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The Effect of Breakfast vs. No Breakfast on Brain Activity in Adolescents when Performing Cognitive Tasks, as Assessed by fMRI --Manuscript Draft--

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Abstract:	<p>Objectives The study examined the feasibility of utilizing functional magnetic resonance imaging (fMRI) with a group of adolescent boys and girls to assess modifications in cognitive function, dependent upon the nutritional state of the participants.</p> <p>Methods Twenty children aged 12 to 14 years, completed two cognitive trials, in a randomized counter balanced order, one in a fasted condition, one after consuming breakfast, during which continuous fMRI data was acquired.</p> <p>Results Although no statistically significant ($P > 0.05$) improvement in task performance was determined, significantly higher activation was recorded in the frontal, pre-motor and primary visual cortex areas in the breakfast trial relative to the fasted.</p> <p>Discussion Such a finding may have important implications in the examination of the role of diet, and specifically breakfast, in determining children's performance within the school environment.</p>

Nutritional Neuroscience ('the Journal')

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Reviewer #1: The revisions to the manuscript address many of the original comments, although somewhat minimally in some cases. For example, whilst the division of errors into misses and false positives is good, it seems inconsistent that some of this data is analysed with t-tests whilst other parts of the error are treated with rank tests. I would like to encourage a consistent statistical approach, preferably using the binomial test as commented previously, especially since the normality of a distribution is difficult to assess reliably in small samples. However, that said I think that the impact on the results will be minimal and the overall pattern would not change. Hence I will leave it to the authors and the editor to decide on the preferred approach here and would not object to publication in the current form.

We have redone the statistical analysis of the different aspects of the cognitive test performance using a repeated measures analysis using mixed models as originally suggested by the reviewer. The methods have subsequently been modified to reflect this.

One other minor change: on page 10, the sub-heading should read "fMRI analyses" and not 'analyzes'.

Changed as suggested.

The Effect of Breakfast vs. No Breakfast on Brain Activity in Adolescents when Performing Cognitive Tasks, as Assessed by fMRI

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Conflict-of-Interest

A grant was received of £18,678.08 from Kellogg Marketing & Sales Company (UK) Ltd to cover MRI scanning costs. Otherwise the research was conducted with the support of internal institutional funds and the authors received no other direct or indirect support, with no further competing interests.

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Abstract

Objectives

The study examined the feasibility of utilizing functional magnetic resonance imaging (fMRI) with a group of adolescent boys and girls to assess modifications in cognitive function, dependent upon the nutritional state of the participants.

Methods

Twenty children aged 12 to 14 years, completed two cognitive trials, in a randomized counter balanced order, one in a fasted condition, one after consuming breakfast, during which continuous fMRI data was acquired.

Results

Although no statistically significant ($P > 0.05$) improvement in task performance was determined, significantly higher activation was recorded in the frontal, pre-motor and primary visual cortex areas in the breakfast trial relative to the fasted.

Discussion

Such a finding may have important implications in the examination of the role of diet, and specifically breakfast, in determining children's performance within the school environment.

Keywords: Cognitive function; Breakfast; fMRI; Nutrition

Introduction

The interaction between diet and cognitive function is an area that has received increasing attention over the last decade. In particular, the impact of diet on performance in children and its role within the school environment has been of interest¹. The most readily controlled aspect of children's diet and potentially the one that has the greatest influence on their performance in school is breakfast. As a result, a number of studies have been specifically aimed at assessing the acute role breakfast has to play in dictating cognitive performance in school²⁻⁶. These have generally revealed a detrimental effect in terms of attention, concentration and memory as a result of missing breakfast with an associated increase in cognitive performance when nutritional requirements are fully met. Likewise, longer term studies have reported regular breakfast consumption results in significantly improved IQ scores⁷ and structural magnetic resonance imaging (MRI) scans have provided some evidence that the long term choice of breakfast leads to alterations in brain gray matter volume with an associated modification in cognitive ability⁸.

Functional magnetic resonance imaging (fMRI) is a technique that is widely utilized in a range of scientific disciplines for the assessment of brain activity⁹. The method relies on detecting changes in local blood flow and oxygen content in those areas within the brain which are active when performing specific tasks. It subsequently provides an ideal tool for examining the degree of brain involvement when faced with cognitive tests and how this may be modified by external factors such as diet.

At present, although there is an extensive body of literature examining fMRI responses to cognitive tests, including a number examining the longer term of effects of dietary supplements¹⁰, very little work has been associated with assessing the acute impact of diet,

and specifically breakfast on cognitive performance. Of those fMRI studies that do exist¹¹⁻¹², the focus has been on adult participants rather than any assessments of the cognitive sensitivity of adolescents to dietary state, although they have shown that for participants conducting N-back tasks, greater activity was observed in the medial prefrontal cortex following a nutritionally balanced breakfast when compared to either no breakfast or only sugar. Although fMRI protocols have been conducted on adolescent participants, these are small in number relative to adult studies¹³ and have mainly focused on more passive tasks, such as examining responses to visual stimuli, although some have been used to examine cognitive responses¹⁴⁻¹⁵. Such an imbalance in relative studies has a number of potential causes. Availability of participants, ethical concerns regarding adolescent populations and potentially reduced compliance to the tasks performed with younger individuals are all possible factors. However, in many respects, an understanding of the functioning of the paediatric brain has greater long-term implications.

Therefore, the aim of the current study was to assess the potential of using fMRI to measure brain activity associated with cognitive tasks in adolescent individuals and examine the sensitivity to acute dietary state by measuring fMRI response differences between when they were satiated or fasted.

Methods

The study was conducted in accordance with the guidelines of the Declaration of Helsinki, the British Association of Sport and Exercise Sciences (BASES) and the British Education Research Association (BERA) and all procedures were approved by the institutional ethics committee. School based consent was firstly obtained from the head teacher and thereafter written informed consent and a completed health questionnaire were obtained from the

parents together with a signed assent from all the participants. Exclusion criteria consisted of the following; participants with any known food allergies, participants with clinically diagnosed learning difficulties, or participants with known counter-indications for MRI scanning e.g. claustrophobia.

Participants

Twenty one adolescent male (n=10) and female (n=11) volunteers (age 12-14 y) were recruited for the study drawn from local schools with whom studies have previously been undertaken. Stature, body mass and sitting stature were measured. Stature and sitting stature were measured using a Holtain stadiometer (Holtain Ltd, Crymmych, UK) accurate to 0.1 cm and body mass accurate to 0.1 kg using a Seca digital scale (Seca, Hamburg, Germany). The average age, stature, sitting stature and body mass of the group of 20 that undertook the fMRI protocol were 13.3 ± 0.7 y, 159.7 ± 6.6 cm, 82.4 ± 4.8 cm and 50.6 ± 8.7 kg respectively.

Study Design

All participants initially visited the centre to practise performing all of the cognitive tasks, both outside of the scanner environment, so that they were fully conversant with the requirements of each test, as well as within a to-scale mock MRI scanner to ensure they were comfortable within the confined space. During this visit all participants also visited the actual scanner so the entire environment was familiar, with the aim of minimizing stress during the testing days and to reduce any differences in responses between the trial days as a result of anxiety. The familiarisation session preceded the actual trials by approximately seven days.

For the testing proper, participants visited the centre on two occasions, when they performed the full cognitive testing procedure within the MRI scanner. The study comprised a randomized cross over design, and was counter balanced, with an equal number of participants having breakfast prior to or after the scanning session on their first visit, with the breakfast order reversed for their second trial visit. For each visit, participants arrived at the centre having had an overnight fast from 10 pm the previous night with them blind to which breakfast regime they would be allocated to on their first visit. The trials were scheduled approximately seven days apart.

Participants arrived in pairs, one of whom was offered only water to ensure adequate hydration levels, while the other was provided with a controlled breakfast. The breakfast consisted of a 40 g cereal serving of wheat biscuits with a sugar frosting together with 125 mL of semi-skimmed milk, resulting in a total calorific load of 207 kcal (carbohydrates 35 g of which starch comprised 22 g and sugar 13 g, fat 3 g of which 1.5 g was saturated, protein 9 g, fibre 3.5 g). Participants were given 15 minutes to consume the breakfast. The participant who was to be scanned without consuming breakfast started the scanning session approximately 30 minutes after arrival, at 9am, equivalent to the typical starting time of the school day. On completion of the testing procedure they were subsequently offered breakfast, without limits on consumption levels. For the participant who was given breakfast prior to the scanning session, scanning took place approximately 1.5 hr after arrival, a time estimated to be equivalent to the typical time between breakfast consumption and the start of the school day. During the interval between breakfast consumption and scanning, the participant waited within the MR centre, during which time they were given access to reading material and other entertainment in order to keep them engaged. Although it was not possible to ensure exactly the same activities were undertaken for each visit, any possible confounding aspects on

subsequent cognitive task performance were attempted to be minimized by providing the same entertainment material for each visit and ensuring that the presence of food images within the literature supplied was minimised.

fMRI protocol

The stimuli were presented to participants in a 1.5 T Philips Gyroscan scanner with an eight element head coil. Within the head coil was mounted a mirror assembly, such that with their head within the coil, participants were able to see a screen at the end of the scanner bed onto which the cognitive tasks were projected. Scanning consisted of initial surveys to determine participant position and a reference scan for coil calibration purposes, with the procedure taking approximately two minutes. The assessment of brain activity during cognitive tasks involved using a standard single shot echo-planar dynamic imaging (EPI) sequence as routinely used for fMRI studies (TR=3 s, TE=45 ms, resolution 2.5 x 2.5 x 3.5 mm, 39 contiguous transverse-oblique slices, scans field of view 230 x 230 mm, 64 x 64 within-plane matrix). Each section of the cognitive tasks required running separate dynamic EPI scans, with the participants given the opportunity to move and get comfortable between each set. This procedure was then followed by a high resolution dynamic scan T1-weighted anatomical image with a resolution of 0.9 x 0.9 x 0.9 mm, taking approximately 3.5 minutes

Cognitive Tasks

The chosen tests were designed to be independent of knowledge, training, practice or educational level, particularly in terms of language or mathematical ability. Instead, they were selected to specifically challenge attributes associated with attention and memory. Other restrictions were imposed by the requirement that the testing took place within the scanner environment, limiting the range of viable participant response options. To ensure ease of

response therefore, tasks were selected that required the pressing of a single button of MRI compatible response boxes held in each hand. All cognitive tests were presented in E-Prime version 2 (Psychology Software Tools Inc, Sharpsburg, USA). All participant responses were recorded to allow for an assessment of accuracy and reaction times and participants were instructed to respond as quickly but as accurately as possible. The first task selected was a simple choice reaction time where participants responded as quickly as possible when presented with an arrow which was either pointing towards the left or the right, in response to which they were required to press the button in the corresponding hand¹⁶. Three sets of the task were run, each of which consisted of the presentation of 45 arrow stimuli. Arrows were presented for 400 ms after which a blank screen was shown for a variable duration between 2000 and 7000 ms. The subsequent tasks were N-Back tests that required the participants to identify occurrences of letters being the same as the previous one (1-back), or two previous (2-back)¹⁷. Letters were presented for 1000 ms followed by a blank screen for 750 ms. Three sets of both 1 and 2-back trials were run, each of which consisted of the presentation of 64 consecutive letters. The choice reaction time examines general alertness and motor speed whereas the N-back task specifically assesses working memory function as a result of participants being required to maintain and update a dynamically changing set of features.

Data pre-processing and analysis

Functional images were analyzed using SPM8 software (The Wellcome Department of Cognitive Neurology, University College London); a suite of MATLAB functions and subroutines with some externally compiled C routines. Pre-processing included slice time correction, spatial processing to correct for head movement and size, and warping to the Montreal Neurological Institute template (MNI305). Images were then convolved with a 3D Gaussian filter with an 8 mm full-width-at-half-maximum (FWHM). The fMRI data was

analyzed based on massunivariate (voxel-by-voxel) testing within the general linear model framework over the whole brain, treating each participant separately and constructing individual maps comparing the differences in response between the two visits. Group analysis was subsequently undertaken combining the individual responses, with significant brain activation defined as arising within a region when the differences in signal intensity between visits gave rise to a P-value < 0.001 after no corrections had been made for multiple comparisons and the cluster size of the activated region was equal or greater than 10 voxels.

When examining cognitive test scores, response times were filtered such that no anticipatory responses, defined as reaction times less than 10 ms, were registered. A maximum response time (2400 ms) was also implemented to prevent mis-assignment of responses. For the decision making task, performance was assessed via the response time and the number of incorrect responses. For the N-back tests, performance was assessed via response time, the number of times participants did not identify matching letters when they arose (no-response) and the number of times they incorrectly considered that a matching pair had occurred (false

positive). In all cases group means $\pm$ standard deviation were determined. To examine any significant changes in reaction times and in the frequency of incorrect responses, no-responses and false positives in the respective tests, data were assessed using a repeated measures analysis using mixed models.~~To assess whether the results were normally distributed Shapiro-Wilk tests were run on all data. In the event of normal distributions, as there were no gender response differences, all participants were grouped and assessed using Student's paired sample t-test. For non-normally distributed data Wilcoxon signed rank sum tests were utilized.~~ To examine any possible trade-off between reaction times and response accuracy, Pearson correlation coefficients were determined for the differences in reaction

times and accuracy between fasted and after breakfast conditions for all individual cognitive tests, with significance defined as $P < 0.05$.

Results

One female, although initially recruited and familiarised to the test protocol decided not to continue and withdrew from the study. Of the remaining 20 participants, 19 contributed to the statistical assessment of the decision-reaction task performance and 18 to the N-back task with 1 and 2 participants respectively removed due to poor task compliance. For the fMRI data, from the subset of participants whose responses had been assessed, the results from 16 participants were examined for both tests, the remaining data sets having been discarded due to excessive movement.

Cognitive Task Performance

For the decision making task, there were no significant differences between reaction time (Fasted: 493.3 ± 51.0 ms, After breakfast: 493.0 ± 76.0 ms), or the number of incorrect responses (Fasted: 8.7 ± 4.1 , After breakfast: 9.7 ± 4.9) ($P > 0.05$). For the 1-Back task, whilst the reaction times were faster following breakfast (Fasted: 548.6 ± 88.2 ms, After breakfast: 543.0 ± 89.0 ms), neither it or the number of incidences of no-response (Fasted: 2.8 ± 3.5 , After breakfast: 2.1 ± 2.3) or false positives (Fasted: 1.5 ± 1.9 , After breakfast: 1.6 ± 1.8) were statistically significant ($P > 0.05$). Likewise, for the 2-Back task although the reaction time was faster (Fasted: 617.0 ± 100.8 ms, After breakfast: 611.6 ± 111.6 ms), and the number of incidences of no-response (Fasted: 8.6 ± 3.6 , After breakfast: 8.3 ± 1.2) and the number of errors was lower (Fasted: 2.8 ± 3.6 , After breakfast: 1.5 ± 1.2) following breakfast, no significant differences were found ($P > 0.05$).

No significant correlations ($P>0.05$) were found between changes in reaction times and response accuracy between fasted and after breakfast conditions for any of the cognitive tests.

fMRI analyses

Figure 1 illustrates the areas of activation which were significantly higher in the breakfast condition compared to the fasted state ($P<0.001$) for the decision reaction task for the 16 participants included in the data analysis. Increased activation was seen in Brodmann area 6, in the frontal/pre-motor area, a region associated with planned motor actions. No areas of significantly increased activation were seen in the fasted state relative to the breakfast condition. In Figure 2 the fMRI areas of increased activation for the breakfast condition relative to fasted state are shown for the N-back 1 and 2 tasks ($P<0.001$). Two main areas of activation were observed, the first of which was the cuneus (Brodmann area 17) the site of basic visual processing. The other was Brodmann area 45 in the frontal cortex which is associated with semantic decision tasks such as that associated with the examination of the composition of words and letters. No areas of significantly increased activation were seen in the fasted state relative to the breakfast condition.

Discussion

The study examined the feasibility of utilizing fMRI with a group of adolescent boys and girls to assess modifications in cognitive function, dependent upon the nutritional state of the participants. By doing so, the aim was to determine whether certain areas of the brain associated with cognitive processing and attention were more active when the individual was satiated, compared to fasted.

No significant modifications were seen in performance in the cognitive tasks as a result of dietary state within the present study. This is in contrast to recent studies by Cooper and colleagues¹⁸⁻¹⁹ where breakfast consumption did significantly improve cognitive function (on some accuracy parameters but not response time) based on a visual search test, the Stroop test and the Sternberg paradigm in an adolescent group of students. However, the lack of significant differences seen in the present study may simply be due to an under powering of the experiment in terms of cognitive task performance, as our post hoc calculations estimate approximately 55 – 70 participants would be needed to establish statistically significant differences, a figure in line with the numbers tested by Cooper *et al*¹⁸⁻¹⁹. However, given that the nature of the current study was an exploratory assessment of differences which could be detected via fMRI methods the recruitment levels selected were based upon the typical sensitivity of this technique. Any under powering of the experiment from the perspective of detecting differences in cognitive test performance scores was not a primary consideration.

The areas of increased activation following the analysis of the fMRI data seen in the breakfast trial relative to the fasted are generally indicative of a state of increased participant attentiveness and are in line with what might be anticipated given the nature of the trials. Brodmann area 6, in the frontal/pre-motor area, is a region associated with planned motor actions and has previously been associated with choice reaction tests²⁰⁻²¹. Likewise, for the N-back tasks the areas of increased relative activation following breakfast consumption link closely with the demands of the task. The cuneus (Brodmann area 17) is the site of basic visual processing and Brodmann area 45, in the frontal cortex, is associated with a number of demands attributed to the N-back task, such as the selection and assessment of the stimuli presented and held in short and long term memory, and has previously been reported when undertaking the N-back protocol²².

The underlying cause of the increased cerebral activity seen following the breakfast consumption may arise simply from an increased metabolic availability of glucose. Indeed, the suggestion that alterations in blood glucose levels lead to modifications in cognitive efficiency are long standing²³, supported by a number of studies reporting improvements in a range of cognitive performance measures following the administration of a glucose drink²⁴⁻²⁷. However, other studies have highlighted differences in acute cognitive performances dependent upon the nutritional content of food, rather than purely the glucose load²⁸. In addition, work by Akitsuki *et al*¹¹ has reported greater brain activation, as assessed by fMRI, when undertaking cognitive tasks following a nutritionally balanced breakfast than sugar alone, suggesting the quality of the breakfast has an impact on subsequent cognitive performance.

fMRI protocols with an adolescent population have a number of intrinsic problems, either not present or reduced in an adult one. Their ability to maintain concentration and focus, and as a consequence their ability to stay still during the course of the experiments, appears limited. We have aimed to minimize these effects by selecting cognitive tasks that require continual feedback on the part of the participant and breaking the tasks up into short periods (approximately 4 minutes) with intervals in between. In addition, extensive practice was undertaken prior to the testing proper so that participants were familiar with the environment and the demands of the testing procedure. Even so, it was still necessary to remove a certain number of participants, approximately 20%, from analysis for each of the tasks, due to either inattention, resulting in very poor task performance, or else excess movement. However, these numbers remain within the range of those that can be accommodated by appropriate

levels of recruitment and illustrate the feasibility of undertaken cognitive fMRI testing with such a population.

In conclusion, the main finding of the study was that significantly increased fMRI signal was observed within Brodmann areas 6, 17 and 45 when performing cognitive tests in adolescents after breakfast consumption of 209 kcal compared to the fasted state, although no associated increases in test performance were detected. Such a finding may have important implications in the examination of the role of diet in determining performance of children within the school environment

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Figure Legend

Figure 1. fMRI scan of the of the brain showing areas of increased activation linked to the decision reaction test for the breakfast trial relative to the fasted state, specifically within Brodmann 6 area.

Figure 2. fMRI scan of the of the brain showing areas of increased activation linked to the N-back 1 (green) and 2 (red) tasks for the breakfast trial relative to the fasted state, corresponding to Brodmann areas 17 and 45.

Figure 1.

Figure 2.