N400 in fMRI: differences between auditory and visual stimuli

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Abstract

The aim of this study was to replicate the findings of the N400 effect in an fMRI experiment with one participant in two modalities. Stimuli consisted of congruent and incongruent sentences presented in either visual or auditory manner, while measuring the participant's reaction time in a semantic decision task. Activation was found in the visual and auditory cortices in the brain for the visual and auditory conditions respectively, in accordance with the hypothesis. However, no significant activation was found in the congruent and incongruent conditions, neither was there a significant interaction effect. The expected slower reaction time in the incongruent condition was also not confirmed.

Introduction

In 1980, Kutas and Hillyard presented participants with written sentences during EEG scanning, and discovered that when ending the sentence on a word that was semantically out of context, a negative event-related potential occurred 400 milliseconds after reading the last word. This has later been dubbed the N400 effect, and is thought to reflect the extra processing required for lexical semantic integration of a semantically incongruent sentence *(Brown & Hagoort, 1993; Holcomb, 1993; Maess et al., 2006).* This would then result in the slower reaction times and higher number of mistakes observed in experimental paradigms with semantically incongruent sentences *(Stanovich, 1981; Holcomb & Neville, 1990; Bentin, Kutas, & Hillyard, 1993; Holcomb, 1993; Niznikiewicz et al, 1997)*. Experimental paradigms for N400 findings usually presents participants with a single word at a time, and findings are found both when using whole sentences as stimuli and even when

there is only a single word to prime semantically before the target word is presented. Some experiments also suggest that the N400 effect in auditory modality is also influenced by non-semantic, physical characteristics like introducing the target in differing voices or rhyming and non-rhyming word pairs *(Praamstra, & Stegeman, 1993*).

The N400 effect was discovered in a paradigm using visual stimuli, but has later also been found in experiments using auditory stimuli *(Kutas & Petten, 1988).* Some evidence suggest that semantic priming effects are stronger and last longer in the auditory modality *(Holcomb & Neville, 1990),* which might be an effect of early processing differences, since late stages of language processing are thought not to be differentiated between modalities *(Gazzaniga et al., 2014. p 489)*.

The N400 paradigms have also been used during fMRI scanning, where a larger BOLD signal response is observed for larger levels of semantic incongruity, again reflecting the greater amount of processing necessary when the semantic priming is counteracted *(Kutas & Hillyard, 1980; Bentin, Kutas, & Hillyard, 1993; Baumgaertner, Weiller, & Büchel, 2002)*. Both MEG/EEG and fMRI experiments find that processing semantically incongruent sentences results in greater activation in non-content specific brain areas, especially the anterior medial temporal lobes, and possibly left lateralized *(Maess et al., 2006; Dien, Michelson, & Franklin, 2010; Geukes, Huster, et al., 2013).*

In this experiment, it is attempted to replicate findings of the N400 in fMRI, and to further test the difference of N400 in visual and auditory modalities. Thus, there are four main hypotheses:

1. It is expected to find activation in the visual cortex when presenting visual stimuli, and in the auditory cortex when presenting auditory stimuli.
2. It is expected to find greater activation in the medial temporal lobes when presenting semantically incongruent sentences, possibly left lateralized.
3. It is expected to find a stronger effect of semantic incongruity in the auditory modality, also expected to be in the medial temporal lobes.
4. It is expected that participants have a slower reaction time on the behavioural task when presented with semantically incongruent sentences.

Methods

Experimental Design

The study used a repeated-measures two-by-two factorial design. The participant was presented with English sentences that were either semantically congruent or incongruent, and presented visually or auditory. We measured the reaction time and the brain activation using fMRI.

Participants

The participant was a 21-year-old Danish male university student. He had an orthodontic device in the lower part of the face.

Materials

Apparatus:

The scanning was performed on a Siemens Skyla 3 Tesla MRI scanner with a repetition time of 2 seconds.
The stimuli were played on fMRI compatible headphones during the auditory condition and shown on a screen through mirror during the visual condition.

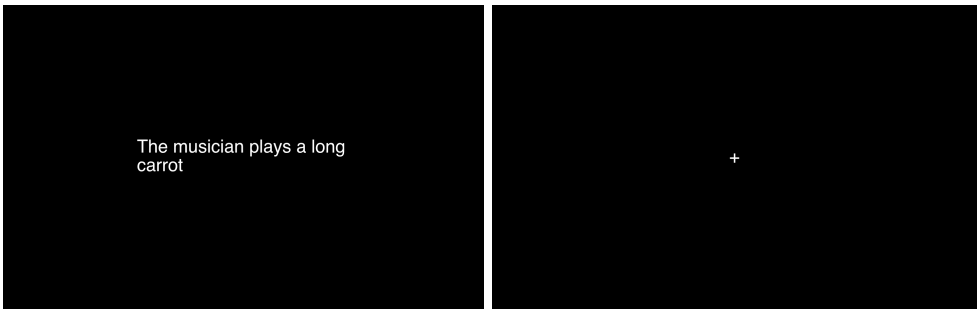
Stimuli:

The stimuli consisted of 88 sentences that were generated with the help of an online random sentence generator (http://www.manythings.org/rs/). The sentence structure patterns that were used can be seen in Figure 1.

Structure:	**Example:**
Subject + Verb + Indirect Object + Direct Object	*I promise her a delicious dinner.*
	His wife forgives him his water.
Subject + Verb + Object	*The doctor prescribed the pills.*
	The volunteers teach the doors.

Figure 1. *The structure of the sentences with examples.*

Sentences were between 20 and 27 characters long excluding whitespaces, and all ended with the target noun that was either semantically congruent or incongruent.
Some of the randomly generated sentences were then edited to avoid random generation mistakes where congruent sentences make as little sense as incongruent sentences.
To reduce experimental noise, the target nouns were chosen with a word length between 3 and 6 letters, with a Thorndike-Lorge written frequency above 1000, and with concreteness ratings between 300 and 700, retrieved from the MRC Psycholinguistic Database.
There were 44 congruent and 44 incongruent sentences. In each condition, half of the sentences were presented auditorily and the other half presented visually, selected randomly.
In the visual condition, the stimuli were presented as whole written sentences on a black background (see Figure 2a). The auditory sentences were made into sound files using Google Translate's sounds and read aloud to the participant from fMRI compatible headphones with a fixation cross on the screen (see Figure 2b).



(a) (b)

Figure 2. (a) *Example of the visual stimuli.* ***(b)*** *Screen during the auditory stimuli.*

Procedure

Prior to the experiment, the participant was instructed, was asked to sign a consent form, and was reviewed for contraindications such as MR unsafe medical devices and implants.
Before starting the scans, and screen display settings and the strength of the sound levels were tested. A substantial part of the stimuli was presented during this testing due to technical error. After receiving confirmation from the participant that he could see the stimuli and that the sound levels were as strong as possible while still comfortable, he was helped into the MRI scanner and the scanning began.

Then a short 30-second pre-scan was made to localize the participant's head, followed by a 6 min structural scan that captured the anatomy of the participant's head.
The scan with experiment was conducted twice, but due to a technical error only the results for the second experiment were measured. The scan lasted for 12 min, of which the paradigm took up 9 minutes.
Between the two experimental scans, the participant was exposed to another 12-minute visual stimuli experimental paradigm. Thus, the complete time the participant spent in the scanner approached 45 minutes.
The participant was instructed to press the blue button with the index finger when the sentence was congruent, and the yellow button with the middle finger when the sentence was incongruent.
In the experimental paradigm, the visual and auditory stimuli alternated consistently, and the incongruent and congruent conditions came in a randomized order.

Data analysis

Preparation

The accuracy of the semantic decision task was 65.9%. Of all the errors, 90.0% belonged to the auditory condition. These data points were included in the analysis in order to keep the necessary statistical power.

Behavioural analysis

Data points with reaction times below 0 were excluded as technical errors from the analysis. Data points without behavioural responses were also excluded.
The behavioural data was weakly but significantly skewed, and thus the assumption of normality was violated.
A model was made to predict reaction times from the congruency conditions, using stimulus type as random intercepts.
All calculations were performed off-line using R version 3.3.2 (2016-10-31).

Scanning analysis

The pre-processing consisted of realignment to remove movement artefacts, coregistration to synchronize changes in structural and functional images, segmentation to discriminate between differ-

ent kinds of brain matter, normalization to make pictures correspond to normalized brain, and finally smoothing to make voxels depend on other voxels using a Gaussian kernel.
The analysis of the pre-processed brain scans tested for general brain activation across all conditions, for differences in brain activation between the visual and auditory conditions and between the congruent and incongruent conditions, and for interaction effects between the two main effects.
The results were corrected for Family Wise Errors (henceforth, FWE) at a 0.05 p-value threshold. An uncorrected 0.001 p-value threshold was used for exploratory results.
When testing the main effect of auditory and visual conditions, it became evident that the labels of the two conditions had been irrevocably switched due to a technical error. For the reporting of the analysis results, it was chosen to attribute the clear activation in the visual cortex to the visual condition, and the activation in the auditory cortex has been attributed to the auditory condition. See the discussion for consequences of this.

All data processing was performed off-line using MATLAB version 9.1 (2016b) and SPM12 version 6906 (2014).

Results

The main effects of visual and auditory conditions

Activation in the auditory cortex (Figure 3) was attributed to the auditory condition, in accordance with the first hypothesis.

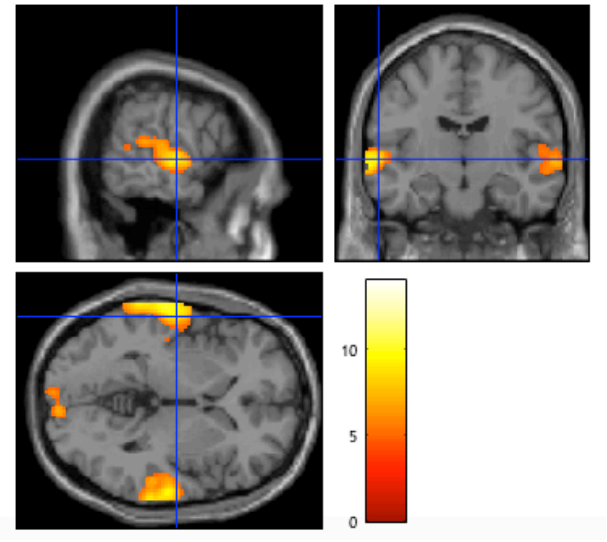


Figure 3. *Activation (threshold at p<0.05) in the auditory cortex (temporal lobes)*

Activation in the visual cortex (Figure 4) was attributed to the visual condition, in accordance with the first hypothesis.

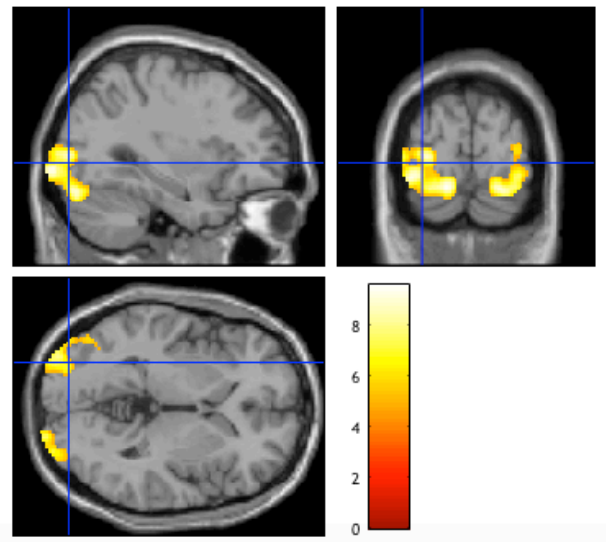


Figure 4. *Activation (threshold at p<0.05) in the visual cortex (occipital lobe)*

The main effect of incongruent condition, uncorrected

In the incongruent condition a slight activation was observed in the midsagittal section (Figure 5) when FWE was not corrected for. The hypothesis predicted greater activation in this condition, but the place of activation is not in accordance with the hypothesis.

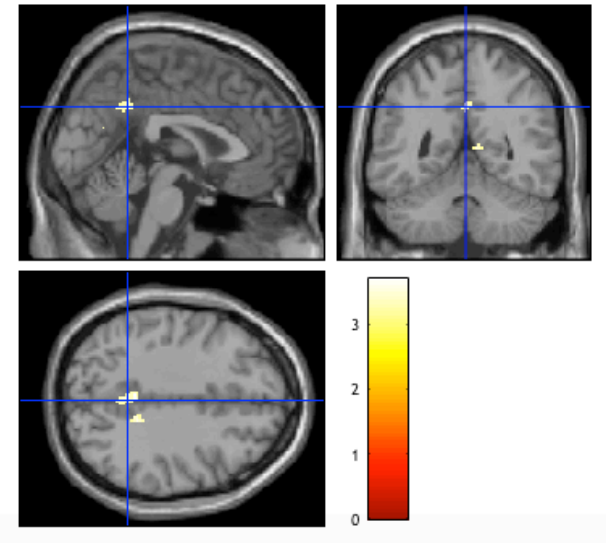


Figure 5. *Activation (threshold at p<0.001 uncorrected) in the midsagittal section*

The interaction effect, uncorrected

As an interaction effect between incongruency and auditory conditions, when uncorrected for FWE there was found spread activation in the occipital lobe and left lateralized activation in the temporal lobe (Figure 6).

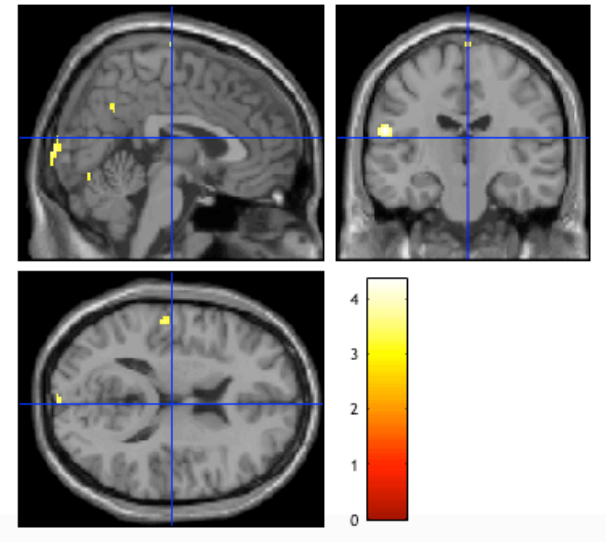


Figure 6. *Activation (threshold at p<0.001 uncorrected) in the occipital lobe and left temporal lobe*

The behavioural task

Even though reaction times were slightly longer in the incongruent condition like the hypothesis predicted, the difference was not statistically significant ($p > 0.05$, $t(84) = 1.08$) (Figure 7).

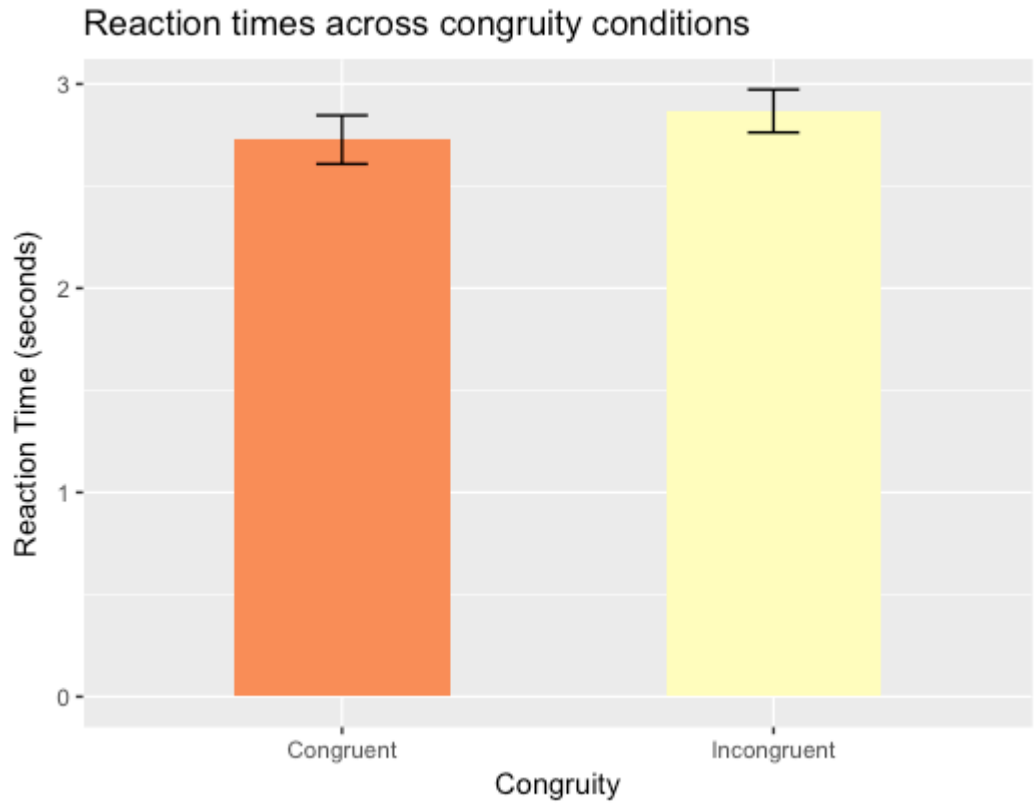


Figure 7. *Behavioural reaction times across modalities for congruity conditions*

Discussion

There are numerous problems with the reliability of the experiment.

Firstly, there was only gathered data from a single participant, which both results in very low statistical power and a questionable representativity of the general population. The participant was also equipped with an orthodontic device, and while this is not thought to have caused much disturbance, it might have introduced enough noise to hide some effects.

Secondly, the results of the analysis initially showed clear activation in the visual cortex for the auditory condition and vice versa. This makes it very likely that an unknown error happened, and that the labels for the conditions were switched. The activation was reattributed to the proper condition, but the fact that the error happened makes it possible that the labels of the congruency conditions were also switched. We see more activation in the incongruent condition, and see activation in the left medial temporal lobes for the interaction between incongruity and auditory conditions. Both are in accordance with existing literature, and makes it plausible that the labels were not switched – but they are only exploratory results in an already unreliable experiment, and so it is impossible to be certain. This means that even if the results of the analysis were statistically significant, the results could still only be used for exploratory purposes.

A third important problem with the procedure of the problem is that, due to technical errors during the test of sound levels, and the fact that the experiment was run twice but only recorded the second time, the participant was exposed once or twice to all the sentences in the paradigm prior to the recorded experiment. That the participant is more familiar with the sentences could make the effect of semantic incongruity less strong, hiding it from an experiment with low statistical power.

It is also important to mention that the participant gave incorrect responses on the very easy behavioural task in almost half the auditory trials. It seems unlikely that the participant lost attention or was answering randomly, or that the task was too difficult, because most of the responses on the visual trials were correct. Most probably, it points to the participant not being able to either hear or understand the sentences, which might be because the sound levels were too low for the participant to make out the stimuli over the sound of the MRI scanner. It is possible that generating the stimuli using sounds from Google Translate – which cannot correctly produce many speech properties like prosody or rhythm – has made the sentences hard to understand. In addition to all this, the difficulty

of the behavioural task on the auditory condition should also be greater, since it is not possible to re-examine the stimuli like it is in visual trials.

Next, the data on the reaction times from the behavioural task was not normally distributed, which further lowers the reliability of the statistically insignificant effect that was indicated by the data.

It is also a question of the method of presenting the visual stimuli. In most the experimental paradigms for investigating the N400 effect, the sentence is shown word for word to make it possible to exactly measure the time of presenting the target word. While this has advantages – the participant would not be able to see the target word before reading the rest of the sentence, and would be likely to spend more time being primed before seeing the target word as well – the greatest disadvantage is that it is a very unnatural way of reading. The choice of maintaining natural reading methods may have introduced noise to the experiment, through which the very low statistical power has not been able to detect an effect. One option for later studies could be to show the whole sentence except the target word, and then show the target word alone. In this way, it is also possible to measure the onset of the stimuli exactly, and it is closer to natural reading methods.

Lastly, the low temporal resolution in fMRI experiments combined with the low statistical power of the current experiment results in potential noise coming from other event-related potentials like surprise effects not related to semantic congruity.

Conclusion

Because of low statistical power and numerous technical and procedural problems, nothing can be firmly concluded from the current experiment. Activation in visual and auditory cortices was observed as a contrast between auditory and visual conditions, but no effect of semantic incongruity on brain activity or reaction times was observed, nor was any interaction effect with modalities confirmed. To be able to make any stronger claim, a less flawed replication of the experiment would have to be done with more participants, possibly altering the way of presenting visual stimuli to require less statistical power.

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