave 30g and 100 g of glucose solution in a random double-blind triple crossover design. They studied 18 healthy normal adults (mean age= 21). Thirty minutes post-glucose, subjects were shown nouns on a computer monitor and then administered recall and recognition tests. There was no effect of glucose on memory tests and plasma glucose measures did not correlate with memory test scores (Azari, 1991). Though similar study designs was shown to reveal gluco-facilitatory effects (Hall, et al., 1989), the failure to observe facilitative effects was attributed to lack of rigorous adherence to pre-experimental dietary requests. Other possible explanations for the lack of effects of oral glucose on performance are task simplicity and younger age of participants. If performance at baseline (or at the sweetener stage) is at ceiling level to task simplicity, it leaves little room for improvement post glucose-ingestion. This has been observed to be a plausible cause for failure to see gluco-facilitation on cognitive

tasks. Addition of motor sequence (interference task) during presentation of a word list, has been shown to expose the facilitative effects of glucose (Foster, 1998; Sunram-Lea, et al., 2002). University graduate students can be considered a skewed cohort of intellectually higher achievers, masking any potential potentiation of performance.

3.6.2 Cognitive tasks

The declarative long-term memory task (or verbal long term memory) consisting of encoding and retrieval (measurable and consolidation and storage (non-measureable) has been repeatedly shown to be sensitive to glucose potentiation effects in various studies (Foster, 1998; Sunram-Lea, et al., 2002).

One of the major limitations of the current study was the unfortunate loss of retrieval data on the performance task. This greatly limited the potential of this study to determine the facilitative effects of glucose (if any) on cognitive performance. The sample size of thirteen individuals was adequate to detect a difference in performance (power calculation gave n = 12).

The working memory is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and problem solving (E. E. Smith & Jonides, 1998). This task has not been convincingly shown to be sensitive to glucose induced facilitation. It was administered to enable correct interpretation of data obtained during performance or other tasks.

3.6.2.1 Continuous performance task

The continuous performance task is a widely used neuropsychological task for assessing vigilance and sustained attention (Nestor, et al., 1991; Nuechterlein, 1991; Rosvold, 1956). It also enables us to evaluate subject's reaction times. It was administered to ensure that any changes observed in the key tasks (encoding and retrieval) were not a simple attentional effect. Existing literature does not suggest this task to be glucose-sensitive. There was no change in performance on this task on either of the study conditions.

Though there are scattered reports of improvement in reaction times to glucose (David Benton, et al., 1987) this test is not considered to be sensitive to glucose manipulation (C. A. Manning, et al., 1990). Benton et al. (David Benton, et al., 1987) studied the effects of 25 g of glucose in 60 six-seven years old children. The children were tested on sustained attention tasks and tasks designed to be intentionally frustrating. Post-glucose children were found to have increase in sustained attention and fewer signs of frustration. However these results have not been reproduced by other researchers. Manning et al. (C. A. Manning, et al., 1990) studied 17 healthy elderly (mean age =73) individuals for cognitive performance following a 50 g glucose drink and placebo. Amongst other tests, the participants were also tested on 'Letter Cancellation Test', an attention task. The researchers found comparable performance results under the different conditions.

3.6.2.2 Working Memory

This is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and problem solving (E. E. Smith & Jonides, 1998).

There was no improvement in the performance, neither on the increasing digit span nor on the alphabetical reordering part of the task, which is inherently more difficult to solve. There was no increase in the number of correct responses; however, there was also no decrease in the number of incorrect answers either.

Existing literature suggests that the working memory task is not amenable to glucose facilitation (C. A. Manning, et al., 1990; Messier, et al., 2010; S. Sünram-Lea, et al., 2001). This task was included in this experiment to ensure that any changes observed in the key tasks were not simply a reflection of general working memory effect. One of the other objectives for inclusion of this task was to observe the brain regions recruited in the performance of this task.

It has been observed by Sunram-Lea et al, that glucose facilitation on memory is fractionated, that is to say that glucose facilitates only certain aspects of memory, such as verbal long term memory as shown in the CVLT task, and that the facilitation is not global. Other factors which have been noted to influence glucose-facilitatory effects on cognition are timing of the drink, the dose of glucose as discussed above and the effect of learning.

Gold et al showed that both pre and posttraining administration of glucose in humans improved memory for a paragraph recall (C. A. Manning, et al., 1992). Studies have employed pretraining (C. A. Manning, et al., 1990), posttraining (C. A. Manning, et al., 1992) or just prior to recall (C. A. Manning, et al., 1998) glucose administration and have observed facilitative effects on learning, memory and retrieval of information.

In the current study, the participants were initially briefed about the upcoming tasks and had one practice run of the cognitive tasks on a laptop computer prior to entering the scanner. The practice runs did not include the words, numbers or

pictures included in the test paradigm. Thus there was minimal learning effect on the observed outcomes. Repeated exposure or practice has been shown to facilitate performance. Studies showing reliable glucose facilitation of long term memory performance following 25 g glucose have generally used repeated exposure to the to-be remembered material following 25 g glucose (Scholey & Kennedy, 2004). Practice and repetition aid in learning episode and the memory trace is strengthened through repetition of stimuli (Owen, et al., 2010b).

All human experiments involve measurement of peripheral glucose levels. Though experiments examining the impact of hyper- and hypoglycemia on extracellular glucose content have yielded variable results in the absolute values of extracellular glucose, in general brain extracellular glucose levels are about 20-30% of the blood glucose levels in the physiological range (Claude Messier, 2004). Rodent studies have verified that extracellular glucose levels value at 0.35 mmol/L (Lowry, et al., 1998). Moreover, neuronal glucose uptake is mainly driven by the neuron's activity (and hence glucose requirement) and not by blood glucose (glucose supply) due to counterregulatory mechanisms, except in the face of severe hypoglycemia (Claude Messier, 2004).

Though unlikely, stress hormones such as epinephrine can sometimes confound the results. Stress hormones such as epinephrine could potentially interact with the action of glucose on memory, by either producing an additive facilitating or impairing effect on memory or contributing to increased variability because subjects do not necessarily react the same way to stressful stimuli (Claude Messier, 2004). The current study was a relatively simple study involving a single venous cannulation prior to the onset of the study. Understandably, cannulation can induce a stress response (with release of catecholamines and

cortisol) and so also stress can be induced by being inside a MRI scanner. However, the drink/ study days were randomised, thus the contribution of stress, if any would be counterbalanced across both days. Stress hormone levels were not measured in this study.

Several other researchers have shown that there is a close correspondence between the rise in blood glucose and the rise in blood insulin following an oral glucose load and it is hard to dissociate the effect of glucose from that of insulin in the mediation of the central effects of ingested or injected glucose. Insulin, in its own right has been shown to have cognitive-enhancing properties. In rodents, small doses of insulin (0.4-0.8 units/kg) to reverse the amnesia produced by a 2 mg/kg scopolamine injection (Blanchard & Duncan, 1997; Claude Messier, 2004) and to facilitate memory following intracerebrovascular injection (Park, et al., 2000). Euglycemic clamps in humans have also been shown to enhance memory, where glucose levels are held constant whilst insulin levels are raised (S. Craft, et al., 1999).

3.6.2.3 Encoding and Retrieval

As described above performance data was not available for this task, so that the only analysis possible was fMRI activation with and without glucose (see later).

3.6.3 fMRI brain imaging

One of the key aspects of the study was to look at and map out areas of the brain engaged in performances of these cognitive tasks. Possible differential patterns of activation following ingestion of glucose during performance of the cognitive tasks were also studied.

3.6.3.1 Continuous performance testing

During performance of the CPT task, robust activation was seen in the regions of the inferior frontal gyrus, superior temporal/inferior parietal cortex on the left and the right sides on both the study days. Activation was also noted in the anterior cingulate/medial prefrontal cortex under both glucose and sweetener conditions. In addition, on the CON days activation was also noted in the anterior cingulate/medial prefrontal cortex, midbrain, cerebellum, right and left thalamus and the left putamen. Similar to our observation, Ogg et al (Ogg, et al., 2008) performed fMRI in 30 healthy adults during performance of Conner's Continuous Performance Test (CPT) (subjects viewed letters on a computer screen and were instructed to respond to every letter except the target letter X). They demonstrated an extensive neural network that was activated during the task and included the frontal, cingulate, parietal, temporal, and occipital cortices; the cerebellum and basal ganglia. The magnitude of activation in several regions correlated with reaction time.

3.6.3.2 Working Memory

As outlined above, activation was noted in the middle/inferior frontal gyrus, inferior parietal cortex bilaterally on both glucose and sweetener days. There was no hemispheric differentiation in the patterns of activation. The hemispheres of the cerebellum and the midbrain were also activated on the glucose days. On the other hand, the middle/inferior frontal gyrus and the superior temporal/inferior parietal cortex on both sides were activated on sweetener days during performance of this task. There was no difference between the drink conditions on the brain activation patterns.

The verbal working memory task has consistently demonstrated activation of the prefrontal and parietal regions in humans, on fMRI imaging (G. D. Honey, et al., 2000). Although frontal lobe activation is often bilateral, the left ventrolateral frontal cortex appears to be primarily concerned with the maintenance of verbal information whereas the right ventrolateral frontal cortex is more involved with maintenance of spatial information (P. C. Fletcher & Henson, 2001). Furthermore, it has been suggested that the ventrolateral frontal cortex is activated during tasks requiring maintenance of information and the dorsolateral frontal cortex is more involved during tasks requiring manipulation of information (P. C. Fletcher & Henson, 2001). In another experiment, prefrontal activation has been shown to increase parametrically in relation to working memory load (Braver, et al., 1997). The prefrontal activations tracking memory load were located in several areas of dorsolateral prefrontal cortex including portions of anterior frontal-operculum and the inferior frontal gyrus that directly overlap with areas activated by deep encoding, as has been described below. Beside the prefrontal cortex, the parietal cortex is also involved in working memory (Suchan, 2008). Most important in a working memory context are connections between the parietal and frontal lobes (Jonides, et al., 1993). A hemispheric dissociation between the verbal (left) and spatial (right) working memory processing in the parietal cortex has been suggested by Smith and Jonides (E. E. Smith & Jonides, 1998).

The observations in this study cannot currently be purely attributed to the effects of glucose. However, given the drinks were effectively matched for sweetness, the results observed are likely to be a post-ingestive effect, rather than the sweet taste in the mouth per se. Indeed post-ingestive peptides (PYY, ghrelin) and

hormones (insulin (Plum, Belgardt, & BrÃ¼ning, 2006)) have been shown to activate similar brain regions (Batterham, ffytche, et al., 2007; Malik, et al., 2008).

3.6.3.3 Encoding

Due to loss of performance data (retrieval), the imaging data during performance of the encoding task was not thought to reveal useful information with regards to successful encoding attempts. Hence these images were not analysed.

The literature describing the brain regions involved during the encoding process have been described in Chapter 1.

3.7 Summary

In summary, following on oral glucose load there was no improvement in the performance of cognitive tasks in spite of having a respectable rise in plasma glucose levels. Though the study was well designed, based on previous work and evidence to demonstrate the facilitative effects of glucose on aspects of cognition, it is unfortunate that the study was rendered less powerful to prove the hypothesis that glucose facilitates cognitive performance, due to adverse technical problems leading to loss of valuable data. There were no differences in brain activation patterns during cognitive performances under different metabolic conditions (glucose vs. sweetener).

3.8 Conclusions

This study did not demonstrate facilitation in performance of cognitive tests, post 25g oral glucose ingestion. Performance of the different cognitive tests mapped out activation of distinct brain regions and the degree of activation was the same

under the two experimental conditions (GLU vs. CON). Thus there were no differences found between condition on either task performance or brain activation in this study.

Chapter 4: Oral Glucose Increases Activation in Reward Areas of Brain in Response to Food Images

4.1 Rationale

The final picture task was designed with the intention of studying the regional brain activation to pictures of food and non-food.

This aspect of the study was subsequently added on to our study design. The topical work done by Farooqi et al. (Farooqi, et al., 2007) locally showing the manipulative effects of leptin on appetite control prompted us to perform this study. Leptin, a key hormone involved in regulation of energy balance, was shown to manipulate the limbic (hedonic) regions of the brain, mainly the nucleus accumbens by changing feeding behaviour and hunger scores in congenitally leptin deficient children. As we were imaging the exact same brain regions with and without the presence of experimental glucose as a part of the study, addition of this task was thought to reveal novel findings in functional brain imaging.

The potential effects of changes in blood glucose on feeding behavior remain unclear 50 years after the glucostat theory first proposed that small changes in blood glucose triggered/ terminated feeding. Recent work suggests that brain areas such as the ventral striatum, involved in reward and motivation, may play a key role in mediating food-related behaviors (Batterham, Fytche, et al., 2007).

4.2 Hypothesis:

Glucose, a nutritive substrate and primary fuel of the brain could also potentially influence energy balance (like leptin) by regulating the appetite pathways in the brain. In overnight fasted (hungry) healthy adults (given a sweetened drink), visualization of food pictures would elicit activation in the limbic regions of the brain. Further, a drink of 25 g of glucose would dampen the activation in these regions, as the individuals would no longer be perceived to be hungry. If this was true, differences in activation patterns to various types of food (appetising vs. bland) would be studied.

This chapter describes the effects of oral glucose on brain activation patterns on functional magnetic resonance imaging (fMRI) to food images.

4.3 Introduction

During the current epidemic of obesity with attendant health and fiscal costs, it has become increasingly important to discover how feeding is controlled and, in particular, how the current obesigenic environment with a ready availability of palatable energy dense food may lead to obesity. Given that the requirement for adequate energy intake is essential for survival, mammals have evolved multiple systems for controlling feeding behavior. A number of nutritional signals including circulating neuropeptides from gut and adipose tissue (such as ghrelin, peptideYY_{3-36} [PYY] and leptin) have been identified as acting on key populations of orexigenic and anorexigenic cells within the hypothalamus and/or brain stem (Bewick, et al., 2005; Gropp, et al., 2005; Luquet, Perez, Hnasko, &

Palmiter, 2005). Alterations in the circulating levels of these neuropeptides in response to a fall or threatened fall in nutrition lead to increased feeding and vice versa with feeding and satiety ((Morton, Cummings, Baskin, Barsh, & Schwartz, 2006; Porte, Baskin, & Schwartz, 2005).

However, in addition to the simple maintenance of energy homeostasis, feeding may also be a rewarding experience controlled by areas of the brain related to motivation and hedonic value (H.-R. Berthoud, 2004). Control of feeding by brain reward pathways may be particularly relevant to the current epidemic of obesity, which has been attributed to the availability of highly palatable energy-dense foods allowing "hedonic" feeding to override homeostatic energy level signaling (Nestle, et al., 1998). Although conceptually convenient to consider homeostatic and hedonic feeding separately, increasing data suggest that the two may be intrinsically intertwined. For example, recent studies using functional magnetic resonance imaging (fMRI), a technique for non-invasively imaging patterns of brain activation in humans (Tataranni & DelParigi, 2003) have shown that ghrelin (Malik, et al., 2008) leptin (Farooqi, et al., 2007) and PYY (Batterham, Fytche, et al., 2007) can all modulate activity in higher brain centers-cortical and limbic areas such as the ventral striatum involved in hedonic responses. Patterns of brain activation in these areas can even predict subsequent "hedonic" food intake (Batterham, Fytche, et al., 2007). Typically, these studies have explored brain responses not to food itself but to stimuli (such as images of highly palatable foods) that might be supposed to induce the thought of, and motivation towards, food and eating. The striking responsivity of reward circuitry to such stimuli suggests that a great deal of the brain's response concerns the motivation towards consumption as opposed to consumption itself.

4 Oral Glucose Increases Activation in Reward Areas of Brain in Response to Food Pictures

As the main brain fuel, glucose was first proposed as a controller of feeding over 50 years ago. The “glucostat” theory postulated that small falls in blood glucose triggered feeding and/or that the post-prandial rise in glucose terminated food intake and although this has failed to gain widespread acceptance, the potential contribution of blood glucose variations across the physiological range to feeding remain unclear (Grossman, 1986; J. Mayer & Bates, 1952). While there is no doubt that a low blood glucose level stimulates emergency feeding, the role of glucose variations in the physiological range as a controller of feeding is unclear. A role for blood glucose in controlling feeding, either by meal initiation being triggered by a small dip in circulating glucose and/or that a prandial rise in glucose leads to meal termination was first postulated several decades ago although experimental data have failed to confirm (or indeed to completely dismiss) the potential influence of glucose (Grossman, 1986; J. Mayer & Bates, 1952; Mobbs, et al., 2005).

Given the consistent effects of neuropeptides on hedonic circuitry, that glucose might alter activity in reward- and motivation-related areas of the brain in response to food-related stimuli. This study describes brain activation patterns in healthy subjects in response to images of foods following a small oral glucose load, independent of taste. In particular, a small dose of oral glucose would dampen appetite and thus reduce the activation of brain regions to pictures of food. Brain activation between appetitive vs. bland food pictures were also studied with the expectation to see increased activation to appetising foods in the fasted (sweetener) state.

4.4 Methods

The overall study methods and materials were similar to that explained in Chapter 2. A separate power calculation was not performed for this study, as it was not part of the initial proposal (see Appendix 1). Essentially subjects viewed pictures of food and non food on a computer screen whilst undergoing MR imaging of the brain. It is noteworthy to emphasize here, that the drinks were matched for taste and sweetness, as detailed in Chapter 2 under 'sweetness matching'. Also, recognizing that palatability and thus liking of food varies on individual basis, the results were interpreted depending on each individual's rating of liking a specific food item. Liking rate was included as a parameter in the model.

In order to maximize sensitivity without unacceptable type I error, we confined our imaging analyses to striatal midbrain, insula, hypothalamic and amygdala regions of interest. OFC showed signal drop-out and hence was not featured in region of interest (ROI) analysis. Hypothalamic and amygdala regions were identified anatomically using the Pick atlas tool implemented in SPM5, with striatal and midbrain regions being identified using criteria described previously (Murray, 2008). Voxels were identified within the regions of interest showing a significantly greater response for food compared to non-food pictures for the glucose and sweetener conditions combined. Each contrast was corrected for multiple comparisons using the False Discovery Rate (Genovese, Lazar, & Nichols, 2002). Parameter estimates were extracted from each of the foci so identified and evaluated stimulus by drink interactions.

4.5 Results

The results of the preliminary sweetness testing are detailed in Chapter 2. The biochemical results of the blood sampling during the experiment (glucose and hormones) are outlined in Chapter 3. It is useful to summarize here, that as expected, following oral glucose ingestion but not sweetener, plasma glucose rose modestly but significantly before returning towards baseline (Figure3.1). In keeping with this rise in plasma glucose, insulin levels also rose in these healthy volunteers on oral glucose study days (Figure 3.2). Glucagon levels remained constant throughout studies, being similar on both study days (Figure 3.3).

The fMRI results obtained during viewing of the food and the non-food pictures are presented below.

4.5.1 Results of fMRI brain activation

The data presented here are for activation on viewing all food pictures. Liking was included as a parameter in the model. At the group level an ANOVA model was used reflecting the 2x2 factorial design with stimulus type (food and non food pictures) and pre-scan drink (glucose and aspartame) as independent factors.

In order to maximize sensitivity without unacceptable type I error, analyses was confined to striatal midbrain, hypothalamic and amygdala regions of interest. Hypothalamic and amygdala regions were identified anatomically using the Pickatlas tool (Maldjian JA, 2003) implemented in SPM5. The striatal and midbrain regions were identified using criteria described previously (Murray, 2008). Initially all study days were looked at regardless of the drink consumed to identify which brain areas were activated by the presentation of food images. Using SPM analysis as described above, viewing images of food (as compared to

non-foods) resulted in a discrete pattern of increased activation in the amygdala (26 -4 -12), midbrain (-10 -10 -4) and the anterior (22 12 -6) and posterior (28 -4 -4) putamen. These data, in healthy volunteers following an overnight fast, confirmed that viewing images of foods resulted in fMRI activation in brain areas involved in reward circuitry (Fig. 4.1). No activation was observed in the insular region and hypothalamus. The threshold was set at $p < 0.05$.

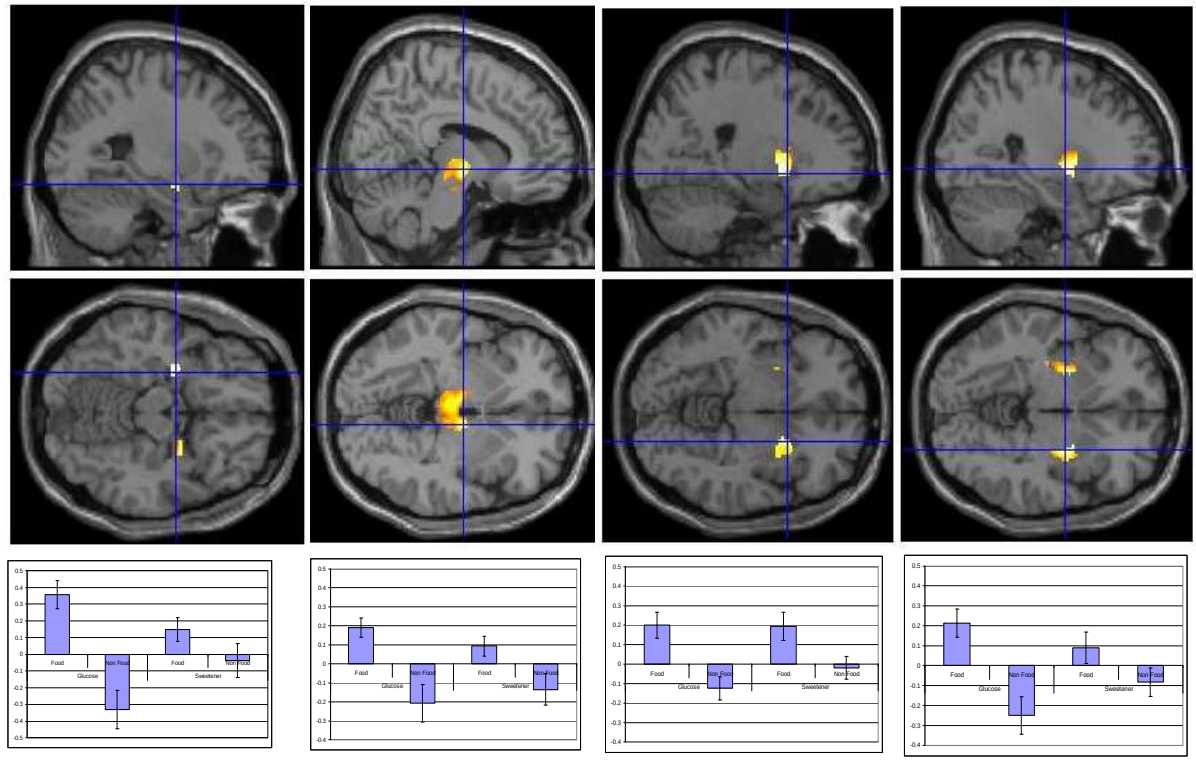

Figure 4.1 Summated fMRI image: Activation with food images

Activation is noted in midbrain, amygdala, and anterior and posterior putamen regardless of type of drink consumed (co-ordinates x y z= 0, 0, 0). Coloured areas indicate significant activation.

Bottom panels show how magnitude of activation in these areas was altered by glucose ingestion * = p<0.05 for interaction indicating difference GLU *vs* CON.

Further, increase in activation was expected in the glucose sensitive areas of the brain following ingestion of sweetener on viewing pictures of the food. This expectation was based on the reasoning that the day the subjects consumed the sweetener (no calories), they were essentially starving and hence their brains would be hungry and light up at the prospect of food. However, we found results to the contrary.

Activation in the areas identified as being responsive to foods was re-examined to study the effects of glucose drink. Contrary to what might be predicted from the glucostat theory, we observed a significant increase in activation induced by food images in the amygdala and posterior putamen with glucose ingestion relative to control drink (Fig.4.2) at $p<0.05$.

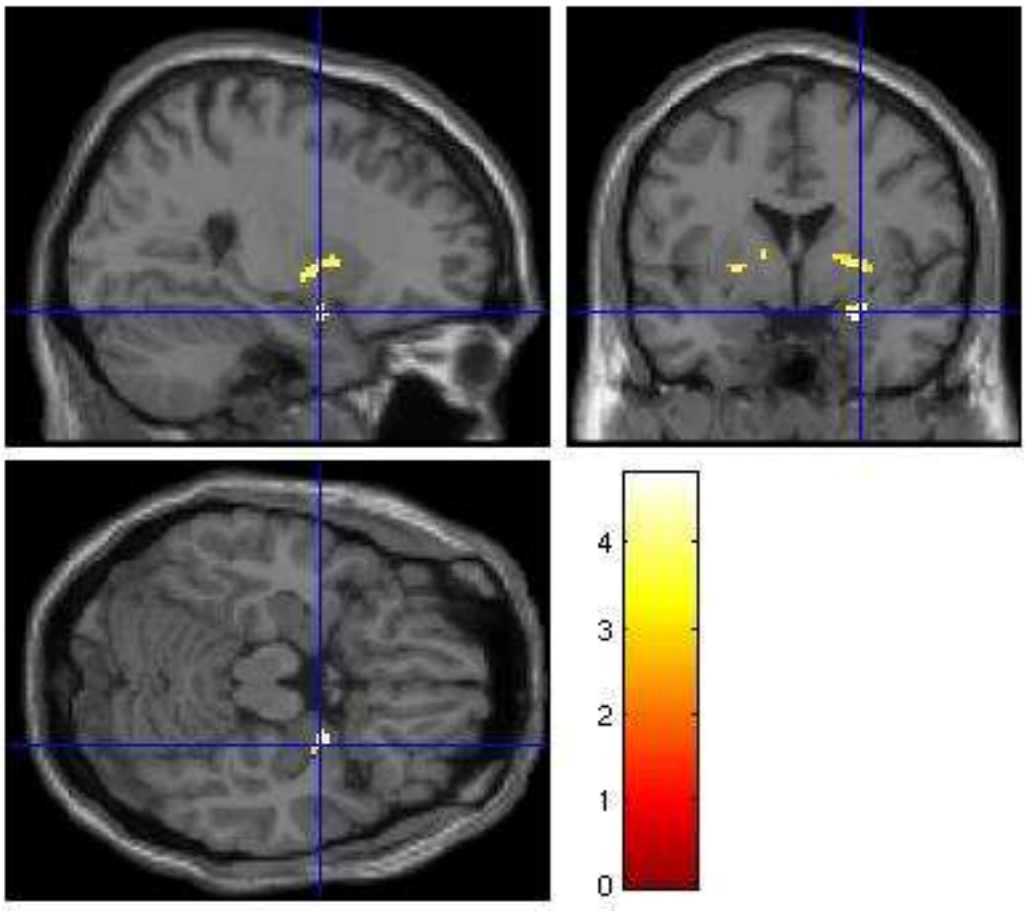

Figure 4.2 Summated fMRI image: Increased activation in amygdala and putamen with food images following glucose (relative to CON)

(Co-ordinates x y z = 26, 0, -17). Coloured areas indicate significant activation.

Finally, the scans were re-examined to identify whether any additional brain regions that were not identified by the initial food *vs.* non-food comparison showed a food-glucose interaction. A further area of activation in the lateral putamen, again a brain area implicated in hedonic circuitry (Fig. 4.3) was identified at $p<0.05$.

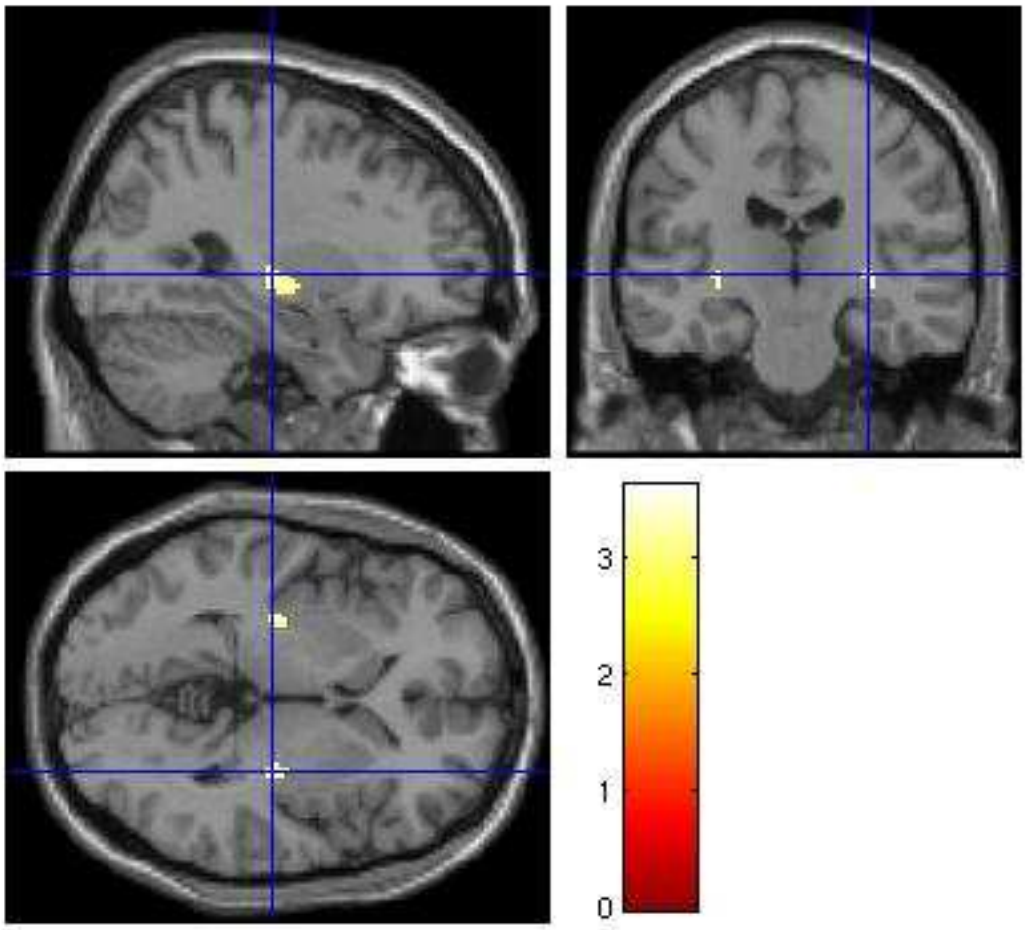

Figure 4.3 fMRI image: Increased activation with food images in lateral putamen following glucose (relative to CON)

(Co-ordinates: x, y, z = 30, -20, 0)

Viewing images of food as opposed to non-foods resulted in a discrete pattern of fMRI activation in the amygdala, midbrain and the anterior and posterior putamen, areas involved in reward processing. Pre-administration of oral glucose was associated with an augmentation of food image-related activation in these areas. There was also a region of lateral putamen that showed a response to such images only when oral glucose had been consumed prior to scanning.

On the other hand, consumption of or priming with sweetener did activate the insular area as expected, as the sweetness was equally matched on both the study days. Thus, though the sweetness of the two solutions were equally matched, the zero-calorie sweetener did not increase the activation of the reward areas of the brain to food pictures any more than that seen on glucose days. This suggests higher assimilation of taste pathways, homeostatic centres judging nutritive content and probably previous knowledge, to drive judgment about further eating.

4.6 Discussion

In summary, the data shows that a 25 g oral glucose load, sufficient to cause a modest rise in blood glucose and insulin in overnight fasted healthy volunteers, resulted in increased responses to food images in the amygdala, posterior and lateral putamen, all areas of brain involved in reward. Given that the drinks were disguised effectively for sweetness, the data suggest that a post-ingestive factor rather than sweet taste is mediating these changes.

What might be the teleological explanation for oral intake of glucose increasing activity in reward pathways when presented with food? Previous descriptions of glucose affecting feeding have focused on the simple initiation of feeding when

hungry and termination when satiated (Grossman, 1986; J. Mayer & Bates, 1952). However, physiological feedback during the act of feeding may affect appetite and nutrient intake. For example, rewarding aspects of the food/ drink being consumed may reinforce nutrient intake. In part this may be mediated by responses, conditioned and unconditioned, generated from the taste, smell and texture of food in the oropharynx (Sclafani, 2006). However, part of the body's ability to gauge the nutritional merit of ingested nutrients may also be "post-ingestive" and related to the energy value. For example, transgenic mice lacking the sweet taste receptor still develop a preference for sucrose ingestion, suggesting the existence of a taste-independent mechanism for detecting the metabolic value of nutrients (Ivan E. de Araujo, et al., 2008). Clearly, the ability to respond positively and rapidly to increase feeding of high-energy foodstuffs might offer an evolutionary advantage.

Goldstone et al studied the interaction between the nutritional status (with and without eating a filling breakfast) and different food stimuli (high calorie and low calorie foods) on brain food reward systems by performing fMRI scans on overnight fasted healthy subjects. In contrast to the results from this study, they demonstrated that fasting (no breakfast) enhanced the subjective appeal of high-calorie foods more than low- calorie foods. This change in appeal was positively correlated with medial and lateral orbito-frontal cortex activation on fMRI scanning. Goldstone et al fed their subjects a 'filling breakfast' consisting of a mixture of nutrients whilst in this study subjects received a fixed dose of 25 g glucose only which makes the results difficult to compare (Anthony P. Goldstone, et al., 2009). Activation was observed in the regions of insula, caudate and putamen whilst Goldstone et al observed increased activation to high

calorific foods in the fasting state in the regions of the medial and lateral orbito-frontal cortex, amygdala, ventral striatum and anterior insular. Whether this variability of regional activation is specific to glucose (rather than a meal effect) cannot be determined from this study.

Central to much reward-driven behavior such as drug addiction (Kalivas & Volkow, 2005) is a mesolimbic circuit in which dopamine-releasing neurons project from the ventral tegmental area to the nucleus accumbens. Palatable foods increase dopamine release in the accumbens (Hernandez & Hoebel, 1988) and ghrelin and leptin probably also exert their actions on food-reward pathways via this circuit (Abizaid, et al., 2006; Holst, 2007). Analogous with this, taste receptor knockout mice described above showed increased dopamine outflow in the nucleus accumbens (ventral striatum) associated with sucrose ingestion (Sclafani, 2006) . If the findings in this study were mediated by increased accumbens dopamine release, how might oral glucose be generating this? By design, direct effects of glucose in GI tract and/or blood stream from indirect effects mediated by glucose induced changes in insulin or incretin signaling were not differentiated, although the available data suggest that insulin or GLP-1 might be expected to decrease appetite (Holst, 2007; Plum, Belgardt, & Bruning, 2006; Porte, et al., 2005). Glucose sensors (glucose-responsive cells) have been identified both outside brain (for example in the portal vein, ideally placed to sense oral nutrients) and within brain in both “homeostatic areas” (hypothalamus and brain stem) and other brain regions including the amygdala (limbic system), and midbrain. In normal weight individuals, in a fasted hungry state, visual food vs. non-food stimuli is reported to produce greater activation in regions including the amygdala, insula and orbitofrontal cortex (Fuhrer, et al., 2008; Gordon, et al.,

2000; Hinton, et al., 2004; Holsen, et al., 2005; LaBar, et al., 2001; Porubská, et al., 2006; Simmons, et al., 2005; St-Onge, et al., 2005; G.-J. Wang, et al., 2004).

It was also considered whether the sweetener used in control studies might exert biological actions to suppress hedonic circuitry, but there is no robust data to support this.

In summary, glucose may have more complex effects on feeding behavior than those originally described, with the data providing evidence for a “feed-forward” mechanism in humans. This work also illustrates the power of fMRI imaging to further the understanding of hedonic circuitry in brain, which may be critical to understand the etiology of human obesity and success of different diets

4.7 Conclusions

The data suggest that a small oral glucose load may increase the perceived reward of foods. One might speculate that this phenomenon may have evolved as a “feed forward” mechanism to switch on feeding rapidly when moving from fasting to intake of high glycemic nutrition which not only ensures further energy intake but also reassures safety (non-poisonous) nature of the food being ingested.

In addition, this study contributes to the growing body of evidence demonstrating an interaction between the homeostatic and hedonic aspects of feeding with various influences, intrinsic and extrinsic influencing the hedonic regions of the brain, which in turn overrides the homeostatic control of energy regulation.

Chapter 5: Discussion

Facilitation of cognitive abilities has been a fascinating subject of research for decades. Memory or cognition is defined as an organism's ability to store, retain and recall information and experiences. Within the long – term memory, memory can be differentiated into two kinds of processes (i) active information processing that is isolated in time and (ii) processes or mechanisms that maintain and consolidate information over extended periods of time.

Encoding and retrieval are active processes that occur at relatively specific points in time; encoding refers to the initial processing of information that potentially instantiates a memory trace, and retrieval refers to newly evolved processing that results from, and often requires access to, prior encoding episodes. Somewhere between these two sets of active processes occur the more temporally distributed processes involved in storage and consolidation, the mechanisms that convert the otherwise transient encoding event into a more enduring form (R. L. Buckner & Koutstaal, 1998).

5.1 How does this study equate with previous reports?

In this study the cognitive effects of a small dose of oral glucose on memory tasks was studied whilst simultaneously studying the regions of brain activation during performance of these tasks, using fMRI. In addition, changes in plasma glucose and hormone levels were also measured.

5.1.1 Dose of glucose

As was expected and as has been reported in various studies (Brandt, et al., 2006; C. Messier, 2004; Sunram-Lea, et al., 2010a), 25 g of oral glucose increased plasma glucose levels significantly well above the baseline and was also significantly higher than those in the aspartame group. For this study, 25 g oral glucose dose was used, rather than a higher dose, as collaborators of the study; Sunram-Lea et al have previously demonstrated facilitative effects on cognition at this dose (see Appendix 2). Moreover, according to the existing literature, glucose facilitative effects in young individuals are observed at lower blood glucose doses than that seen in healthy, older individuals (Claude Messier, 2004). As the average age of the participants was 24 years, 25 g of glucose was thought to be appropriate. There is further thought that higher doses of glucose show beneficial effects in healthy elderly individuals and in people with Alzheimer's disease, where as lowers doses of glucose have been found have facilitative cognitive effects on healthy younger individuals (Claude Messier, 2004).

Human studies comparing glucose effects on cognition using different doses, such as 25 g and 60 g have found variable results with regards to change in blood glucose levels, some reporting significantly different whilst some reporting no significant difference in blood glucose levels (Azari, 1991; Owen, et al., 2010b; Sunram-Lea, et al., 2010a) over similar time periods. These variable results have been attributed to reflect differences in the glucoregulatory control within the sample (Owen, et al., 2010b). There have been other studies that have failed to demonstrate the enhancing actions of glucose. Azari et al gave 30 g and 100 g of glucose solution in a random double-blind triple crossover design. Thirty minutes post-glucose, subjects were shown nouns on a computer monitor and then

administered recall and recognition tests. There was no effect of glucose on memory tests and plasma glucose measures did not correlate with memory test scores (Azari, 1991).

5.1.2 Cognitive performance and glucose

The study was designed to test the effects of glucose (facilitative) on long term verbal recall which has been shown to be enhanced following glucose (Gonder-Frederick, et al., 1987; Messier, et al., 1997; L. M. Riby, et al., 2006; Sunram-Lea, et al., 2002). Encoding has been extensively shown to be facilitated by glucose ingestion (Bernard, et al., 2004; Paul C. Fletcher, et al., 2003). Also the amount of hippocampal activity at the time of encoding has been shown to predict how well that item is subsequently remembered (Brewer, et al., 1998; Kirchhoff, et al., 2000; A. D. Wagner, et al., 1998).

The hypothesis was to see improved performance on the retrieval task following ingestion of glucose However, with loss of data on the 'retrieval' task due to technical issues, it was difficult to interpret the results from the encoding task.

The cognitive performance task (CPT) was included to test for attention and to mark it as a reference to assess the attentiveness on the other tasks. According to the existing literature, this test is not considered to be sensitive to glucose manipulation (C. A. Manning, et al., 1990) and the results from the current study were equivocal.

The working memory is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and

problem solving (E. E. Smith & Jonides, 1998). This task has not been shown to be sensitive to glucose induced facilitation of task performance.

Various studies have demonstrated the importance of task choice and difficulty while studying the effects of glucose on cognitive performance. Unless the task at hand is challenging enough, glucose fails to show its facilitative actions. In fact, researchers have included dual tasks such as hand movement during memorization of lists of words to try and dissociate the concentration at the time of encoding and then study the performances in the presence and absence of glucose (Sunram-Lea, et al., 2002). This tactic has been shown to uncover the facilitative effects of glucose, suggesting that the possible "depletion" of episodic memory capacity and/ or glucose-mediated resources in the brain due to performing a concomitant task might be crucial to the demonstration of a glucose facilitation effect. Messier et al, did not find a differential effect of glucose when university students were asked to remember high-imagery words versus the more difficult low-imagery words (Messier, et al., 1999).

As this study included fMRI brain imaging, it was difficult to administer a concurrent motor task, as not only was there minimal room for movement in the scanner, but significant movement would have deteriorated the image quality of the brain scans. It has been observed by Sunram-Lea et al, that glucose facilitation on memory is fractionated, that is to say that glucose facilitates only certain aspects of memory, such as verbal long term memory as shown in the CVLT task, and that the facilitation is not global.

Other factors which have been noted to influence glucose-facilitatory effects on cognition are timing of the drink, the dose of glucose and the effect of learning. Gold et al showed that both pre and posttraining administration of glucose in

humans improved memory for a paragraph recall (C. A. Manning, et al., 1992). Studies have employed pretraining (C. A. Manning, et al., 1990), posttraining (C. A. Manning, et al., 1992) or just prior to recall (C. A. Manning, et al., 1998) glucose administration and have observed facilitative effects on learning, memory and retrieval of information.

Repeated exposure or practice has been shown to facilitate performance. Studies showing reliable glucose facilitation of long term memory performance following 25 g glucose have generally used repeated exposure to the to-be remembered material following 25 g glucose (Scholey & Kennedy, 2004). Practice and repetition aid in learning episode and the memory trace is strengthened through repetition of stimuli (Owen, et al., 2010b). In this study, the participants were initially briefed about the upcoming tasks and had one run of practice of the cognitive tasks on a laptop computer prior to entering the scanner. The practice runs did not include the words, numbers or pictures included in the test paradigm. Thus the perception was that there was minimal learning effect on the observed outcomes, if any.

Though it is unlikely, stress hormones such as epinephrine can sometimes confound the results. Stress hormones such as epinephrine could potentially interact with the action of glucose on memory, by either producing an additive facilitating or impairing effect on memory or contributing to increased variability because subjects do not necessarily react the same way to stressful stimuli (Claude Messier, 2004). The current study was a relatively simple study involving a single venous cannulation prior to the onset of the study. Understandably, cannulation can induce a stress response (with release of catecholamines and cortisol) and so also stress can be induced by being inside a

MRI scanner. However, the drink/ study days were randomised, thus the contribution of stress, if any would be counterbalanced across both days. Levels of stress hormones were not measured in this study.

Finally, as is well known, there is a close correspondence between the rise in blood glucose and the rise in blood insulin following an oral glucose load and it is hard to dissociate the effect of glucose from that of insulin in the mediation of the central effects of ingested or injected glucose. Insulin, in its own right has been shown to have cognitive-enhancing properties. In rodents, small doses of insulin (0.4-0.8 units/kg) to reverse the amnesia produced by a 2 mg/kg scopolamine injection (Blanchard & Duncan, 1997; Claude Messier, 2004) and to facilitate memory following intracerebrovascular injection (Park, et al., 2000). Euglycemic clamps in humans have also been shown to enhance memory, where glucose levels are held constant whilst insulin levels are raised (S. Craft, et al., 1999).

Though this study was well designed, based on previous work and evidence to demonstrate the facilitative effects of glucose on certain aspects of cognition, it was disappointing that the study was rendered less powerful to prove the hypothesis that glucose facilitates cognitive performance, due to adverse technical problems leading to loss of valuable data.

Moreover due to the simplicity of the task, performance at baseline (or at the sweetener stage) was probably at a ceiling level, leaving little room for improvement post glucose-ingestion. This has been quoted to be a plausible cause for failure to see gluco-facilitation on cognitive tasks. The cohort of graduate students from the University of Cambridge was probably, a skewed cohort of intellectually higher achievers.

5.2 Cognitive performance and brain activation patterns on fMRI

Neuroimaging allows us to observe the subset of memory processes described as active memory processes (encoding and retrieval) that can be isolated in time (R. L. Buckner & Koutstaal, 1998). On the other hand, neuroimaging is unlikely to demonstrate areas involved in temporal distribution of processes related to storage and consolidation (R. L. Buckner & Koutstaal, 1998). Apart from the previously alluded to limitations of functional neuroimaging, it is unlikely to resolve directly flow of information processing that occurs over very brief time scales, such as the order of 10s or milliseconds. However, these processes can be observed with electrophysiological recording techniques (R. L. Buckner & Koutstaal, 1998).

One of the aspects of the study was to look at and map out areas of the brain engaged in performances of these cognitive tasks and to investigate possible differential patterns of activation following ingestion of glucose to reflect differences in cognitive performances.

During performance of the working memory task, activation was noted in the regions of the middle/inferior frontal gyrus, inferior parietal cortex bilaterally on both glucose and sweetener days, similar to that observed by other researchers. The hemispheres of the cerebellum and the midbrain were also activated on the glucose days. The superior temporal cortices on both sides were additionally activated on sweetener days during performance of this task. There was no difference between the drink conditions on the brain activation patterns. The verbal working memory task has consistently demonstrated activation of the

prefrontal and parietal regions in humans, on fMRI imaging (G. D. Honey, et al., 2000). Although frontal lobe activation is often bilateral, the left ventrolateral frontal cortex appears to be primarily concerned with the maintenance of verbal information whereas the right ventrolateral frontal cortex is more involved with maintenance of spatial information (P. C. Fletcher & Henson, 2001). Furthermore, it has been suggested that the ventrolateral frontal cortex is activated during tasks requiring maintenance of information and the dorsolateral frontal cortex is more involved during tasks requiring manipulation of information (P. C. Fletcher & Henson, 2001). In another experiment, prefrontal activation has been shown to increase parametrically in relation to working memory load (Braver, et al., 1997). This was not observed in this study. The prefrontal activations tracking memory load were located in several areas of dorsolateral prefrontal cortex including portions of anterior frontal-operculum and the inferior frontal gyrus that directly overlap with areas activated by deep encoding, as has been described below. Beside the prefrontal cortex, the parietal cortex is also involved in working memory (Suchan, 2008). Most important in a working memory context are connections between the parietal and frontal lobes (Jonides, et al., 1993).

During performance of the CPT task, robust activation was seen in the regions of the inferior frontal gyrus, superior temporal/inferior parietal cortex on the left and the right sides on both the study days. Activation was also noted in the anterior cingulate/medial prefrontal cortex under both glucose and sweetener conditions. In addition, on the CON days activation was also noted in the anterior cingulate/medial prefrontal cortex, midbrain, cerebellum, right and left thalamus and the left putamen. Similar to our study, Ogg et al (Ogg, et al., 2008)

performed fMRI in 30 healthy adults during performance of Conner's Continuous Performance Test (CPT) (subjects viewed letters on a computer screen and were instructed to respond to every letter except the target letter X). They demonstrated an extensive neural network that was activated during the task and included the frontal, cingulate, parietal, temporal, and occipital cortices; the cerebellum and basal ganglia.

Due to loss of performance data (retrieval), the imaging data during performance of the encoding task was not thought to reveal useful information with regards to successful encoding attempts. Hence these images were not analysed.

The literature describing the brain regions involved during the encoding process have demonstrated robust left pre-frontal activation overlapping with the regions activated by the word generation tasks (E. Tulving, Kapur, Markowitsch, et al., 1994). Fletcher et al have demonstrated activation of the hippocampal system during memory tasks such as encoding of faces, words, scenes or objects (Bernard, et al., 2004). Fletcher et al have also previously demonstrated differences in brain activation seen at different levels of encoding, with deep encoding during learning trails resulting in left prefrontal cortex and medial temporal lobe activation (Paul C. Fletcher, et al., 2003). Kapur et al have suggested that when subjects process verbal stimuli in a semantic manner either under experimental or real life conditions, this involved increased neuronal activity in the left inferior pre-frontal cortex. Increased activity in this region, irrespective of the individual's intention to remember, lead to a more readily retrievable memory trace (Kapur, et al., 1994).

Buckner and Tulving (R. Buckner, & Tulving, E. , 1995) proposed that these functional neuroimaging studies demonstrate how multiple kinds of information

processing might interact to promote long-term memory, as is evidenced by overlapping areas of encoding and working memory processes in the brain. Effortful word generation tasks, verbal working memory tasks, and long term memory encoding tasks all activate similar brain pathways including left prefrontal regions and related structures. Processing that requires verbal elaboration (deep processing) appears to activate left prefrontal cortex selectively whereas well automated tasks involving verbal information (shallow tasks) do not (Demb, et al., 1995). Shallow encoding tasks thus may lead less often to the formation of explicit long term memories because they do not initially require representation of the information in prefrontal cortex, the anatomical substrate that supports higher level representations necessary for conscious retrieval.

5.2.1 Food imagery and brain activation patterns

The final aspect of the study introduced a new dimension to the overall focus of the study. It looked at the brain activation patterns following visualization of food images under different physiological conditions (glucose vs. sweetener). It was commenced following seminal work by Farooqi et al showing the modulating effects of leptin on limbic regions on the brain regulating appetite. Mayer proposed the ‘glucostat theory’ 50 years ago, suggesting the role of glucose in the initiation and termination of meals. Results from the leptin study facilitated the hypothesis that glucose too could potentially influence hedonic regions of the brain such that it would manipulate appetite.

Glucose being the primary fuel for the brain is ideally placed to manipulate appetitive behaviour. Indeed, as limbic regions of the brain were being imaged as part of the glucose-cognitive study, it was possible to study the effect of glucose

on appetite by using visual stimuli in the form of pictures of food with little change in the study design. It is important to point out that though this aspect of the study was prompted by the leptin work they were not identical with respect to the study design. Farooqi et al studied two children with congenital leptin deficiency under fasted and fed (meal) conditions. The current study looked at the brain activation patterns to food images following 25 g glucose and sweetener conditions in healthy adults. Though one might argue that the sweetener condition could equate to a fasted state, the 25 g glucose state would not equate to a fed state or post-meal situation. Thus though the food imagery paradigm used for this part of the study was identical to that used by Farooqi et al, the protocol of the study was distinct. The task included pictures of food and non-food (neutral, everyday) items to study the brain activation patterns on viewing pictures of food (as compared to neutral non-food items) under different physiological states (glucose vs. no glucose).

Recognizing that palatability and thus liking of food varies on individual basis, the data were interpreted depending on each individual's rating of liking a specific food item. Following glucose ingestion, increased responses to food images was observed in the amygdala, posterior and lateral putamen, all areas of brain involved in reward.

Goldstone et al studied the interaction between the nutritional status (with and without eating a filling breakfast) and different food stimuli (high calorie and low calorie foods) on brain food reward systems by performing fMRI scans on overnight fasted healthy subjects. In contrast to our results they demonstrated that fasting (no breakfast) enhanced the subjective appeal of high-calorie foods more than low- calorie foods. This change in appeal was positively correlated

with medial and lateral orbito-frontal cortex activation on fMRI scanning. Goldstone et al fed their subjects a 'filling breakfast' consisting of a mixture of nutrients whilst we gave our subjects a fixed dose of 25 g glucose only which makes the results difficult to compare (Anthony P. Goldstone, et al., 2009). In the current study, activation was observed in the regions of insula, caudate and putamen whilst Goldstone et al observed increased activation to high calorific foods in the fasting state in the regions of the medial and lateral orbito-frontal cortex, amygdala, ventral striatum and anterior insular. Whether this variability of regional activation is specific to glucose (rather than a meal effect) cannot be determined from this study.

What might be the possible reason for oral glucose increasing activity in reward pathways when presented with food? Previous descriptions of glucose affecting feeding have focused on the simple initiation of feeding when hungry and termination when satiated (Grossman, 1986; J. Mayer & Bates, 1952). However, physiological feedback during the act of feeding may affect appetite and nutrient intake. For example, rewarding aspects of the food/ drink being consumed may reinforce nutrient intake. In part this may be mediated by responses, conditioned and unconditioned, generated from the taste, smell and texture of food in the oropharynx (Sclafani, 2006). However, part of the body's ability to gauge the nutritional merit of ingested nutrients may also be "post-ingestive" and related to the energy value. For example, transgenic mice lacking the sweet taste receptor still develop a preference for sucrose ingestion, suggesting the existence of a taste-independent mechanism for detecting the metabolic value of nutrients (Ivan E. de Araujo, et al., 2008). Clearly, the ability to respond positively and rapidly

to increase feeding of high-energy foodstuffs might offer an evolutionary advantage.

The results of this study probably provide some merit to the feed-forward theory. Mook et al have shown that a rat that is satiated for glucose in solution, and will drink no more of it when access is prolonged, will promptly return to ingestion when offered laboratory chow, milk, or even glucose itself in powdered form (D. G. Mook, et al., 1986). The feed-forward component appears necessary because this happens only when, and if, the chow or powder is actually encountered.

Central to much reward-driven behavior such as drug addiction (Kalivas & Volkow, 2005) is a mesolimbic circuit in which dopamine-releasing neurons project from the ventral tegmental area to the nucleus accumbens. Palatable foods increase dopamine release in the accumbens (Hernandez & Hoebel, 1988) and ghrelin and leptin probably also exert their actions on food-reward pathways via this circuit (Abizaid, et al., 2006; Holst, 2007). The observatiosn in this study cannot be solely attributed to the effects of glucose, as has been alluded to before.

On balance, this study was better designed matching for sweetness than comparing with unsweetened water for example. The study design might also extrapolate better to real world where sweetener might be the substitute for glucose, and our data suggest use of sweeteners in place of glucose might offer an advantage beyond simple caloric substitution for those looking to curb appetite and lose weight. In summary, the visual food-stimulus study found that glucose may have more complex effects on feeding behavior than those originally described, with the data providing evidence for a “feed-forward” mechanism in humans. The work also illustrates the power of fMRI imaging to

further the understanding of hedonic circuitry in brain, which may be critical to understand the etiology of human obesity and successes of different diets.

5.3 Study limitations

Though our study was well designed, based on previous work and evidence to demonstrate the facilitative effects of glucose on certain aspects of cognition, it is unfortunate that the study yielded negative results with respect to the hypothesis set at the outset for cognitive performance.

One of the contributing factors for this was the loss of valuable performance data (encoding-retrieval) due to adverse technical problems. Other possible explanations for failure to observe gluco-facilitatory effects on cognition include insufficient task difficulty, ceiling effect of performance in the cohort of Cambridge University students. As performance difference was not documented between the 2 drink conditions, it is not surprising that brain activation patterns were not different in the two groups. It is also possible that the study was subjected to limited power of n = 13.

Albeit the results of the second part of the study looking at brain activation patters on viewing food imagery did not comply with our hypothesis, it produced fascinating results. This study finding might also explain the reported efficacy of low glycemic index or low carbohydrate diets such as the ‘Atkins diet’. One of the mechanistic explanations offered is that high glucose leads to high insulin and rebound fall in glucose leading to further feeding. This data suggests an alternative explanation that the glucose load associated with ingestion of high glycemic-index carbohydrates triggers hedonic circuits, although it is important

to emphasize that different diets were not directly examined in this study and that further work would be needed to detail this.

The results from this study have prompted further exploration of the appetite pathways to try and decipher the results of the study. Possible investigative questions arising from this study has been explored in the following chapter.

Chapter 6: Future work

This study was set out to investigate the effects of a small dose of oral glucose on cognitive performance. In addition, the brain activation patterns on viewing images of food both in the presence and absence of a small dose of oral glucose was also studied.

Differential brain activity patterns on viewing pictures of food versus non-food, more so following ingestion of glucose was observed. Though, this is a novel finding on its own, it is difficult to solely attribute the brain activation to the ingestion of glucose per se. It is well known that post-ingestive factors, such as insulin, PYY_{3-39} influence the limbic regions in a similar fashion. The current study design does not exclude this possibility. Thus, it would be interesting to study this observation further by performing a pancreatic clamp during fMRI and measuring the levels of metabolic hormones, such as PYY_{3-39}, insulin. This would facilitate delineating the effects of insulin from those of glucose.

Though 25 g glucose, as used in this study resulted in a modest but significant rise in blood glucose, it does not by any means equate to a full meal. Rational thinking might prompt one to expect reduced brain activation to food pictures in the presence of glucose as substrate, as glucose would reduce the homeostatic drive to feed. Instead, glucose seemed to "prime" the hedonic regions of the brain, such that consumption of glucose actually made food pictures more appealing, as evidenced by more intense brain activation in the limbic regions. Further, no difference in brain activation patterns on viewing foods with high

caloric value versus those with low caloric value, in the presence of glucose was observed.

Based on these preliminary results, it would be interesting to see if there was differential brain activation in the limbic regions on viewing high vs. low calorific food pictures when the individual was calorie replete. If this was the case, it would potentially indicate overriding of the homeostatic system of energy control (intake) by the hedonic systems. To be able to decipher this, one could image the brains of candidates whilst they were sipping drinks of varying caloric content inside the scanner, for example, 25g, 50g and 75 g glucose (or equivalent sweetness) and view food and non-food pictures, simultaneously. Would the brain regions be as active on viewing high calorific foods in presence of 75 g of glucose instead of 25 g of glucose? Would there be a switch in brain activation areas when the individual was calorie replete? Questions such as these need further explanation and testing.

These studies could potentially further our understanding of the most sought after explanation for the drive to eat. With the growing incidence and prevalence of obesity, its associated co-morbidities and implicit health costs, advancement in biology of appetite regulation and controllers of feeding behavior is of paramount significance. Lessons learnt from these and similar studies could be translated to benefit patient management over a wide spectrum of disorders. For example, knowledge of contribution of the limbic system in the control of appetite and food choices, could suggest a role for psychotherapy in the treatment of obesity. As the limbic regions have traditionally been shown to be implicated in addiction disorders, measures used to tackle substance abuse, could also be implemented to address obesity disorders.

Glossary

Abbreviation	Full form
IFR	Immediate Free Recall
DFR	Delayed Free Recall
AC	Anterior Cingulate Cortex
Acb, NAc	Nucleus Accumbens
AIC	Agranular Insular Cortex
AMY/ AMG	Amygdala
AP	Area Postrema
aPFC	Anterior Prefrontal cortex
ARC	Arcuate Nucleus
aTL	Anterior Temporal Lobe
aTL	Anterior Temporal Lobe
BLA	Basolateral Amygdala
BMI	Body Mass Index (Kg/m^2)
BOLD	Brain Oxygen Level Dependant
CCK	Cholecystokinin
CeN	Central Nucleus of Amygdala
CNS	Central Nervous System
CPT	Continuous Performance Testing
CRH	Corticotrophin-releasing Hormone
CVLT II	Californian Verbal Language Test II

DLPFC	Dorsolateral Prefrontal Cortex
dmnX	Dorsal Motor Nucleus of Vagus
DS	Dorsal Striatum
ECF	Extra-cerebrospinal fluid
EEG	Electroencephalogram
FDG-PET	Fluro-deoxyglucose Positron Emission Tomography
fMRI	Functional magnetic resonance imaging
GABA	Gamma amino butyric acid
GLUT 1	Glucose uptake transporter 1
GLUT 3	Glucose uptake transporter 3
GLUT 4	Glucose uptake transporter 4
GP	Globus Pallidus (D, dorsal; V, ventral)
HbA1c	Haemoglobin A1c
HIP/ Hipp	Hippocampus
HPA	Hypothalamo-adrenal axis
IOFC	Inferior Orbitofrontal Cortex
IX	Glossophrangeal Nerve
K-ATP	Potassium-Adenosine Triphosphate channel
KO	Knock-out
LH	Lateral Hypothalmus
LHA	Lateral Hypothalamic Area
LTM	Long term memory

MEG	Magnetoencephalogram
MNI	Montreal Neurological Institute
mOFC	Medial Orbitofrontal Cortex
MoN	Motor Nuclei for Oromotor Control
mPFC	Medial Prefrontal Cortex
NAc	Nucleus Accumbens
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide-Y
NTS	Nucleus of Tractus Solitarius
OFC	Orbitofrontal cortex
OLF	Olfactory Bulb
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PIR	Piriform Cortex
PIT	Pituitary Gland
PRL	Prelimbic Cortex
PVN	Paraventricular Nucleus
PVN	Paraventricular Nucleus of the Hypothalamus
PYY/ PYY_{3-36}	Polypeptide YY
rCBF	Regional Cerebral Blood Flow
RF	Medullary reticular Formation
RVLM	Rostroventrolateral Medulla
SAM axis	Sympatho-adrenal axis

SNc	Substantia Nigra pars compacta
SNS	Sympathetic Nervous System
STM	Short Term Memory
STS	Superior Temporal Sulcus
T1DM	Type 1 diabetes
Thal	Thalamus
V	Trigeminal Nerve
V1/V4	Visual Processing Areas 1, 4
VII	Facial Nerve
VMH	Ventromedial hypothalamus
vmPFC	Ventromedial Prefrontal Cortex
VS	Ventral Striatum
VTA	Ventral Tegmental Area
VTA	Ventral tegmental Area
WM	Working Memory
WT	Wild-type

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Appendices

Appendix 1 Research Plan

Specific Aims

1. To examine the effects of changes in blood glucose on memory function and upon accompanying patterns of brain activity.
2. To identify memory differences between non-diabetic subjects, and patients with type 1 diabetes (T1DM) with and without exposure to severe hypoglycemia. These differences will be characterized in terms of performance deficits and changes in brain activation.
3. To examine whether differences occurring as a consequence of exposure to severe hypoglycemia in T1DM are reversible.
4. To examine also fMRI activation of glucose-sensing areas of brain at different glucose nadirs in T1DM patients with and without hypoglycemia unawareness

b) **Background and Significance:**

Intensive lowering of blood glucose levels in type 1 diabetes (T1DM) reduces risk of chronic complications of diabetes but is associated with an increased risk of suffering from severe hypoglycemia (DCCT, 1993). This is, in large part, a consequence of the non-physiological nature of current insulin replacement regimens with subcutaneous injections or pumps, so that even the most careful and best-controlled T1DM patients will experience fluctuations in blood glucose levels throughout the day, being exposed to blood glucose levels that may be higher and/ or lower than normal. Glucose is essential for the normal functioning

of the central nervous system (CNS) with conventional wisdom being that brain contains relatively small amounts of stored carbohydrate fuel. Brain metabolism and neurocognitive functioning are thus dependent upon a continuous supply of circulating glucose. The outcome of this work will significantly advance our understanding of the effects of recurrent hypoglycaemia on cognition in humans and may ultimately aid in the prevention or retardation of cognitive impairments associated with diabetes and/or hypoglycaemic episodes."

The deleterious effects of acute hypoglycemia on the brain are well known to T1DM patients and their carers and families, with low blood glucose levels frequently resulting in slowed and muddled thought processes, and even lethargy or frank coma if levels fall low enough. In keeping with this, psychological tasks requiring attention and concentration and those engaging psychomotor skills and mental tracking (e.g. 4-choice reaction and Stroop and trail making tests) are sensitive to acute experimental hypoglycemia (Evans, Pernet, Lomas, Jones, & Amiel, 2000). Certain other aspects of cognition, however, are relatively resistant to the effects of hypoglycemia. Some studies have reported that memory is resistant to the effects of acute hypoglycemia, with performance on a variety of memory tasks being unaffected by a moderate decrease in blood glucose (Mellman, Davis, Brisman, & Shamoon, 1994; Meneilly, Cheung, & Tuokko, 1994). Most of the more recent studies, however, suggest that a number of memory domains (discussed in more detail later) are sensitive to acute hypoglycemia. (Sommerfield, Deary, McAulay, & Frier, 2003). Whether recurrent hypoglycemia results in long-term effects on brain and cognition remains uncertain. A number of case reports have documented cognitive problems- and in particular problems with memory- in T1DM subjects

with a history of recurrent severe hypoglycemia (A. E. Gold, Deary, I.J., Jones, I. J., O'Hare, J.P., Reckless, J.P., Frier, B.M., 1994). Very deep and prolonged hypoglycemia/ aglycemia undoubtedly results in irreversible structural brain damage, with certain brain areas being particularly susceptible to damage. These include areas involved in memory such as the hippocampal CA1 region and dentate gyrus (Auer, 1984). Such serious and prolonged hypoglycemia is fortunately rare in clinical practice but we do not know if more modest hypoglycemia as may be experienced by some T1DM patients may result in cumulative structural and/or functional damage to medial temporal lobe areas, including the hippocampus resulting in permanent cognitive impairment. Young rats exposed to recurrent moderate hypoglycemia (more representative of that experienced by T1DM patients) demonstrate more subtle changes in the hippocampus, with no apparent neuronal necrosis but with a loss of neuronal synaptic plasticity (Yamada, et al., 2004). A number of cross-sectional studies have reported decrements in a variety of cognitive tasks including memory in T1DM patients with a history of recurrent severe hypoglycemia (Langan, Deary, Hepburn, & Frier, 1991; Lincoln, 1996). In particular, the developing brain seems to be especially sensitive to hypoglycemia (Hannonen, Tupola, Ahonen, & Riikonen, 2003; Hershey, Lillie, Sadler, & White, 2004). In contrast, other studies, including prospective follow up analysis of patients from the DCCT and Stockholm studies did not find such a relationship (DCCT, 1996; Kramer, et al., 1998; Ryan, Williams, Finegold, & Orchard, 1993).

Where reported, all of these assessments of cumulative effects of recurrent hypoglycemia were performed with subjects at euglycemia. A number of studies have also looked at the effects of antecedent hypoglycemia on

subsequent cognitive performance during hypoglycemia in humans. Reports have been mixed with studies demonstrating that previous hypoglycemia may worsen (Lobmann, et al., 2000), have no effect on (Maran, Lomas, Macdonald, & Amiel, 1995), or mitigate (Veneman, Mitrakou, Mokan, Cryer, & Gerich, 1994) cognitive deficits during subsequent hypoglycemia. A recent study examining both healthy and diabetic rats exposed to recurrent hypoglycemia (RH) showed an interesting anomaly, with RH animals performing better than controls on maze testing- a memory task- during euglycemia but significantly worse than control rats during hypoglycemia (McNay & Sherwin, 2004). One possible interpretation of this work is that the hippocampus adapted to prior occurrences of hypoglycemia by upregulating the transport of glucose from blood into brain. This hypothesis is supported by microdialysis measurements of hippocampal glucose, which normally show a dip in control studies during maze testing (McNay, Fries, & Gold, 2000). RH rats were protected against the task associated dip in hippocampal glucose- a finding that was associated with better maze performance when tested at euglycemia. During hypoglycemia, however, hippocampal glucose levels plummeted during maze testing in diabetic RH rats, consistent with the marked fall in performance. If such a phenomenon occurs in humans, it is possible that the damaging effects of hypoglycemia on memory may be missed by studies that test subjects only at euglycemia. Our current proposal (described later) includes the intention to look specifically for this in T1DM patients exposed to recurrent severe hypoglycemia.

Although less clinically apparent than the effects of acute hypoglycemia, there is now considerable evidence that acute hyperglycemia may also affect neurocognitive functioning. A number of studies have demonstrated that

intraperitoneal glucose administration improves cognitive performance in rodents (Croul, 1986; Ferguson, et al., 2003; P. E. Gold, 1991, 1992; Wenk, 1989; White, 1991). Microdialysis measurements of rat brain glucose have shown that hippocampal ECF glucose levels fall during maze testing (a memory task), suggesting that metabolic demands associated with cognitive performance in the hippocampus are limited by glucose supply, even in non-diabetic rats at euglycemia. The fall in ECF glucose can be prevented by (intraperitoneal) administration of glucose, correlating with enhanced performance on maze testing (McNay, et al., 2000). Similarly, studies performed in Sünram-Lea's and Foster's laboratories and elsewhere have shown that oral glucose administration enhances memory, in particular declarative long-term memory (LTM), in healthy non-diabetic humans (Gonder-Frederick, et al., 1987; Messier, Gagnon, & Knott, 1997; Sunram-Lea, Foster, Durlach, & Perez, 2002; Sünram-Lea, Foster, Durlach, & Perez, 2004). Declarative LTM is memory for events or materials - such as word lists or stories - which can be consciously brought to mind and described some time after the materials were originally learned, and after a sufficient delay has elapsed (or sufficient competing mental activity has taken place) for the information to be no longer held in short-term memory (STM). Declarative LTM is essentially memory for past events or materials and there is clear evidence that it is highly dependent upon the hippocampus with the left hippocampal region differentially mediating memory of verbal materials and the right hippocampal region mediating non-verbal (e.g. spatial) memory (Aggleton & Brown, 1999).

Finally, although not a classical end-organ for diabetic damage, there is also evidence that chronic hyperglycemia may adversely affect the brain,

resulting in a “diabetic encephalopathy” (Ferguson, et al., 2003; Ryan, et al., 1993). In keeping with this, a number of studies have suggested that T1DM subjects perform less well than non-diabetic patients on a variety of cognitive assessments, although it is worth emphasising that in general the differences were relatively subtle (reviewed by Brands 2004). Suggested mechanisms for this have included oxidative stress, microvascular disease and the effects of brain insulin receptors. Rodent studies have demonstrated increased apoptosis in the hippocampus of BB/ Wor rats- a model of T1DM accumulating over time with diabetes (Li, Zhang, & Sima, 2002).

To summarise the above, memory tasks are probably sensitive to acute hypoglycemia. Recurrent severe hypoglycemia may cause memory problems, possibly because of the sensitivity of neurons in areas such as the hippocampus to hypoglycemia. Acute hyperglycemia improves certain aspects of memory (LTM), probably by delivering fuel to key brain areas including the hippocampus. Finally, chronic hyperglycemia may also result in cognitive deficits.

However, there is much that remains unknown about the influence of different patterns of hypo- and hyperglycaemia upon human cognitive function in healthy and in diabetic subjects. In this work, we plan to examine the effects of diabetes and glycemia on memory by combining cognitive testing with functional magnetic resonance imaging (fMRI). This will enable us to examine brain areas mediating the effects of changes in glucose levels on memory performance. Functional MRI is dependent on coupling of neuronal activity with energy metabolism and blood flow, and uses non-invasive blood oxygenation level dependent (BOLD) activation to indicate areas of altered blood flow and

thus increased brain activity. One published study used fMRI to examine brain activation during cognitive function during acute hypoglycemia, but looking at non-memory tasks such as finger tapping and simple and choice reaction time in healthy volunteers. Hypoglycemia was associated with task-specific localized changes in brain activation (Rosenthal, et al., 2001)

Studies conducted by Fletcher and others have used fMRI to show activation of the hippocampal system during memory tasks such as encoding of faces, words, scenes or objects (Bernard, et al., 2004). The amount of hippocampal activity at the time of encoding predicts how well that item is subsequently remembered (Brewer, et al., 1998; Kirchhoff, Wagner, Maril, & Stern, 2000; Wagner, et al., 1998), termed the 'subsequent memory effect' or the 'difference due to memory' (Dm) effect (Paller, Kutas, & Mayes, 1987). Our protocol will examine Dm effects during the encoding phase within the glucose memory facilitation framework. Fletcher has previously demonstrated differences in brain activation seen at different levels of encoding, with 'deep' encoding during learning trials resulting in left prefrontal cortex and medial temporal lobe activation (Paul C. Fletcher, Stephenson, Carpenter, Donovan, & Bullmore, 2003). As described later, by implementing the same 'level of processing' approach, we intend to investigate the brain regions that are differentially sensitive to glucose administration under different encoding conditions ('deep' versus 'shallow' encoding). In addition to declarative LTM, we also intend to examine working memory. Working memory is a limited capacity system for the simultaneous maintenance and manipulation of information which is fundamental to a broad range of cognitive processes, including reasoning, language comprehension, and problem solving (Smith &

Jonides, 1998). Previous fMRI studies have consistently demonstrated involvement of prefrontal and parietal regions in verbal working memory in humans (G. D. Honey, Bullmore, & Sharma, 2000). Although frontal lobe activation is often bilateral, the left ventrolateral frontal cortex appears to be primarily concerned with the maintenance of verbal information whereas the right ventrolateral frontal cortex is more involved with maintenance of spatial information (P. C. Fletcher & Henson, 2001). There may also be anatomical divisions within the frontal cortex that subserve different processes with the ventrolateral frontal cortex being activated during tasks requiring maintenance of information, and the dorsolateral frontal cortex being more involved during tasks requiring manipulation of information (see Fletcher and Henson 2001 for review).

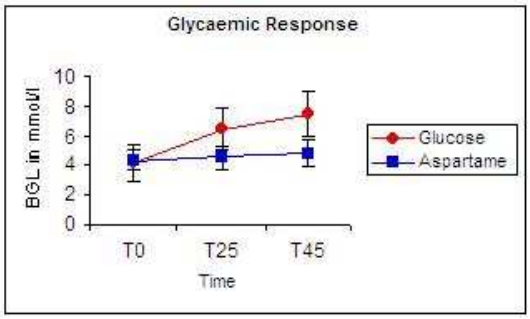
Finally, although not the primary aim of our work, it is also possible that we may be able to identify areas of brain activation associated with changes in glucose levels. For example, hypothalamic activation has been reported following an oral glucose load (Liu, Gao, Liu, & Fox, 2000; Matsuda, et al., 1999). It is also possible that hypoglycemia may also result in changes in brain activation patterns in glucose-sensing areas in the hypothalamus and brain stem. Comparing these patterns in T1DM patients with/ without hypoglycemia unawareness in study 2 below may allow us to visualize differences in brain activation occurring as a consequence of changes in brain glucose sensing associated with the syndrome of hypoglycemia unawareness/ impaired counterregulation. A previous report used positron emission tomography (PET) brain imaging to identify a subthalamic brain region with a significantly different response pattern between the aware and unaware groups (Iain Cranston, Reed,

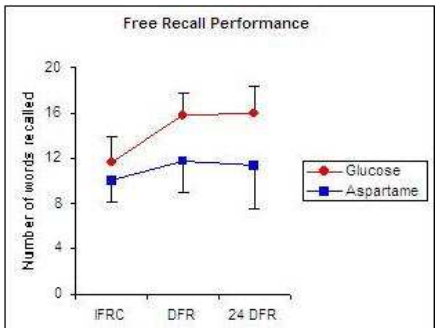
Marsden, & Amiel, 2001). We are aware that other groups, including some funded by the JDRF are currently using fMRI to look at this. It is possible that information collected from our study might complement the attempts of others to look at this phenomenon.

Significance of this work for type 1 diabetes: This work will significantly advance our understanding of how fluctuations in blood glucose as experienced by patients with T1DM affect memory. In particular, the work may describe effects of recurrent hypoglycaemia in T1DM on memory and may ultimately aid in the prevention or retardation of cognitive impairments (and/or fear of such problems) associated with diabetes and/or hypoglycaemia. In addition, the data may allow insight into the etiology of defective counterregulation/ hypoglycemia unawareness as described in the preceding paragraph.

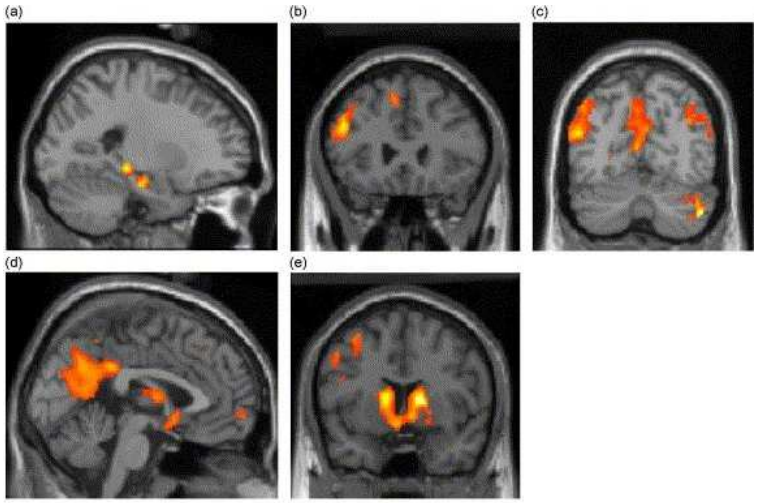
c) Preliminary Studies

As outlined above, a number of studies in the applicants' laboratories have demonstrated the effects of glucose administration on memory. As an example, the figures below show blood glucose on the left and performance on a verbal memory task (Free Recall Performance on the right). The data show that administration of a 25 g oral glucose load in healthy subjects resulted in a rise in blood glucose (T0 baseline, T25and T45 are 25 and 45 mins after ingestion, respectively) and an improvement in recall performance up to 24 hours later (immediate recall averaged over 5 trials (IF), delayed recall within 45 mins of ingestion (DFR) and 24 hours later (24DFR) (Sunram-Lea, et al., 2002).





Also as outlined above, Dr Fletcher's team have considerable experience in using fMRI to examine brain areas activated during memory tasks. As an example, the figures below shows regions of significant activation during a "famous faces" recognition task superimposed on a T1-weighted anatomical image in standard space. Panels show activation in (a) the left hippocampus, (b) the left frontal cortex, (c) the bilateral temporo-parietal junction and the right cerebellum, (d) a region including the precuneus/cuneus and the retrosplenial cortex, and (e) the head of caudate bilaterally (Bernard, et al., 2004).



d) Research Design and Methods

Study 1: Regional brain activation and cognitive performance following glucose administration in healthy non-diabetic volunteers

Background/ Rationale: Ingestion of glucose improves declarative long-term memory (Sunram-Lea, et al., 2002), but the neuroanatomical basis for this phenomenon remains unexplored. We therefore aim to identify the effects of glucose ingestion on fMRI brain activation engendered by a series of relevant cognitive tasks. This will serve as a critical prelude to an exploration of the effects of altered glucose levels on memory performance in diabetic subjects (study 2 below). Study 1 will also allow fine-tuning of important methodological aspects, such as the level of task difficulty and scanning parameters prior to more interventional clamp studies described in study 2.

Methodology: We will recruit 12 healthy adult participants (aged 18 to 60 with no neurological/ psychiatric illness or diabetes) using a within-subjects design. Local ethical approval (LREC) will be obtained in keeping with national guidelines and subjects, having given fully-informed written consent, will attend on 2 occasions. Visits will be separated by at least a week. After an overnight fast, subjects will come to the scanner at 09 00 am. A cannula will be placed retrogradely in the non-dominant arm for blood sampling. At least 60 minutes will then be allowed for stress hormone/ plasma glucose equilibration during which time subjects will practice cognitive function tests. Participants will then drink 150 mls of water containing, on one occasion, 25 g of glucose (SUG) and for the other study a similar volume sweetened with aspartame (ASP). Studies will be

performed in random order with participants blinded to the order of study and conditions. Subjects will then immediately enter the scanner for cognitive testing and fMRI scanning as described below.

Cognitive Tasks:

i) Long-term memory task: Participants will be visually presented with a list of nouns and required to perform one of the two encoding tasks as previously described: [Fletcher 03]

a) Deep Encoding Task: The word is accompanied by an instruction to pay attention to whether or not it is living/animate as opposed to non-living/ inanimate. Participants will be instructed to press one of two buttons on a conveniently located keypad to indicate their choice (yes or no).

b) Shallow Encoding Task: The word is accompanied by an instruction to pay attention to whether there were an odd or even number of syllables in the word (e.g. STREAM … odd?). A keypad response indicates their yes/no decision.

For both tasks, the chosen button for 'yes' and 'no' responses will be held constant within participants to avoid confusion, but counterbalanced across subjects. A total of 144 nouns will be presented in a pseudo-randomised order, 72 for each of these two tasks.

As described below, after scanning has finished (approximately 30 minutes after initial list presentation/encoding) participants will be presented with a recognition memory task (the 'remember-know' procedure (J. M. Gardiner, 1988)). Subjects will be presented with 200 words (2 seconds appearance with inter-stimulus interval of 1.5 seconds) including the 144 words that they have seen during scanning and instructed to indicate, by pressing one of four buttons, whether it was (i) remembered from the study list ('remember' response), (ii)

known to be on the list but not remembered ie. an item feels familiar from the study list but they cannot consciously recollect its earlier presentation, ('know' response) (iii) "new" if not recognized or (iv) "guess" if they think they are guessing. The guess response option decreases the tendency for subjects to use remember and know responses for higher and lower confidence responses respectively (John M. Gardiner, Ramponi, & Richardson-Klavehn, 1998, 2002).

ii) Working memory task (R. A. E. Honey, et al., 2004): Subjects will be presented with a set of letters for 2.5s, followed by an instruction cue ('FORWARD' or 'ALPHABET') for 1.5s. Following a 7s delay (during which a fixation cross will be presented on screen) a probe will be displayed for 4s during which participants are required to respond with a 'yes' or 'no'. If the cue was 'FORWARD', participants are required to remember the letters in the order they were presented and the response cue is a question about the position of the letter (e.g. was 'N' 2^{nd}?) and participants are to give a 'yes' response if that letter had been presented in that position in the initial display, and a 'no' response if it had not. If the cue was 'ALPHABET', participants are required to mentally rearrange the letters presented into alphabetical order and to remember the letters in the new order. The response cue is again regarding the position of the letter (e.g. was 'N' 2^{nd}?) but in this condition participants have to give a 'yes' response if that letter has that position alphabetically, and a 'no' response if it has not. For both the 'FORWARD' and the ALPHABET' conditions, participants will be presented with three (low-load condition) and five (high-load condition) letters.

iii) Continuous performance task: this task engages sustained attention and also enables us to evaluate subjects' reaction times. Subjects are required to monitor the computer screen upon which numbers flash up at a rate of roughly one per

second. They are instructed to respond with a key press, as rapidly as possible, every time they see a "0" and to withhold responses to all other digits. We have previously run the task at two levels of difficulty, one in which stimuli are clear and easily visible and one in which they are degraded and a greater level of visual attention is required to pick out targets. Performance will be measured in terms of ability to discriminate targets from non-targets and the reaction time when responding to a target. We will explore brain activity in terms of the main effects of performing the task compared to a resting baseline and in terms of the impact of stimulus degradation.

The cognitive tests will take 30 mins to complete with fMRI scanning being performed during encoding of LTM tasks and during performance of the other three cognitive asks (working memory task, reaction time and visual processing). Baseline plasma glucose and insulin will be drawn immediately prior to drinking fluid and then at regular intervals during studies (5 mins glucose, 15 mins insulin). Retrieval of LTM (the “remember know” procedure as described above) will subsequently be performed outside the scanner 30 mins after the initial encoding task.

Scanning will be performed on a Bruker MedSpec operating at 3 Tesla. Data will be analysed using Statistical Parametric Mapping (SPM2: Wellcome Dept of Cognitive Neurology, London, UK). Essentially, this involves a voxel by voxel implementation of the general linear model with individual subjects' responses to activation tasks identified and carried through to a group analysis identifying the common regional brain activations through a series of standard t tests. Group analyses will be used to evaluate brain responses to the task and to identify the impact of the glucose drink upon these activations.

Anticipated Results:

Plasma glucose and insulin will rise in SUG tests but not ASP. Cognitive performance on LTM tests will be enhanced in SUG compared with ASP studies as previously described. We anticipate that this will be mirrored by differences in fMRI activation patterns in brain areas including the hippocampus. Group analysis may also allow us to identify areas of brain activation associated with glucose ingestion (hypothalamus and perhaps brain stem).

Sample size: Assuming CV of 25% and glucose mediated difference in recall of 25% (from Sünram-Lea 2002) then n of 12 gives 90% power to detect similar difference with alpha of 0.05.

Study 2: Effects of recurrent hypoglycemia on cognitive performance and brain activation.

Background/ Rationale: The cumulative effects of recurrent severe hypoglycemia in T1 DM on brain function including memory remain controversial. We aim to examine whether T1DM patients with a history of severe hypoglycemia demonstrate altered performance on memory testing at either low and/or high blood glucose levels.

Methods: We will study three groups of 12 subjects:

a) [SH-DM] T1DM patients with a lifetime history of more than 5 severe hypoglycemic episodes (requiring third party rescue) AND currently suffering from hypoglycemia unawareness (assessed by standard questionnaire asking about frequency of hypos and symptoms experienced). The rationale for

recruiting subjects with both a history of severe hypoglycemia and with current hypoglycemia unawareness is detailed later in the application but in short might allow to examine whether any changes in this group are reversible or not.

b) [DM] T1DM patients with no episodes of severe hypoglycemia and good awareness of hypoglycemia (assessed as above)

c) [CON] Non-diabetic age- and gender-matched controls

In addition to the above characterization, T1DM subjects will also have 2 further baseline measurements performed to document symptomatic awareness:

1. Prospective home glucose diary for 3 weeks recording blood glucose and episodes and related symptoms of hypoglycemia.
2. Subjects will also wear a CGMS subcutaneous glucose sensor to identify episodes of (asymptomatic) hypoglycemia.

Clamp Studies: Diabetic subjects will attend for 2 study days, being tested under conditions of hypoglycemia (Lo) and hyperglycemia (Hi) respectively. Healthy volunteers (CON) will attend on a single occasion only to examine effects of clamped hypoglycemia as described below. Our plan is to perform CON studies first to allow comparison with study 1 (see discussion below) but then to perform studies in T1DM groups in random order. Studies in individual subjects will be performed in random order separated by at least 1 week, with subjects blinded as to the order and study conditions on each occasion. Studies will be performed in the morning after an overnight fast. T1DM subjects will be admitted to the clinical research facility overnight prior to study morning(s) with blood glucose being controlled carefully overnight by continuous intravenous infusion of insulin with regular monitoring of plasma glucose in order to avoid fluctuations in blood glucose in the few hours prior to studying- in particular hypoglycemia.

On the study morning(s), two cannulae will be inserted into non-dominant arm, one in antecubital fossa for infusion and one inserted retrogradely into a distal hand vein for sampling. After at least 60 mins for equilibration, a primed continuous infusion of 1.5 mU/kg/min regular insulin (actrapid) in 2% autologous blood/ saline will be started, together with a variable infusion of 20% dextrose. The latter will be adjusted according to regular measurements of plasma glucose by the bedside. Using this glucose clamp technique, plasma glucose will be maintained at 5 mmol/l for 60 mins. Subjects will then be moved into the scanner and an anatomical scan obtained. Infusion pumps will remain in an adjacent room but will be connected to the subject through a dividing wall so that magnetic material does not enter the scanning room. Previous experience has shown that this can be performed safely and without disrupting by increasing the dextrose infusion rate for a few seconds prior to transfer. Following stabilisation, in LO studies, plasma glucose will be allowed to fall rapidly down over 10-15 mins to 2.5 mmol/l as previously described [Lo] (Rosenthal, et al., 2001). Once the glucose nadir is achieved in LO studies (probably about 15 mins), cognitive testing and fMRI scanning will be performed as described above. Following cognitive testing and scanning, delayed LTM recall will be tested as above with subjects maintained at the same glucose level.

Hyperglycemic studies (Hi) will be identical from the subject's perspective, but using a lower dose insulin infusion (0.3 mU/k/min) in T1DM subjects. After equilibration in the scanner, dextrose will be infused to elevate plasma glucose to 10 mM. The differences in insulin infusion from Lo studies are because the higher dose insulin infusion necessary to lower rapidly plasma

glucose during Lo studies would prevent plasma glucose from being elevated rapidly in Hi studies.

In addition to glucose sampling, extra samples will be drawn at baseline and during studies for measurement for later measurement of counterregulatory hormones (epinephrine, glucagon, cortisol, growth hormone and insulin). Symptoms of hypoglycemia will be assessed by a standard questionnaire at baseline (outside the scanner) and then again at 10 minute intervals throughout the clamp studies. On completion of LTM recall testing, subjects will be removed from the scanner, plasma glucose levels will be restored to normoglycemia (if necessary), the insulin infusion will be stopped and the participant will be given a meal and plasma glucose monitored carefully until stable at euglycemia.

Data Analysis:

As reviewed above, similar effects are seen with intra-peritoneal (rat studies) and oral (human studies) glucose administration suggesting that the route of administration of glucose is not key to the mechanism of action. We therefore anticipate that intravenous glucose (Hi) will have a similar effect to boost declarative LTM in T1DM as that seen with oral glucose administration in study 1. We also anticipate that hypoglycemia will result in impairment in both LTM, working memory and reaction time tasks. Functional MRI testing will allow us to examine the patterns of brain activation associated with these tasks at different glycemic levels and allow comparison with Con and between diabetic groups below. We anticipate that we will see changes in brain activation associated with cognitive tasks during hypoglycemia similar to those seen with different cognitive tests by Rosenthal et al (Rosenthal, et al., 2001).

Similar comparisons will be performed in diabetic groups and allow us to determine whether declarative LTM is improved by glucose administration in T1DM analogous to the patterns seen in non-diabetic subjects. In addition comparison of DM and SH-DM will allow us to examine whether there are changes in cognitive performance during hypoglycemia induced by antecedent hypoglycemia. For example, one possibility is that, analogous to the pattern seen in rat studies of McNay (McNay & Sherwin, 2004), SH-DM will perform better than DM on memory testing at euglycemia but perform worse during hypoglycemia. Alternatively, exposure to antecedent hypoglycemia in SH-DM patients may have resulted in an adaptation so that memory performance is protected against hypoglycemia, analogous to changes reported in some studies for other cognitive function tests. Differences between diabetic groups SH-DM vs DM on cognitive function testing may be mirrored by differences in brain activation. It is also possible that differences in brain activation may be seen without changes in cognitive performance- for example a compensatory recruitment of other areas. Finally it may be possible to identify changes in brain activation during hypoglycemic studies in other areas of brain associated with hypoglycemia-sensing such as the hypothalamus and brain stem, and to compare this pattern of activation in DM and SH-DM groups to identify possible changes associated with hypoglycemia unawareness.

Possible Future Direction:

Are effects of recurrent hypoglycemia on cognitive performance and brain activation reversible?

If we find in study 2 that memory performance and associated brain activation is different in SH-DM subjects when compared with DM, we plan to examine whether these effects are a reversible change or reflect irreversible structural alterations in brain circuitry as a consequence of hypoglycemia. As an analogy, it has been suggested that the hypothalamic glucose-sensing mechanisms that detect hypoglycemia and initiate counterregulation may adapt dynamically depending on prior glycemic experience. Antecedent hypoglycemia results in an adaptation of the brain's "hypoglycemia sensor" so that it becomes less sensitive to subsequent hypoglycemic challenges, with antecedent hyperglycemia doing the reverse. Various suggested mechanisms include changes in local brain glucose transport (Kumagai, Kang, Boado, & Pardridge, 1995), blood flow (Boyle, et al., 1994) and/ or expression of key intracellular mediators of glucose metabolism in response to altered glycemic experience. It is possible that such mechanisms may also underpin changes in areas of brain involved in cognitive processes including memory domains following antecedent hypoglycemia. Indeed an upregulation of blood-brain glucose transport in the hippocampus is supported by McNay's rat microdialysis studies as discussed above (McNay & Sherwin, 2004). If we identify changes in brain performance and activation in the recurrent hypoglycemic group in study 2, we would plan to examine whether these changes are reversible following a period of avoidance of hypoglycemia (similar to the adaptation believed to occur in glucose-sensing areas of brain) (I. Cranston, Lomas, Amiel, Maran, & Macdonald, 1994; Mitrakou, et al., 1993). As discussed in study 2, this is the rationale for recruiting T1DM patients with both a history of recurrent hypoglycemia and current hypoglycemia unawareness in study 2 above.

Suggested Methods: Following study 2 above, SH-DM patients will undergo a period of intensive diabetes education with the aim of trying to achieve a period of 3 weeks free of hypoglycemia as previously described (I. Cranston, et al., 1994). It is important to point out that this process will occur in these patients whether they continue to be involved in the study or not in accordance with normal clinical practice. Following a period of 3 weeks without hypoglycemia (assessed by home blood glucose monitoring and where possible supported by repeat CGMS) subjects will attend for one further hypoglycemic clamp study with assessment of cognitive and brain activation patterns as described above. We would anticipate seeing a partial recovery of impaired counterregulatory hormonal responses and symptomatic awareness, consistent with previous reversibility studies (I. Cranston, et al., 1994). As discussed above, changes/ recovery in memory performance and or brain activation when compared with first studies would indicate whether changes were reversible or not.

This follow-on study would clearly be an important addendum to our main body of work as clearly T1DM patients and their families, carers and physicians would wish to know whether changes in brain occurring as a consequence of insulin-induced hypoglycemia are reversible or not.

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Appendix 2 Sweetness match

(Unpublished work by Sandra Süram-Lea)

The possibility that memory enhancement by glucose may be due to some strengthened association through reinforcement has generally been dismissed since saccharin solutions that have been found to be equally preferred and pleasurable to glucose solutions have not been shown to produced the same memory-improving effects (Messier and White, 1984). Furthermore injections of glucose which do not produce taste stimulation but provide the same amount of glucose as drinking sucrose solutions produced memory improvement (Messier and White, 1987). One experiment demonstrated that vigilance (but not memory) was improved when people were told that they were drinking glucose (whether or not they were receiving glucose), suggesting that the placebo effect could contribute to the improvement in cognition observed (Green et al., 2001). However, most of the human studies that examined the effect of glucose on memory used placebo solutions that were not readily identifiable by the subjects. One study compared the effect of drinking water, saccharin and glucose solutions and found that saccharin did not enhance memory compared to water (Messier et al., 1998), again suggesting that the placebo effect, if present, has limited impact when subjects are blind to the type of solution they drink.

Clearly it is necessary that participants are unable to distinguish between placebo and active drink solutions, therefore the present pilot study seeks to derive placebo solution of equal sweetness to an active 25g glucose solution.

Method and Results

Aspartame was favoured as a placebo as it has a sweet taste without the bitter chemical or metallic aftertaste reported in other artificial sweeteners. Aspartame is an artificial sweetener that is 180 times sweeter than sugar in typical concentrations, without the high energy value of sugar. It has a caloric value similar to sugar (4 kcal/g), but the amounts used are small enough to consider aspartame essentially free of calories. Analogous to previous research the drinks were also flavoured with lemon juice to improve palatability (Foster *et al.*, 1998, Sünram-Lea *et al.*, 2001; 2002a; 2002b; 2004 & Green et al., 2001)

Seven independent judges (4 males, 3 females) were provided with six 300ml drinks (labelled 1-6) containing five dosages of Canderel aspartame tablets and one drink containing 25g of dextrose glucose. Tasting was carried out with uniform containers (disposable plastic cups) to ensure that participants will not be influenced by colour or other characteristics of the receptacle. The ratings were carried out in a laboratory setting and drinks were refrigerated for 30 minutes and then taken out of the fridge 10 minutes before the experiment. The experimenter and those participating in the sweetness matching test were blind to the drink contents. All drinks were flavoured with lemon juice Participants were first asked to taste and experimental liquid and rate all drinks for how sweet they were on a five point scale (1 being the least sweet and 5 being the most sweet) and then match it to another liquid of perceived equivalent sweetness The Canderel dosage which was rated as most similar in sweetness to the solution containing 25g of glucose was 5 aspartame tablets (See table 1).

Table 1; Sweetness Rating of Glucose and Aspartame Flavoured Drinks.

Drink	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5	Judge 6	Judge 7	Total
Glucose 25g	3	4	3	5	3	3	3	24
Aspartame 3 tablets	2	1	3	2	1	2	3	14
Aspartame 5 tablets	3	4	3	5	4	3	3	25
Aspartame 7 tablets	5	3	5	4	4	5	5	31
Aspartame 9 tablets	5	5		4	5	5	4	33
Aspartame 11 tablets	4	4	5	5	5	5	4	32

In terms of comparable sweetness, 1 tablet of Canderel is formulated to be as sweet as 5g sucrose, therefore 5 tables would be equivalent of 25g of common table sugar. While sucrose is considered sweeter than glucose, the addition of lemon juice most likely impacted on the sweetness levels of the drinks. Aspartame has previously been observed to react with other food flavours and reaction to food flavours may be altered by citric acid (Chi & Ho, 1088).

Aspartame also has a longer lasting sweetness flavour than glucose (Bahoshy, 1976) so in order to determine whether individuals could discern the aspartame drink from the glucose drink the same seven participants were asked to perform a second taste test 10 minutes later.

Using a double-blind procedure, participants were asked to swill their mouths with water and were then presented with two new drinks (labelled A or B) one containing 25g glucose the other containing 5 aspartame tablets. The participants were told that one drink contained glucose and the other contained an artificial sweetener. Participants were then asked to taste both drinks and identify which drink contained glucose. They were given a sheet of paper on which to make their response by circling one of three responses; Drink A, Drink B or Don't Know.

Three participants circled a 'Don't Know' response, of the remaining four participants two correctly identified the glucose drink and 2 incorrectly believed that the aspartame drink was glucose. Therefore 43% of the sample admitted that they could not tell the difference between the two drinks and the remaining participants identified the drink at chance levels.

Conclusion

Two taste tests were conducted. The first test demonstrated that participants rated a drink containing 5 Canderel tablets mixed with lemon juice as similar in sweetness level to 25 g glucose mixed with lemon juice. The second taste test demonstrated that participants were unable to distinguish between a these two drinks.

Therefore 5 Aspartame tablets dissolved in 300ml of water with lemon juice appears to be matched in sweetness to 25g dexterous glucose dissolved in 300ml water with lemon juice.

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Appendix 3 Instructions for cognitive tasks

MEMORY TASK/ ENCODING (1)

- You will see words every few seconds.
- Each word will have an accompanying instruction, telling you what you should think about for that word.
- If instruction is **"Pleasant?"** decide if the word has pleasant connotations or not. If it does, press the **middle finger for "Yes",** if not, press the **index finger for "No"**.
- Sometimes, it may feel a bit difficult deciding if the word is pleasant or not but just remember there is no right or wrong answers. We are really using this task to encourage you to pay attention to the meaning of the word.
- If the instruction is **"Even Syllables?"** just decide whether the word has an even number of syllables. If it does press the **middle finger for "Yes"**, if not, press the **index finger for "No"**.

{Run Practice Task}

ATTENTION TASK/ CPT (2)

- In this task there will be rest periods, preceded by the instruction **RELAX (in red)** and there will be task periods when you should press with your **middle finger** whenever you see a **zero** on the screen. These will be preceded by the instruction **READY (in green)**

- Sometimes the screen will be fuzzy, making it a bit more difficult; sometimes the screen will be clear, making it easier.

- The task lasts about 8-10 minutes.

WORKING MEMORY (3)

- In this task you will see some letters on the screen
    - I would like you to remember the letters by repeating them over in your head (please don't say them out loud)
- The next thing you see will be an instruction
    - This might be the word **FORWARD**, in which case I would like you to remember the letters you have just seen in the same order as you saw them
    - Sometimes the instruction will be **ALPHABET, i**n this case I would like you to rearrange the letters in your head into alphabetical order
- Next you will see a cross **(+)** on the screen, I would like you to remember the letters in the order as per the instruction
- Finally you will be asked a question about the letters that you have remembered
- If the answer to the question is **YES** please press with your **right (middle) finger**
- If the answer to the question is **NO** please press with your **left (index) finger**

PICTURE TASKS (4)

You will be shown a series of pictures, which will be of food and non- food items. I would like you to **press the second button with your middle finger,** every time you see the picture come up again. Smiley faces in the tasks are rest periods.

No practise session for this task.

RETRIEVAL (post-scanning)

- You will be presented with some words that you saw in the scanner and some that you didn't.
- We would like to find out if you remember seeing the word and, if so, whether you remember which task you were asked to carry out on it (i.e. did you decide if it was pleasant or if it had an even number of syllables).
- You will have 3 options:

    - If you don't think that you saw the word press button **"Z".**

    - If you remember seeing the word and think that you were asked to decide if it was **'Pleasant'** during scanning - **press "B"**. *Press down longer if you are sure about your answer.*

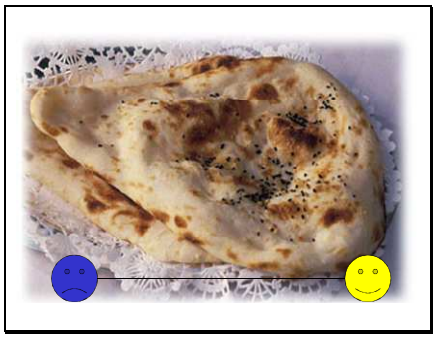
    - If you saw the word and were required to decide if it had **'Even syllables'**, press **"N"**. *Press down longer if you are sure about your answer.*

- Please use the **left hand to press 'Z'** and the **right hand** to press **'B' & 'N'.**

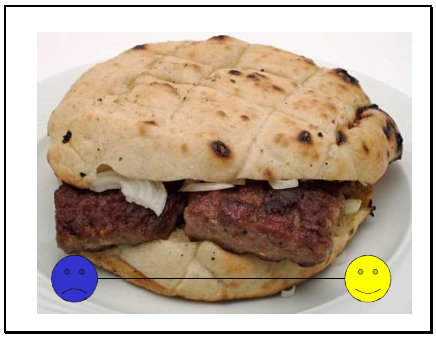
- You do not have to make a new decision of whether the word is 'Pleasant' or has 'Even syllables'. You merely have to remember which task you carried out and not remember whether you called the word pleasant or not or had even syllable or not.

Appendix 4a Food and non-food images used during fMRI imaging

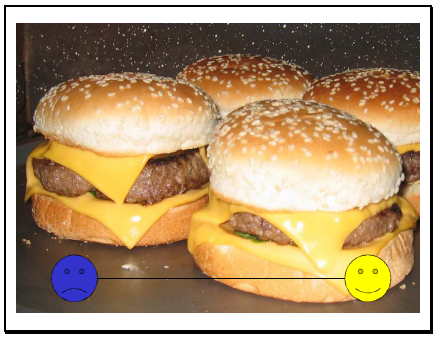
Slide 1



Slide 2



Slide 3



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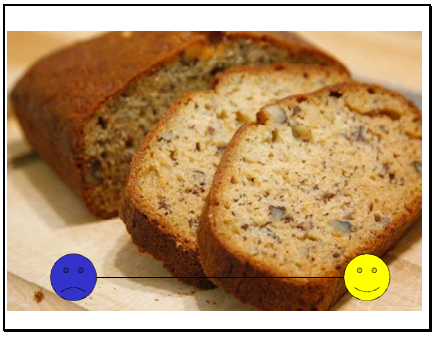

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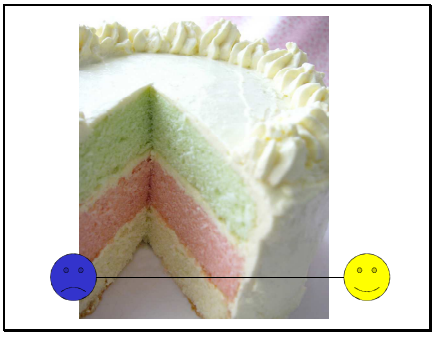

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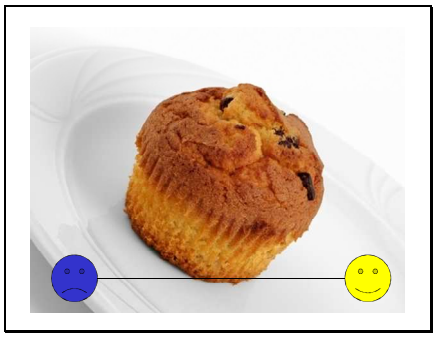

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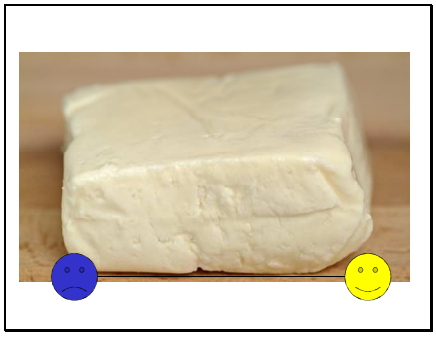

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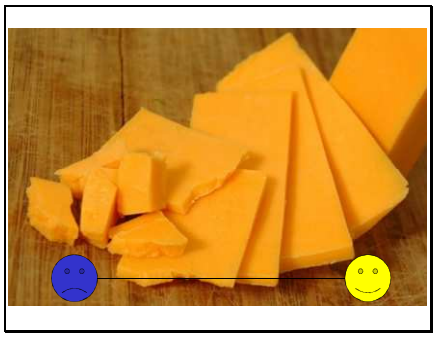

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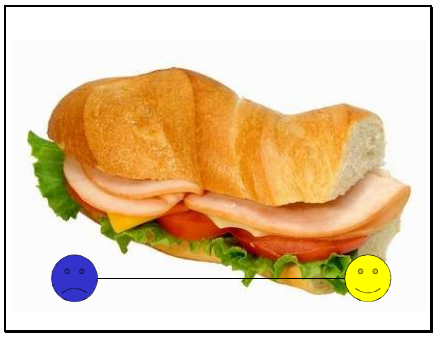

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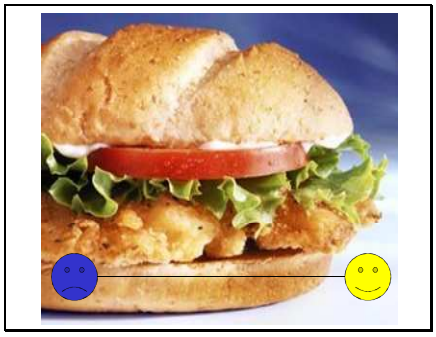

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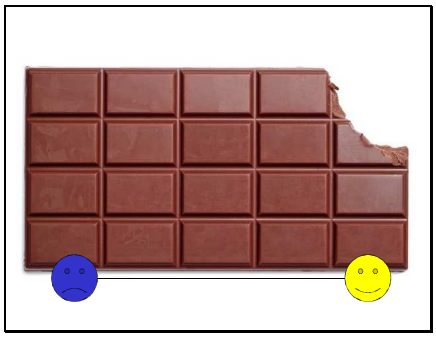

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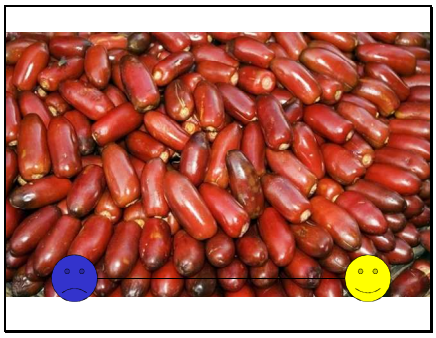

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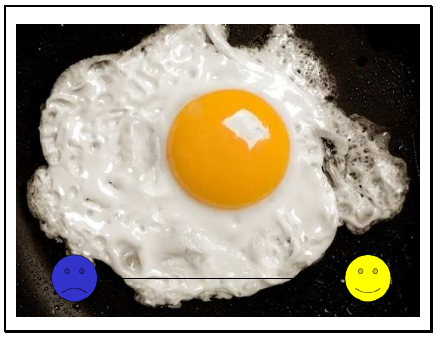

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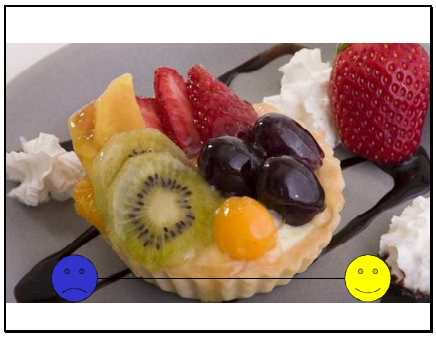

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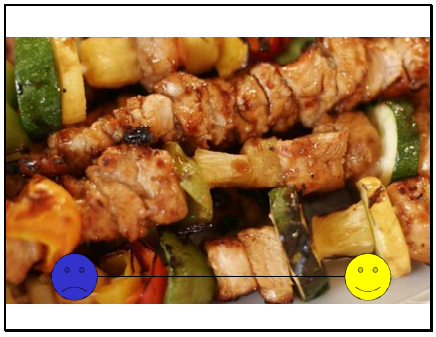

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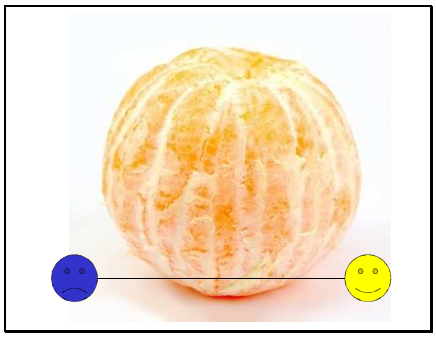

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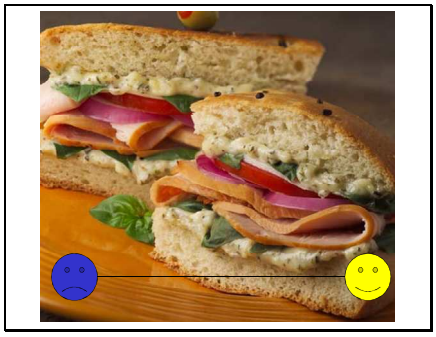

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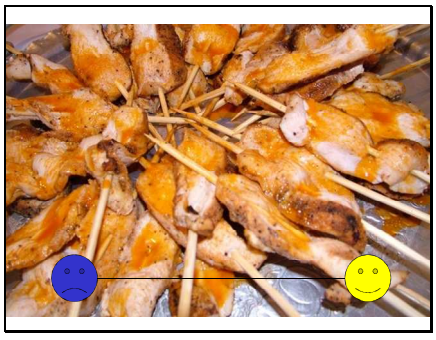

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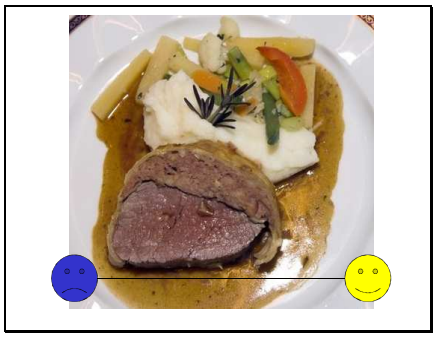

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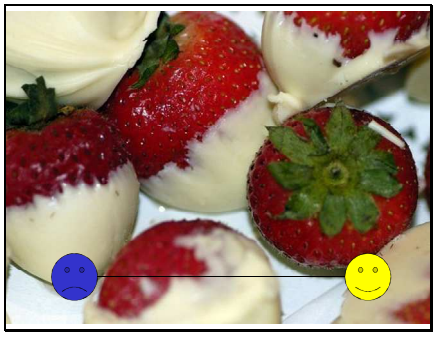

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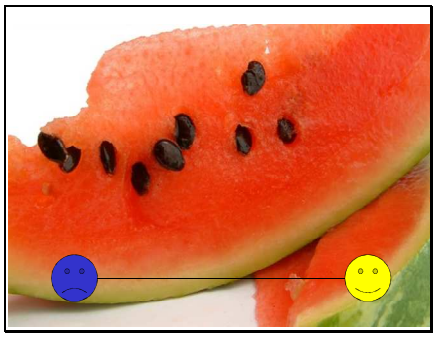

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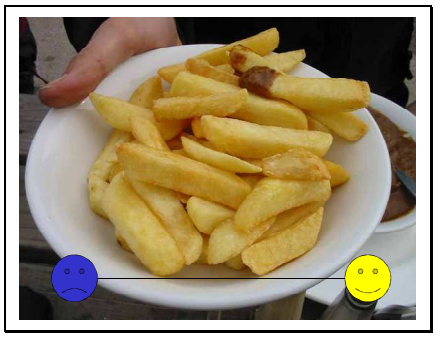

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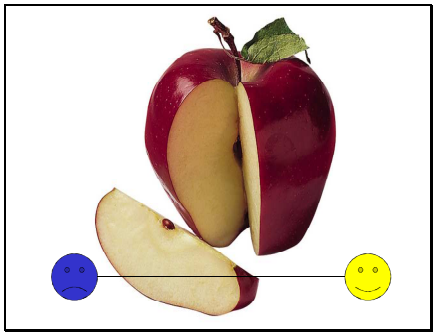

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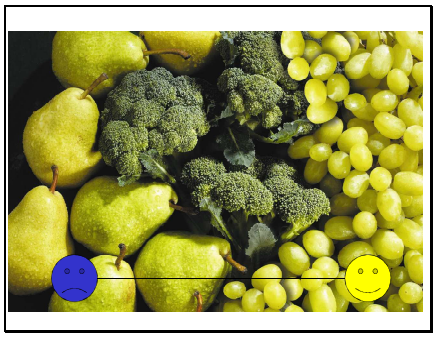

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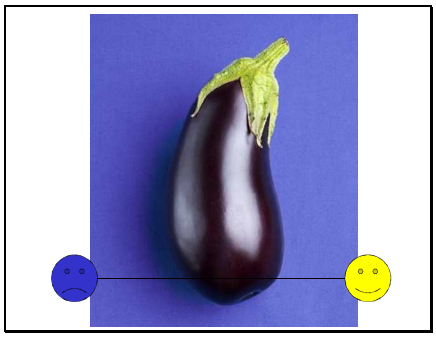

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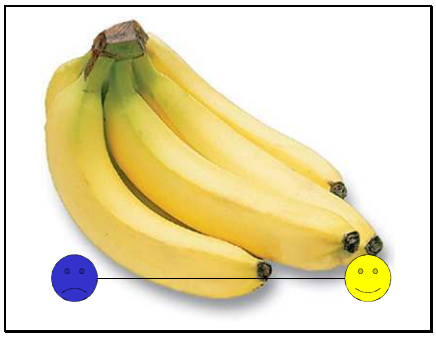

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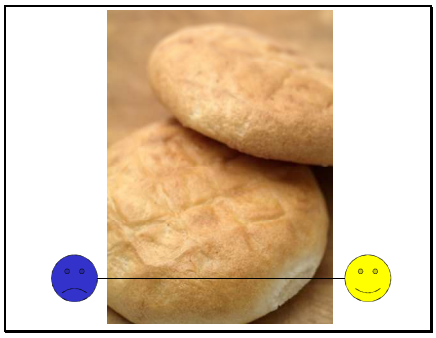

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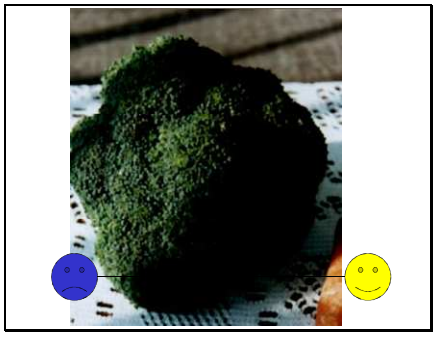

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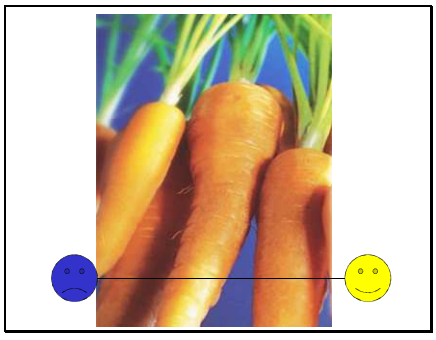

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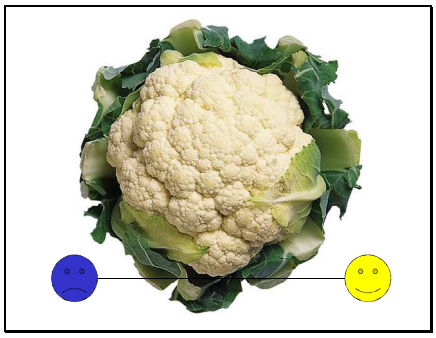

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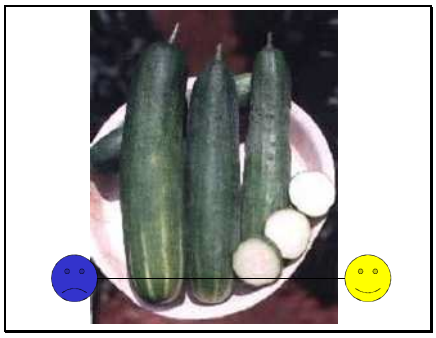

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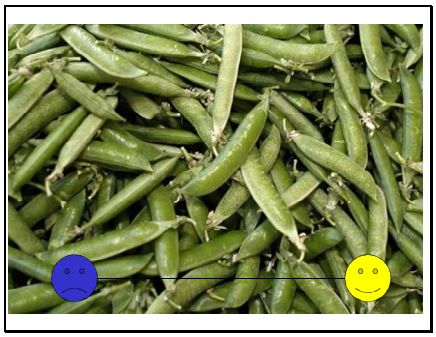

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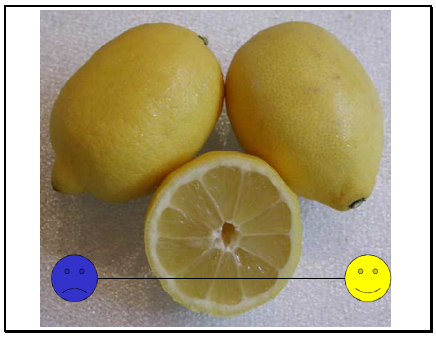

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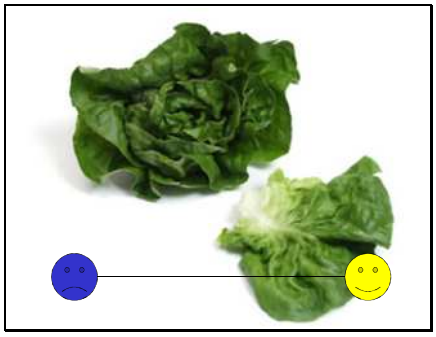

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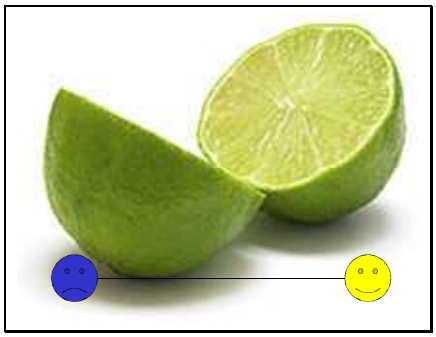

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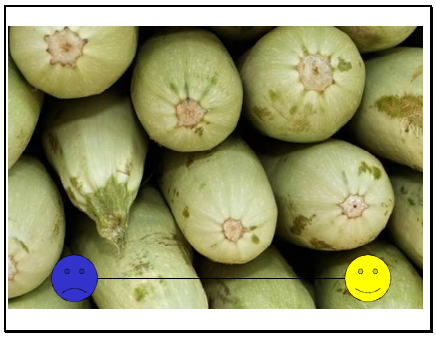

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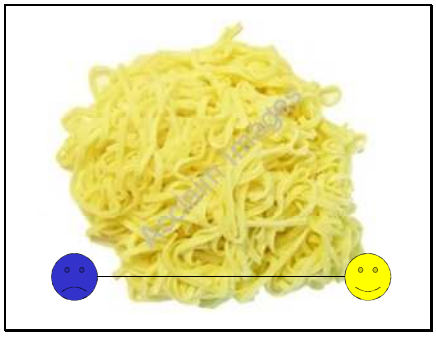

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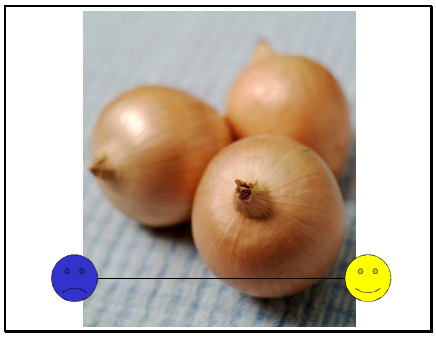

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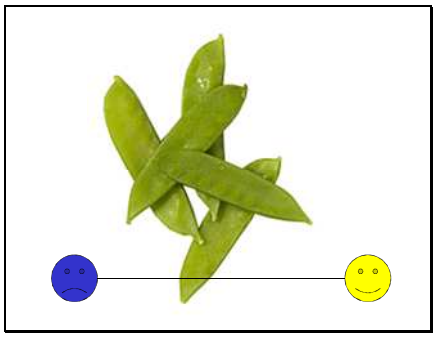

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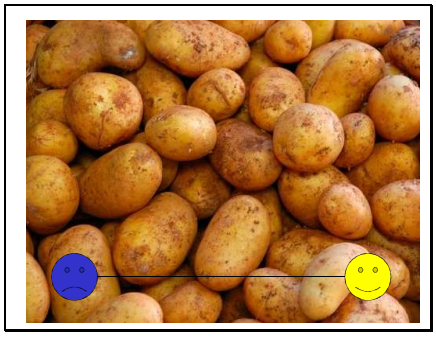

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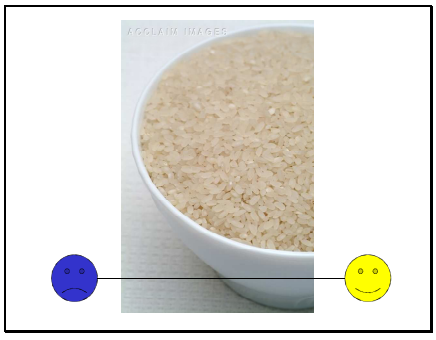

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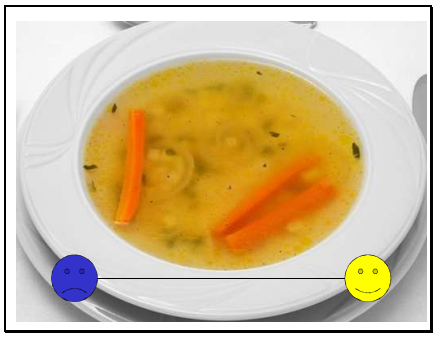

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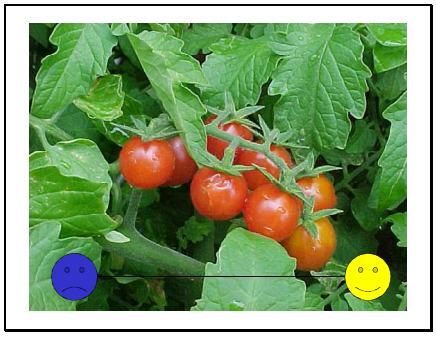
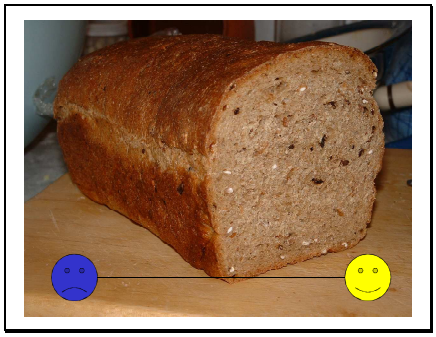
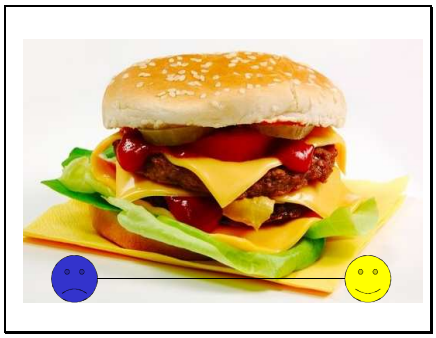


Appendix 4b Food and non-food images used during fMRI imaging

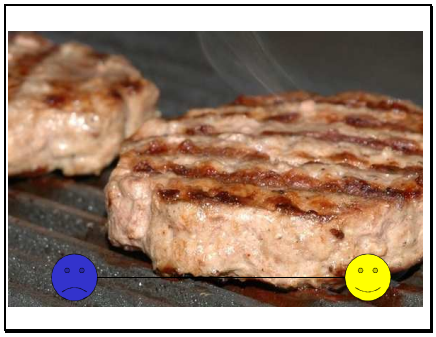
Slide 1



Slide 2



Slide 3



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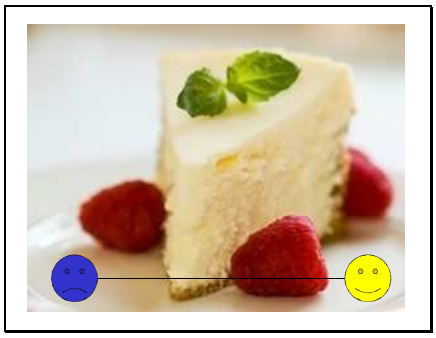

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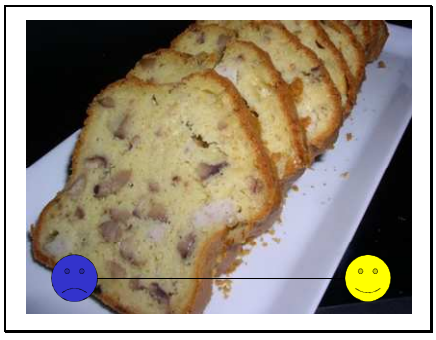
Slide 6



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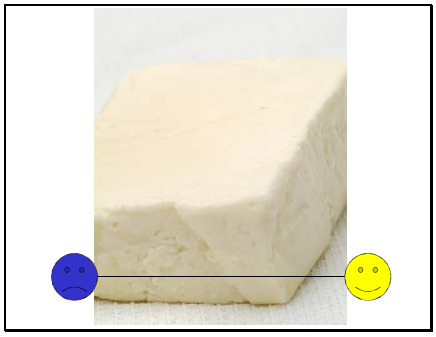
Slide 8



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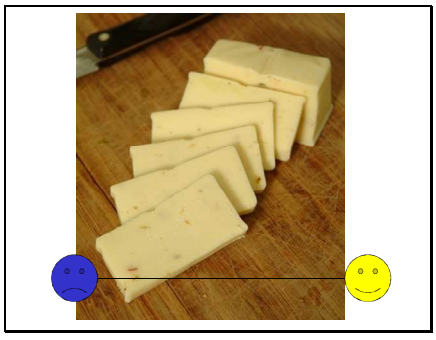

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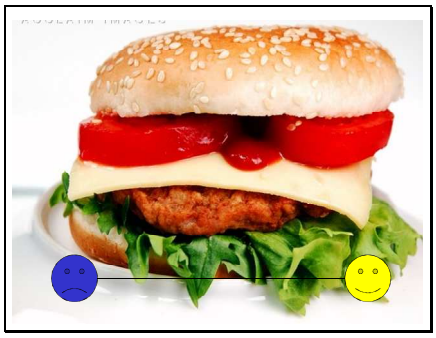

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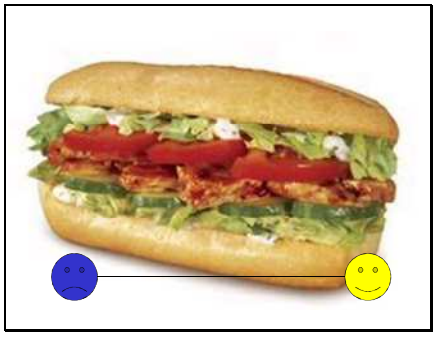

Slide 12

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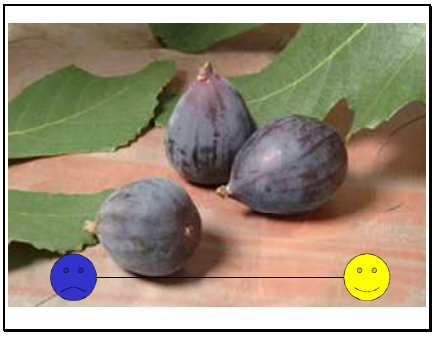

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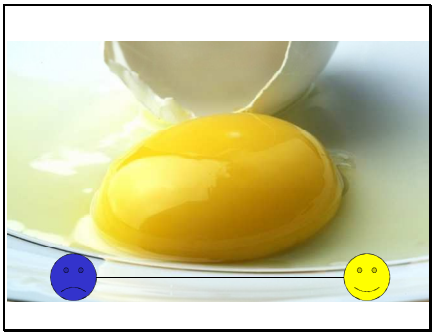

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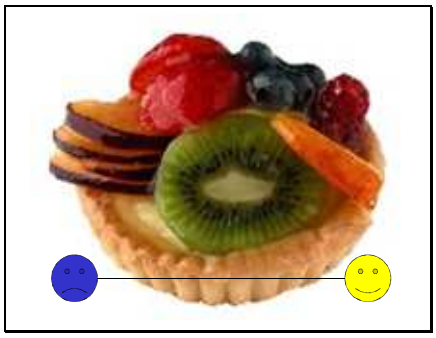

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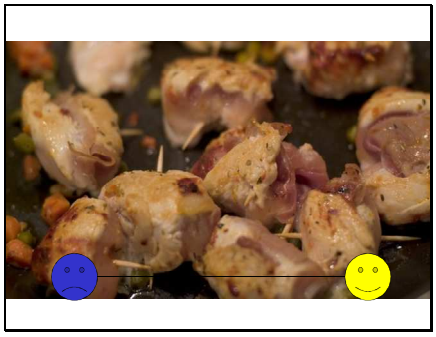

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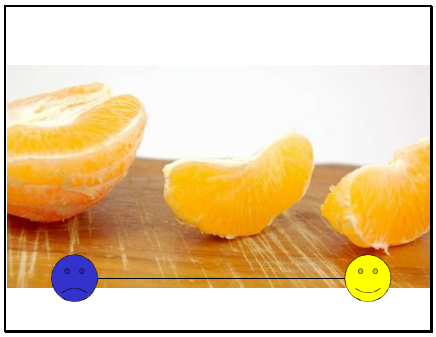

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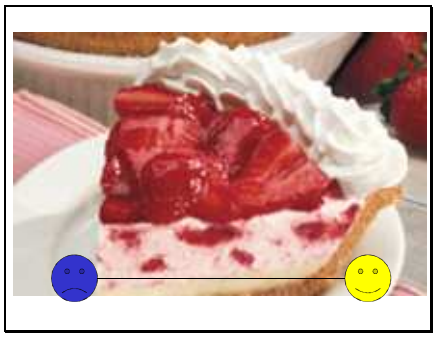

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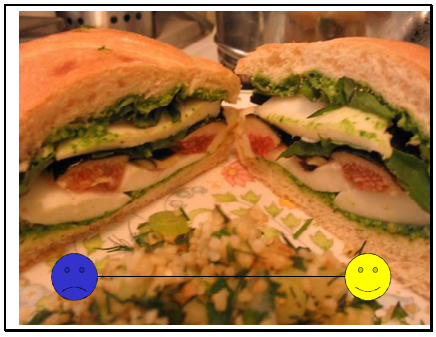

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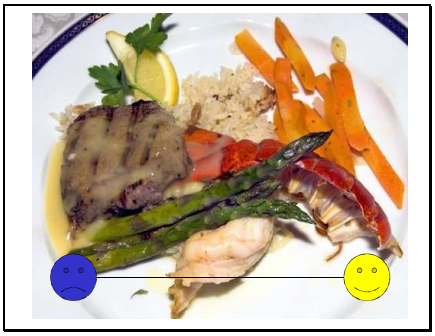

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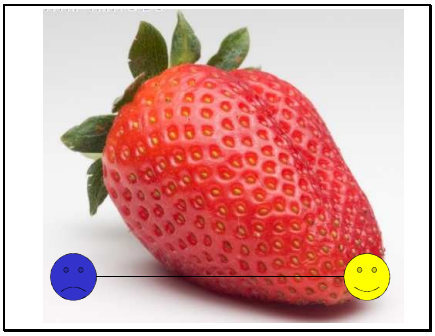

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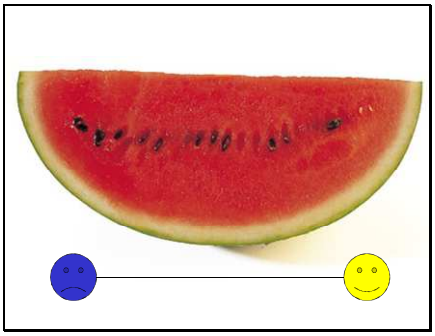

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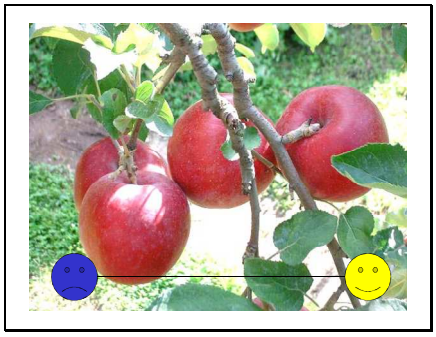

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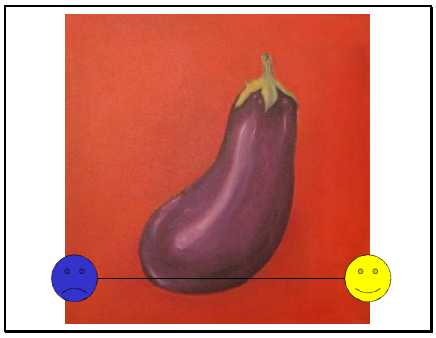

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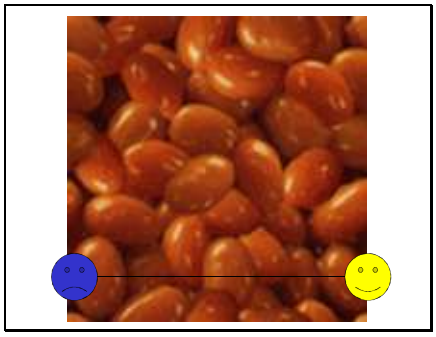

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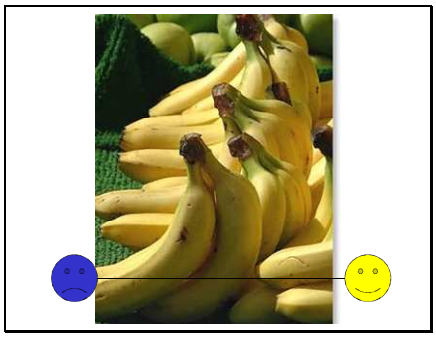

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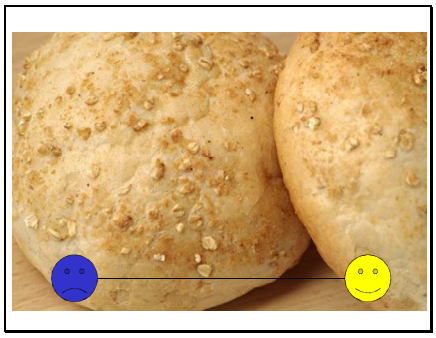

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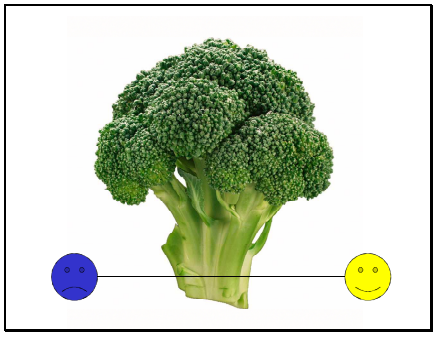

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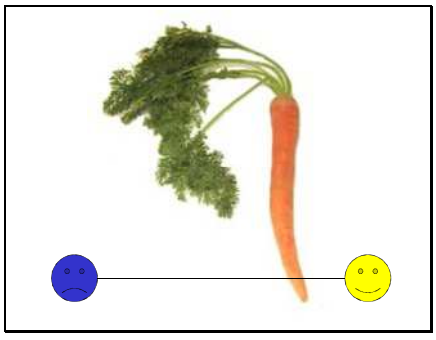

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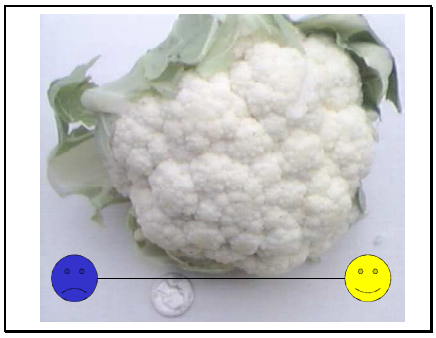

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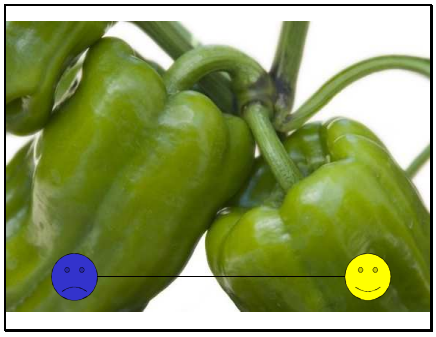

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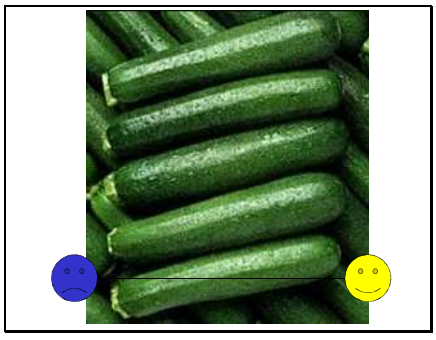

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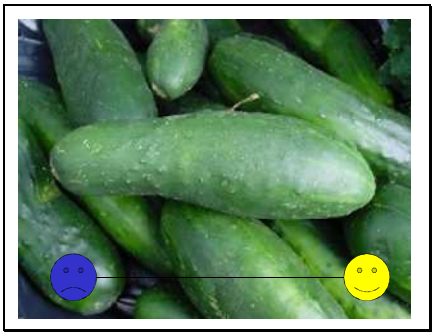

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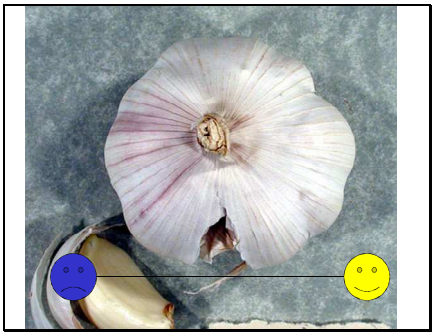

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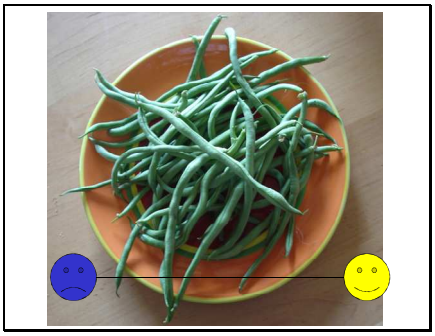

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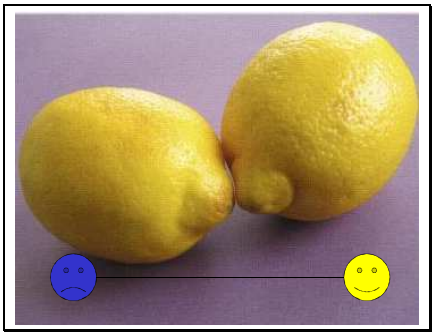

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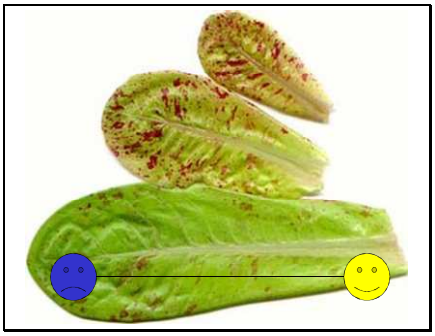

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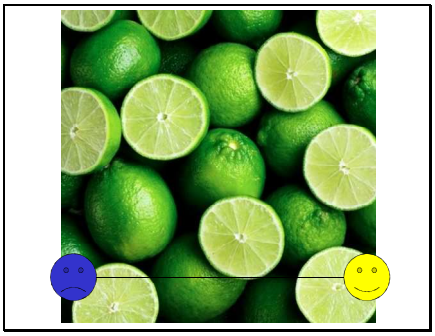

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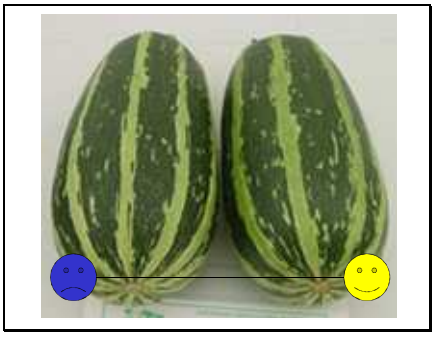

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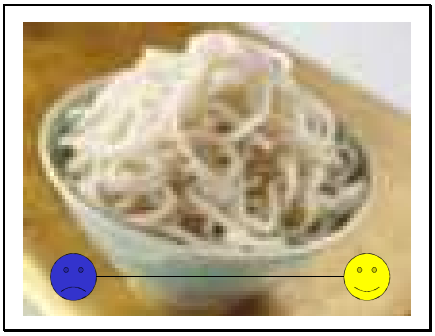

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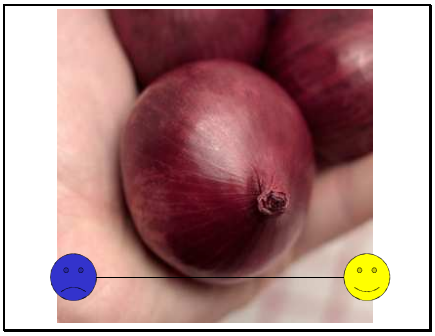

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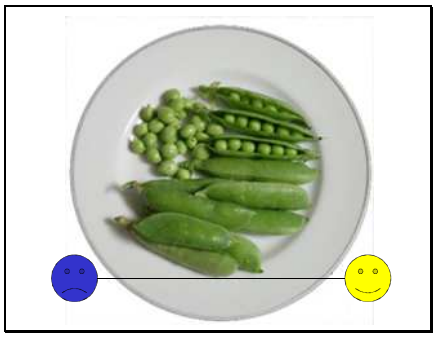

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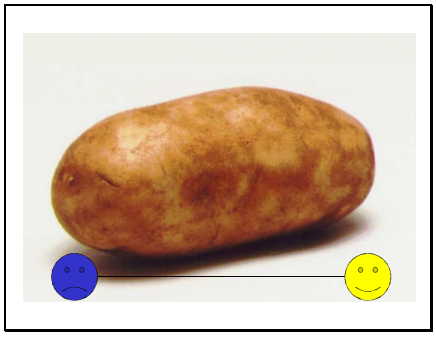

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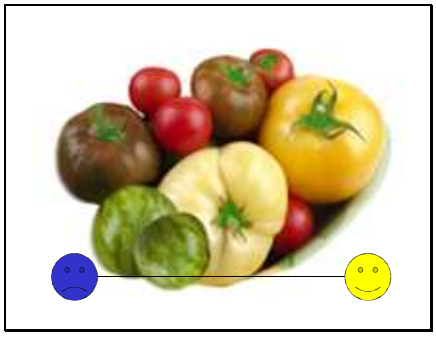

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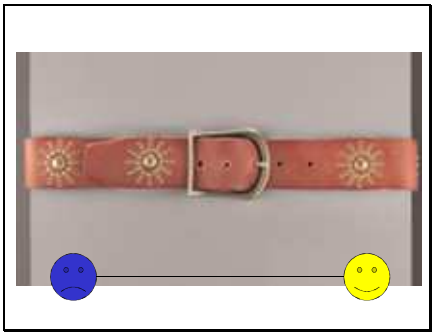

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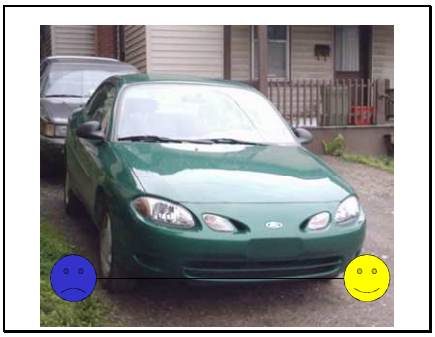

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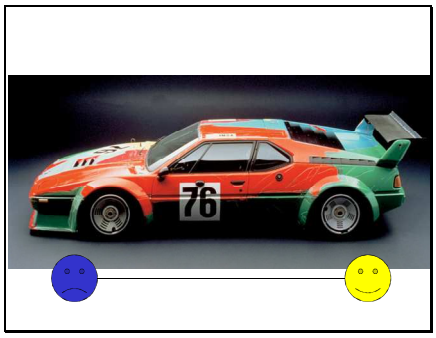

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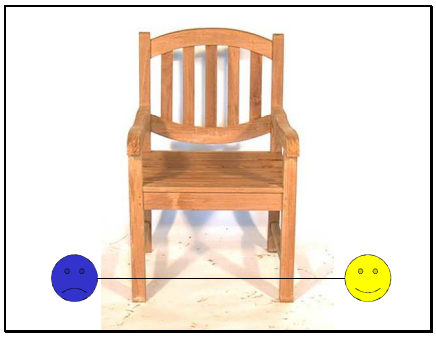

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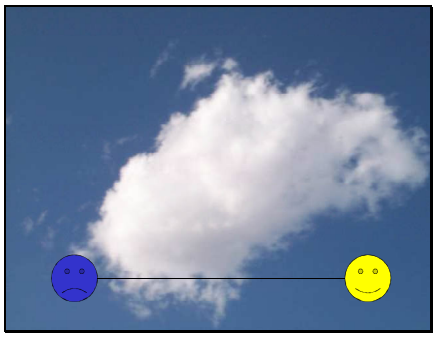

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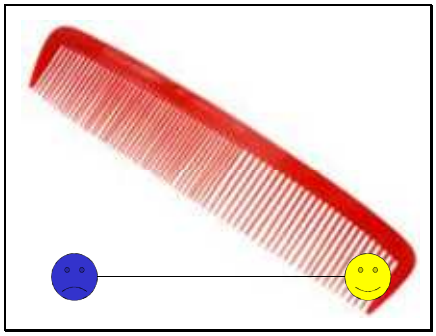

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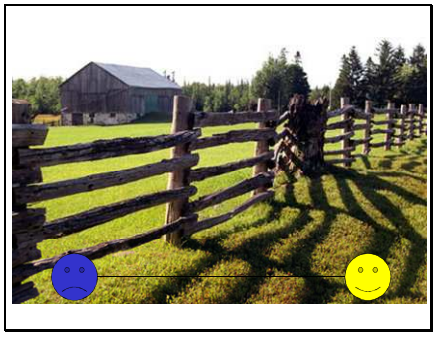

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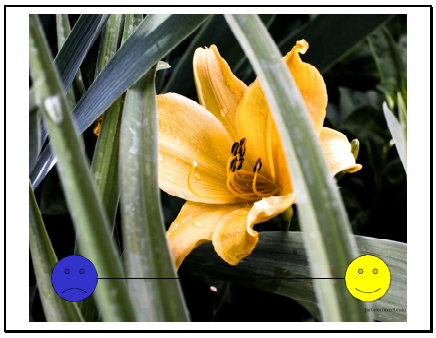

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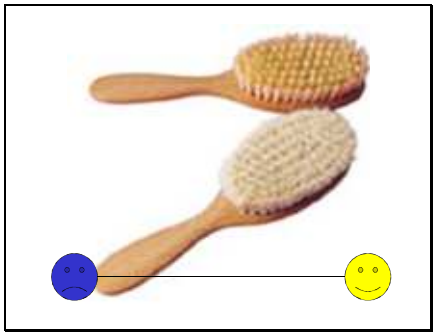

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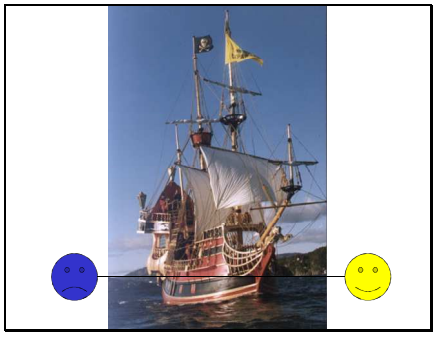

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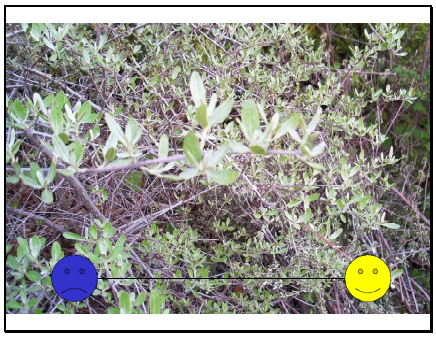

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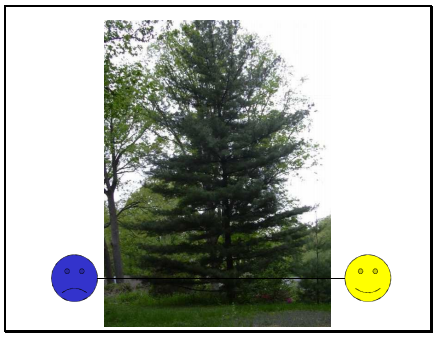

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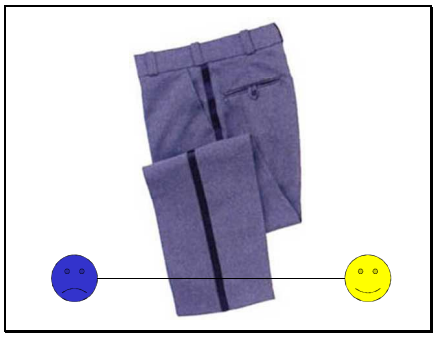

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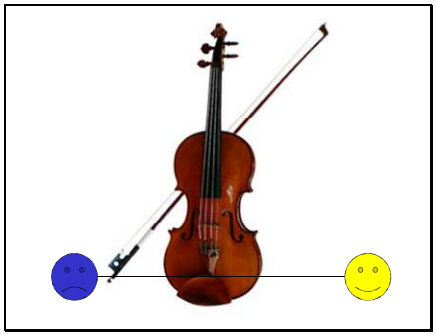

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