Investigation of face processing mechanisms in the brain using fMRI

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Abstract

Expertise in face processing seems confined to the upright orientation: face inversion causes deterioration of performance in face discrimination tasks - the face inversion effect (FIE). Functional magnetic resonance imaging (fMRI) techniques are a powerful tool in investigating the neural correlates of face perception, but they have not yet clarified the neural mechanisms underlying the FIE. In this Thesis, the main objective was to implement methods for conducting and analysing behavioural and fMRI experiments to study the effects of face inversion in a parametric fashion, by using faces at multiple orientations. Appropriate face stimuli were generated and a face discrimination task was implemented at orientations between 0° and 300°, using both a block and an event-related design. Additionally, the possibility of acquiring expertise in a non-canonical direction was also explored, by implementing a training protocol at 120° and then testing for transfer of learning to 240°. Behavioural tests on a group of subjects and fMRI pilot experiments were conducted to assess the effectiveness the methods developed. It was found that block designs yield an overall better result, by showing a significant effect of face orientation on reaction times. An effect of session with Learning was observed, but it did not reach significance. Although the results obtained clearly lack statistical power, probably due to the small number of subjects, they do indicate trends in accordance with previous results. Moreover, the fMRI pilot experiments demonstrated the feasibility of the paradigms and suggest that visual brain areas are indeed modulated by face orientation.

Keywords: fMRI, FIE, paradigm, learning, implementation, face processing.

1. Introduction

Functional magnetic resonance imaging (fMRI) is a technique developed in the early 1990s that allows for the detection of short-term physiological changes associated with brain activity (Huettel et al. 2004). fMRI acquisition sequences are tuned to detect changes in the concentration of the haemoglobin molecule, present in blood. This molecule has magnetic properties that depend upon its binding to oxygen (Webb 2003): oxygenated haemoglobin (Hb) is diamagnetic, i.e. has zero magnetic moment; deoxygenated haemoglobin (dHb) is paramagnetic, with significant magnetic moment (Huettel et al. 2004). The presence of a paramagnetic molecule in the blood stream creates magnetic susceptibility between blood vessels and the surrounding tissue. If the field differences created by the susceptibility occur within a voxel of the image, then water resonance frequencies shift, producing phase dispersion. This will cause loss of signal and the voxel will appear darker; these intensity changes are the basis of the BOLD (blood-oxygenation-level dependent) contrast (Ogawa et al. 1990).

The analysis of fMRI data is aimed at detecting the brain regions where the BOLD signal time course is correlated with the stimulus/task of hemodynamic interest. For this purpose, fMRI data may be expressed by a general linear model (GLM, equation 1).

$$y = G \times \beta + \varepsilon \qquad (1)$$

In this equation, data (observed response) is represented by a matrix, y, of N time points by V voxels (re-arranged in one dimension, for simplicity). G is the design matrix, which specifies the model to be used (in terms of design factors and confounds that need to be used). As each of these factors corresponds to a column, G is of dimensions: N time points x M model factors. The parameter matrix, β, manages the relative (weighted) contributions of each regressor present in G to response y. As such, β is of the following dimensions: M model factors x V voxel; thus, each cell of this matrix indicates parameter amplitude for a specific voxel. The model is adjusted to the data y in order to obtain the combination of weights that minimizes ε. (Friston 2005).

The most relevant parameters for any fMRI experiment are detection and estimation. The first pertains to identifying active voxels and the latter to knowing the time course of the activated voxels. However, it might not be possible to create an fMRI design that enables simultaneously good detection and good estimation (Huettel et al. 2004). Two main types of stimulation/task paradigm designs are usually employed: Event-Related (ER) and Blocked (BD) designs. The former is characterized by grouping together stimuli with the same level of the independent variable (stimulus category / task condition) which remains constant throughout the block. It has a high detection power (Huettel et al. 2004). In the latter, each event corresponds to one level of the IV (independent variable) and the estimation power is high. fMRI designs may also be parametric. These are designs that are employed to study the effects of the modulation of a given stimuli, e.g., by creating different levels of difficulty associated with a given task, but without changing the nature of the stimuli (Amaro and Barker 2006).

Some regions of the brain specialize in a category of stimuli, i.e. respond more to a given category of stimuli, such as places, objects or faces. Namely, a central complex of stimuli-selective activation in the lateral occipital cortex (LOC) (Grill-Spector and Malach 2004), has been reported to respond strongly to objects (Grill-Spector and Malach 2004). One other region has been reported to respond more strongly to places and scenes than to other object categories (Peelen and Downing 2005); as such it is known as the parahippocampal place area or PPA (Grill-Spector and Malach 2004).

Specific face processing seems to be restricted to three main regions, all of them bilateral. These encompass the inferior occipital gyrus (IOG, also known as the posterior fusiform gyrus or OFA, for occipital face area), the lateral middle fusiform gyrus and the superior temporal sulcus (STS) (Rossion and Gauthier 2002). The most relevant area, the one that presents the largest difference in activation for faces and objects, is the lateral middle fusiform gyrus, where the fusiform face area or FFA is located. The object-selective region, LOC, has also been reported to respond to upright and inverted faces (Epstein et al. 2005).

Further proof of specialization of the brain is in the fact that inversion has a disproportionate effect on face recognition (Rossion and Gauthier 2002), a phenomenon that is known as the face inversion effect (FIE). The effect of processing faces in orientations different than 0° or 180° has not been widely studied. However, a recent study, using faces in 6 orientations (0° to 300°, 60°increments), reported higher response times and error rates, for orientations up to 180° and decreasing values for both variables for orientations 240°-300° (Gomes et al. 2009).

The main objective of the work presented in this Thesis was the development of the methodological tools required for the performance of a brain imaging experiment to study the mechanisms of face processing and learning in the human brain using fMRI. For that, 3 paradigms were developed, Block, Event Related (Face Rotation paradigms) and Learning (divided into two phases training and transfer). Each of these was applied in behavioural testing; Block and Event Related were also used in fMRI acquisitions, to assess in which areas these paradigms elicited activation. An additional paradigm, Localiser (LOCA), was developed to identify which areas are more responsive to faces, houses and objects.

2 Implementing the Paradigms

Faces to be used as stimuli were generated using a demo version of FACES4.0 (IQ Biometrix, www.iqbiometrix.com/products_faces_40.html*)*. A total of 54 faces were generated, grouped in 18 sets of 3. Each of these sets has one specific combination of external features (chin, face shape, hair, ears and neck). For each type of external feature, there are 3 sets of internal features; their distribution was thought out so as to be as harmonious as possible. Generated faces were exported and edited in Photoshop 6.0 (Adobe Systems Incorporated) and rotated into the desired orientations, from 0° through 300°, every 60° (6 orientations). Since each of the 54 faces occurred in each orientation, the total number of faces generated was thus 324. A mask was designed for each face, by shuffling the pixels in the face, with the aid of a MATLAB (MathWorks) script. This way, the overall image power is maintained but the face is not recognisable.

The 4 paradigms implemented were defined in Presentation (Neurobehavioral Systems, http://www.neurobs.com/) languages PCL and SDL.

Three of these paradigms, Block, Event Related and Learning paradigms consisted of a series of trials of a face discrimination task: two faces are presented sequentially and then the subject has to decide whether they are identical or different and press one of two buttons according to this decision. These trials have the same structure in all experiments and follow the design shown in Figure 1. Half of the trials presented in each paradigm are *same trials*, trials in which stimuli 1 and stimuli 2 are the same face. In the remaining trials, the stimuli presented are different, making them *different trials*.

Each trial begins with a 100 ms-long fixation cross, to focus the subject's attention. This is followed by an interval that separates the fixation from the first stimulus (blank interval), lasting an average 250 ms (randomized between 200 ms and 300 ms). The first stimulus lasts 400 ms and immediately after, the corresponding mask is shown (for 200 ms). This is followed by an interval, lasting an average 800 ms (randomized between 700 ms and 900 ms) which separates the mask from the second stimulus (interstimulus interval). This stimulus is always 10% bigger than the first and lasts only 200 ms. Each trial is separated by an inter trial interval, with an average duration of 1550 ms (randomized between 1400 ms and 1700 ms). The averaged duration of a trial, including inter-trial interval, is 3500 ms (Jacques and Rossion 2007). The two faces shown in each trial always share external features and are displayed in the same orientation. The mask is also displayed in the same orientation as the stimuli. Every face appears in one same trial and one different trial. Total number of trials is 216, for all paradigms except the transfer phase of Learning, which has only 72 trials. In the blocked paradigm, the 216 trials are divided into 6 blocks resulting in a total of 36 blocks, each containing 6 trials. Each block corresponds to one orientation. In Event Related, the 216 trials occur individually, with no relation to the previous or the consecutive trial. The occurrence of orientations is pseudorandomized. In the Learning paradigm, only one orientation is shown in each phase (120° in training and 240° in transfer). In each of these paradigms, null trials (null blocks in the case of block design) were introduced, in a ratio of 1:6 (in transfer, the ratio was 1:3). Figures 2-4 describe the basic time course of these 3 designs. Learning Paradigm had pauses dividing it into 3 equal parts, while BD and ER were defined as 3 individual runs. The reason for this was that the paradigms would be too demanding on the subject if they were presented without interruption. The fourth paradigm, FFA-PPA-LOC Localiser (LOCA) required no task to be performed and contained 4 runs. 16 blocks would present gray scale pictures falling into one of 4 categories: *Houses (H)*, *Faces (F)*, *Objects (O)* or *Scrambled objects (S)*. 5 more blocks were added as fixation blocks. Block order was symmetrically counterbalanced within each run; run 1 and run 3 had the same block order, as had run 2 and run 4 (Peelen and Downing 2005).

To analyse the results obtained, a multistep methodology was employed. Firstly, Presentation was programmed to generate a logfile in each experiment recording the following information for each trial: relation between the faces shown ("same" or "different"); pair of faces shown; response button pressed (1 or 2); response times (delay between presentation of the second face stimulus and response).

Secondly, a MATLAB script was written to load the Presentation logfiles and extract the relevant information for the analysis of the experiments. Here, trials were grouped into sets – named *blocks* – each containing 6 trials. Grouping of trials was done by orientation and by occurrence for face rotation paradigms and by occurrence only for Learning paradigm. Two parameters were extracted from this analysis: error rates and response times, for each factor. These were statistically analyzed by SPSS (www.spss.com) and the respective charts were built using a MATLAB script. To better enable access to all features of this methodology, an interface was built using MATLAB.

3. Materials & Methods

3.1. Behavioural Experiments

5 subjects were selected for the BD experiment (4 males), with an average age of 37.80±21.41 years and an average education of 11.20±5.85 years. 3 subjects used the left mouse button to identify faces as "same". 5 subjects were also selected for the ER experiment (1 male), with an average age of 39.4±20.66 years and an average education of 14.60±3.29 years. 7 unpaid volunteers participated in the Face Learning experiment, 3 males, with the average age of 23.3±3.20 years and an average education of 17.4±3.05 years. None was paid. 3 subjects used the left mouse button to answer "same". All subjects were right handed and had normal or corrected to normal vision. Subjects were applied the paradigms described after the respective instructions were given. The subject's responses (same/different) were given using a computer mouse, in which one button (left or right) would correspond to a "same" answer and the other to a "different" answer. Buttons were counterbalanced across subjects. Test was preformed once on each subject, at natural light levels. The Training phase of the Learning paradigm was applied on each subject for 4 consecutive days, once a day, at approximately the same time every day. At the end of the fourth day, the subjects were asked to perform the Transfer phase.

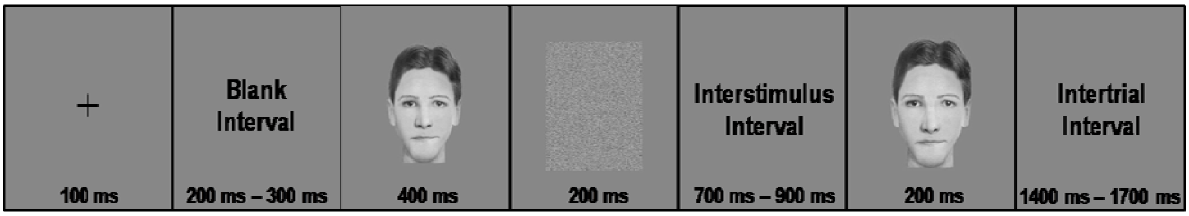

Figure 1: Trial structure diagram.

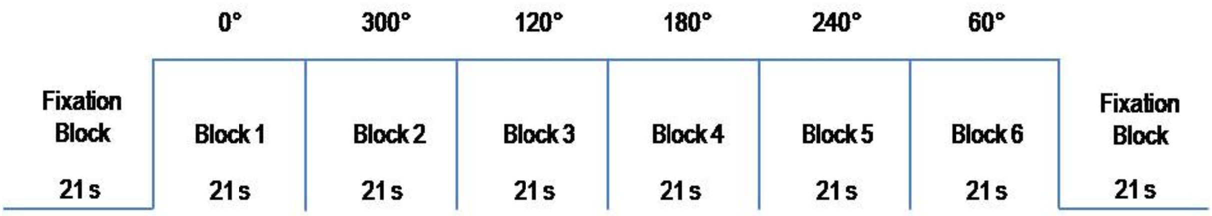

Figure 2: Block Design paradigm diagram. 6 consecutive blocks of stimuli are followed by a fixation block, of the same length. This pattern is repeated 6 times throughout the paradigm, with the orientation order changing each time. The orientation order shown corresponds to the first 6 blocks.

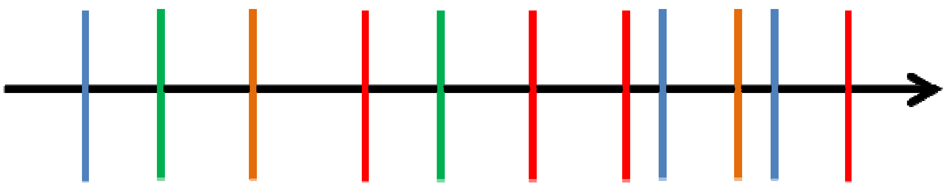

Figure 3: Example of an Event-Related design diagram. Red lines represent fixation trials; the remaining lines represent task trials: each colour identifies a different stimuli level. Notice that the distance between each bar is not uniform, indicating the presence of jitter. The ratio (task trials)/(fixation trials) is not the same as was used in the current paradigm. [Based on a figure in (Mechelli et al. 2003)]

3.2 *fMRI Experiments*

Two pilot fMRI experiments were carried out at Sociedade Portuguesa de Ressonância Magnética (SPRM), with the collaboration of Dr. Martin Lauterbach. One healthy female subject aged 23 was studied in a pilot experiment. The subject was right handed and had corrected-to-normal vision. An informed consent form was signed prior to the scan. 2 fMRI sessions were conducted, 2 weeks apart. On the first session, an anatomical image was collected and the subject was submitted to the block design. On the second session, subject was submitted to localiser scan, event-related design and another instance of block design paradigm. Functional data were acquired on a 3 Tesla Philips MRI system using a GE-EPI (gradient-echo echo planar imaging). Stimuli were shown to the subject via a double-mirror screen, attached to the Radio Frequency coil; the projector was a computer in the outer room. In both sessions, the subject lied in the scanner in supine position, with the head aligned and a clear view of the double mirror. The subject used an MR-compatible button box device to respond during the face discrimination task. No responses were recorded in the pilot experiment. Data was processed using FSL (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl) (Smith et al. 2004; Woolrich et al. 2009), specifically FEAT (model-based and fMRI analysis, version 5.98). A T1-weighted 3D high-resolution image was acquired to provide morphological information of the whole brain with good spatial detail. A number of functional imaging runs were then acquired throughout the two sessions. Resulting data were registered to the subject's high-resolution T1-weighted image and to the standard MNI (Montreal Neurological Institute) space (Collins et al 1995), using FLIRT (Jenkinson et al. 2002; Jenkinson and Smith 2001).The parameters used in each acquisition are shown in Table 1, discriminated by session.

To analyse fMRI data, a set of explanatory variables (EVs) was defined for each paradigm. These were combined in contrasts used for lower level analysis of data. Following this step, all paradigms (except block design from session 2, acquired in a single run) were subjected to higher level analysis where the averaged results of all runs were analysed for each contrast. As BD from Session 1 had too much noise, its analysis was replaced by BD from session 2. For Face Rotation paradigms, EVs were built according to orientation (0, 60, 120, 180, 240, 300); in Localiser, EVs were based on stimulus type (Faces, Houses, Objects, Scrambled). Higher-level results for LOCA were extracted from 3 out of the 10 contrasts, which were considered to yield the most significant: results: contrast 4 for faces (F>S), contrast 8 for houses (H>S) and contrast 10 for objects (O>S). For both FR-BD and FR-ER the most significant contrast (of the 13 defined) was contrast 3, pertaining to quadratic modulation of activation. LOCA results for contrast F>S were added to the FR results, to better identify areas where overlapping occurred.

For fMRI results, only the relevant activation clusters are shown and both the respective maximum (or peak) coordinates and the centre of gravity (COG) coordinates are indicated.

Parameters	Session 1	Session 2		
	FR-BD (3 runs)	LOCA	FR-ER (3 runs)	FR-BD (single run)
TR (ms)	2000	3000	3000	3000
TE (ms)	35	35	35	35
FWHM (mm)	8	5	5	5
Number of volumes acquired	165	114	106	325
FOV (mm^2)	230x230	230x230	230x230	230x230
Matrix (image) dimensions	96x96x29	80x80x38	80x80x38	80x80x38
Flip angle	90°	90°	90°	90°
Resolution (mm^3)	2.396x2.396	2.875x2.875	2.875x2.875	2.875x2.875
Number of slices	29	38	38	38
Slice Thickness (mm)	4	3	3	3
Dummies	6 vols (12 s)	4 vols (12 s)	4 vols (12 s)	4 vols (12 s)

Table 1: fMRI parameters for each acquisition

4. Results

4.1 *Behavioural Results*

For BD, repeated measures ANOVA found no significant effect of block on either measure (F(0.761), p> 0.05 for error rates; F(0.570), p> 0.05 for response times). Although the effect of orientation on error rates was not significant (F(1.967), p>0.05), a significant effect was observed for response times (F(3,899), p<0.05). Further analysis demonstrated that no trend was found for either measure, whichever factor considered.

The global average reaction time was 590.83±23.3 ms and the global average error rate was 0.17±0.03. Both reaction times and error rates exhibited a quadratic pattern of variation as a function of orientation: results present an initial rise that reaches a maximum value at 120°, beginning then to decrease. This decrease does not occur in a successive manner, as orientation 5 registers higher values of response times than orientation 4. Nevertheless, orientations 4-6 (180° - 300°) all register smaller values of response times than those registered for orientation 3. Factor block elicits no discernible trend in either error rates or response times.

Regarding Event-Related Design, repeated measures ANOVA found no effect of orientation or block on either measure ($p>>0.05$). Additionally, cubic trends were found for the effect of orientation on response times ($F(11.865)$, $p<0.05$) and the effect of block on response times ($F(42.755)$, $p<0.05$). Despite the lack of statistical significance, some trends could be observed in the data.

Considering error rates only, factor orientation seemed to have a more pronounced effect than factor block, as it caused a higher variability in values, which were as low as (approximately) 0.20 and larger than 0.35. Error rates associated with orientation presented less variability, with value oscillating between 0.25 and 0.30, approximately. Response times presented a similar behaviour, as results for factor "block" presented a higher amplitude of values, ranging from a minimum of less than 550 ms (block 1) to a maximum of over 650 ms. Orientation yielded a smaller amplitude, but elicited more variability. For this factor, response times register a successive increase up until the 2nd orientation (60°), but orientation 3 (120°) caused a decrease in this variable. The maximum value corresponds to orientation 4 (180°), with orientation 5 eliciting a similar, but smaller, value. The lowest value for this variable is registered for orientation 6. None of the factors shows any level of consistency, which translates in the absence of a pattern of results for both error rates and response times, with either factor. For this paradigm, the global average reaction time was 621.13 ± 30.04 ms and the global average error rate was 0.27 ± 0.03.

Learning paradigm considered only one factor, block. However, given the nature of the paradigm, a "session" parameter will also be included in the overall results. However, repeated Measures ANOVA yielded no significant effect of "Block" or "Session" ($p>0.05$). Still, a linear trend ($F(11.200)$, $p<0.05$) was found for the effect of block on error rates. For response times, the trend yielding significant results was a 4th order trend ($F(4.444)$, $p<0.46$). In spite of these results, a clear trend for an effect of session on Response Times could be observed both in the results. This variable seems to decrease with the effect of session, yielding a global difference (between the first and last values) of approximately 100 ms. For error rates, the effect of session was less clear, but it is still noticeable. No clear trend was found for the effect of "block" on either response times or error rates. This is true for the two instances of learning considered: training phase and transfer phase.

Figure 4 displays results considered most significant for each of these paradigms: error rates/response times *vs.* Orientation for Face Rotation paradigms and error rates/response times *vs* Session for Learning.

4.2 fMRI results

Considering Localiser, for the contrast O>S (Objects), only 2 clusters of activation were considered significant. These clusters are bilateral, and can be found in LOC, in the left (x=-42, y=-82, z=0; COG: x=-27.6, y=-42.2, z=12.6) and the right hemisphere (x=44, y=-72, z=-12; COG: x=39.7, y=-66.8, z=-1.28). These clusters had values of Z of respectively 12.6 and 12.7 ($2.3<Z<13.3$).

Regarding contrast H>S (Houses), only two clusters were regarded as significant. One was located in the right hemisphere (x=28, y=-50, z=-12; COG: x=36.3, y=-66.9, z=3.81) and the other on the left (x=-28, y=-52, z=-10; COG: x=-38.6; y=-69.6, z=0.0), but both pertain to the temporal occipital fusiform cortex. The right cluster registered a value of Z=10.6 and for the left one Z= 9.26 ($2.3<Z<11.0$).

For Faces (contrast F>S), 3 clusters were considered relevant. Contrary to what happened for houses and objects, one of these clusters displayed lateralization, occurring only in the right hemisphere (x=40, y=-44, z=-24; COG: x=42.4, y=-45.5, z=-23.7), specifically in the temporal occipital fusiform cortex. For this cluster, Z= 6.55. The remaining clusters are indicative of activation in LOC in both the left (x=-42, y=-76, z=-16; COG: x=-51.1, y=-70.2, z=-4.55) and the right hemisphere (x=44, y=-74, z=-8; COG: x=53.7, y=-69.2, z=1.95). Z values are Z= 6.51 for left cluster and Z= 9.26 for right cluster ($2.3<Z<9.3$).

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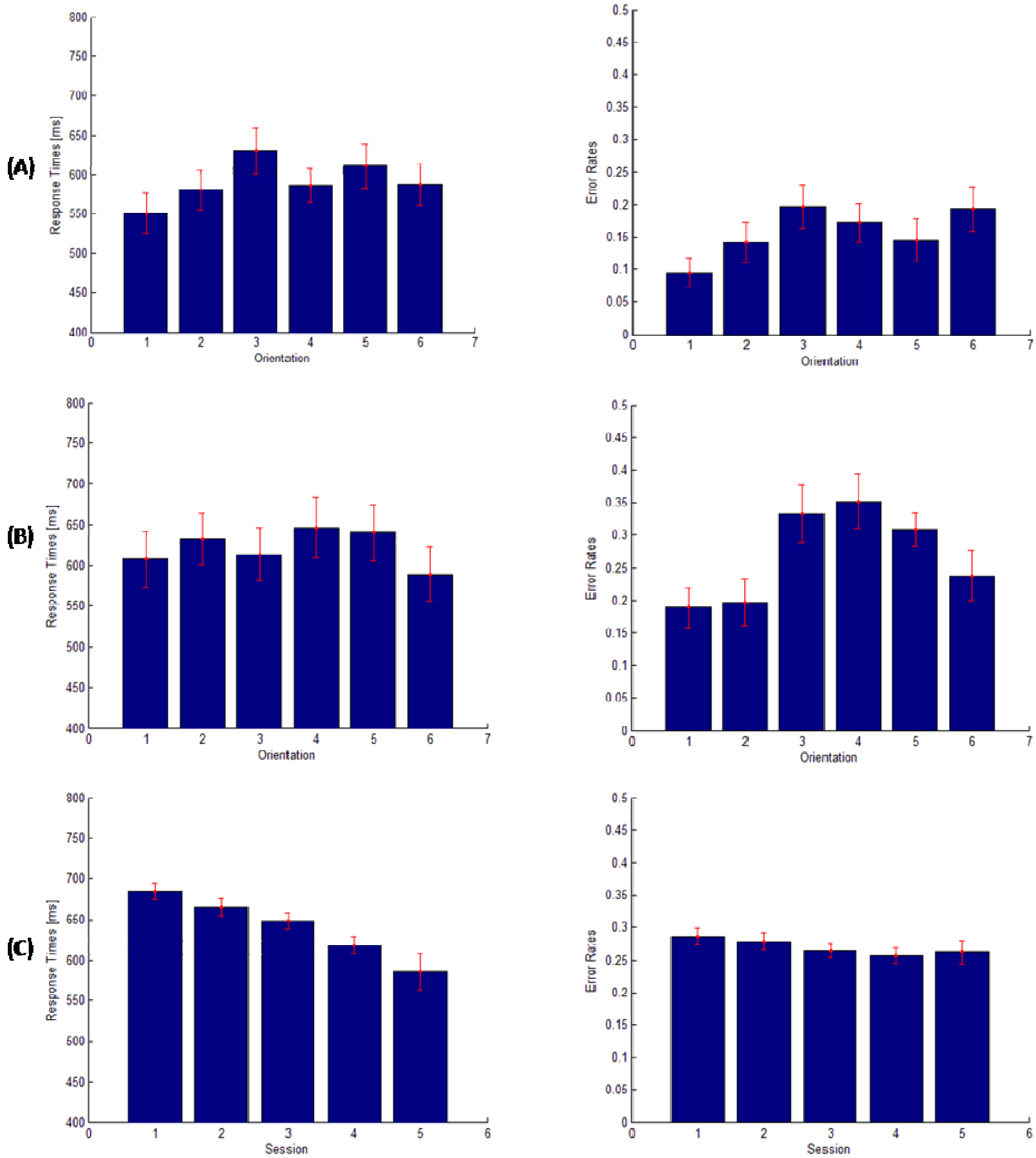

Figure 4: Overall results for behavioural experiments, for response times (left) and error rates (right). Only results for orientation (in the case of Face Rotation paradigms) and Session (in the case of Learning) are shown. (A) BD; (B) ER; (C) Learning. Orientations 1-6 correspond respectively to 0°, 60°, 120°, 180°, 240°and 300 °. Session 5 corresponds to Transfer Session. Bars represent overall averages and error bars represent standard error of mean.

On the 6 clusters identified by contrast 3 for FR-BD, the following regions were identified: right LOC (peak coordinates: x=44.4, y=-75.4, z=-5.65), with maximum Z=.8.86; left LOC.(peak coordinates x=-46, y=-78, z=-6); right (x=48, y=-50, z=-22) and left (x=-44, y=-46, z=-22) temporal occipital fusiform cortex (including FFA), with maximum Z values, respectively, Z= 3.87 and Z= 4.93 (3.5<Z<8.9).

Regarding FR-ER, some runs presented an artifact known as Gibbs Ringing. This effect polluted the results and hindered a proper higher-level analysis. As the first run was unaffected by this artifact, its results were usable and were thus analysed. The contrast used yielded 7 clusters of activation, including the following areas: left LOC (peak coordinates x=52.9, y=-65.1, z=21), with maximum Z= 4.46; right LOC (peak coordinates -57.8, -69.4, 8.24), with maximum Z= 5.17; right inferior temporal gyrus, temporo-occipital part (peak coordinates x=48, y=-56, z=-16), with maximum Z= 2.67 (2.3<Z<5.3). No similar activation was found in the left hemisphere.

Results for these 3 experiments are displayed in Figure 6.

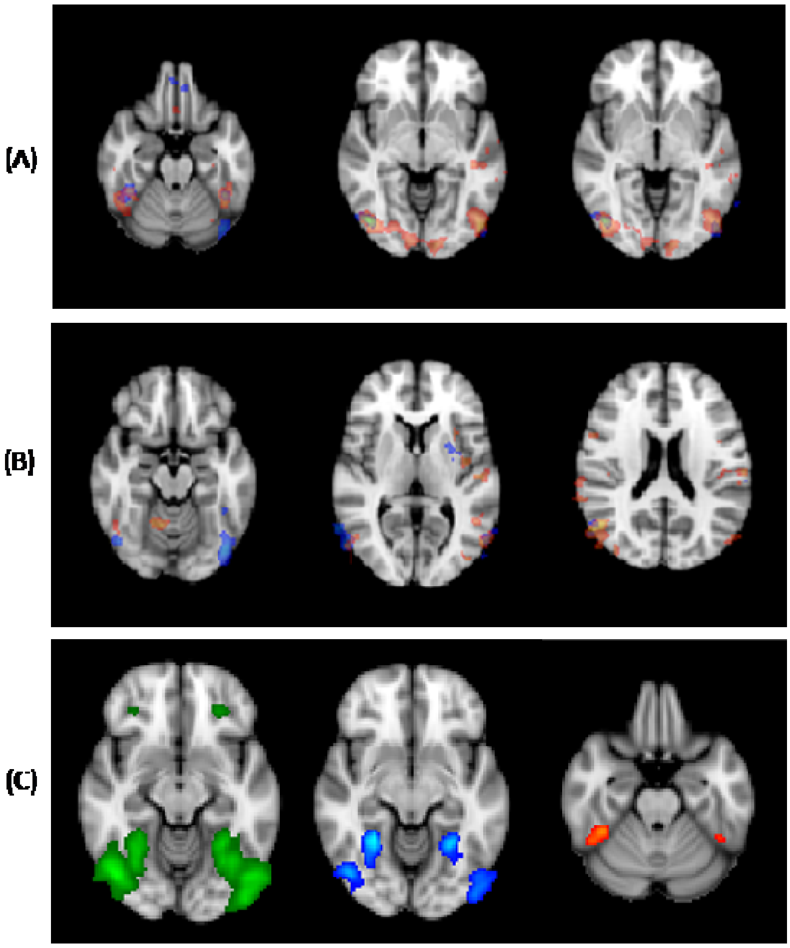

Figure 6: Results for fMRI pilot experiments: (A) Results for BD (3.5<Z<8.9). *Left:* FFA, both hemispheres (slice z= -22). *Middle:* Right hemisphere activation for LOC (slice z= -6). *Right*: Left hemisphere activation for LOC (slice z= -4); (B) Results for ER (2.3<Z<5.3). *Left:* FFA, right hemisphere (slice z= -16). *Middle:* Left hemisphere activation for LOC (slice z= 8). *Right*: Right hemisphere activation for LOC (slice z= 20); (C) Results for LOCA. *Left:* Bilateral activation of LOC, contrast O>S (3.5<Z<13.3, slice z= -12). *Middle:* Bilateral activation of PPA, contrast H>S (3.5<Z<11.0, slice z= -10). *Right:* FFA activation, right hemisphere, contrast F>S (3.5<Z<9.3, slice z=-24). Background image is MNI template. Right hemisphere is on the left.

5 Discussion

5.1 Behavioural results

The behavioural tests employed both the block and the event-related designs of the multiple face orientation experiment. The practice and transfer experiments on a non-canonical orientation were also tested. The results showed the expected effects of orientation on reaction times, with a quadratic pattern. However, these results were not statistically significant, which was attributed to the low statistical power due to the small group of subjects used.

Overall results for BD show a larger effect of orientation than of block: effects of the latter are negligible, as no coherent pattern of behaviour was generated. While these results for block are not unexpected, the effect of orientation is different than anticipated: it was expected of error rates and response times an approximately quadratic modulation. This way, 0° would correspond to the minimum (canonical orientation) and 180°to the maximum (inverted position). From 0°-180° there would be a successive increase, while orientations 240°and 300° would stand for a decrease, but never to lower values than those registered for orientation 0°. While the overall form of the response is not far from this model (0° is the minimum value in both cases), orientation 180°registers a decrease in the value of response times. Thus, the value obtained for this orientation is smaller than what would be expected. This is an intriguing effect and one that is common to both error rates and response times. It seems to suggest subjects had less difficulty in processing inverted faces than faces at 120°or 240° orientations, as both error rates and response times registered low values for these orientations. It is possible that the occurrence of such results is related with the low statistical power of the experiment (only 5 subjects were used). Expansion of the subject pool, by conducting further experiments, might help clarify this situation and generally improve experimental results.

Repeated measures ANOVA found results that were almost statistically significant ($p<0.05$) for the effect of orientation on response times. Regarding the effect of block, no significance effect was found, but this factor is expected to have a less considerable effect.

Generally speaking, behavioural results for FR- BD show that the observed effects of face orientation on reaction times over a face discrimination task are still present when a block design is employed - in place of an event-related design, as previously used, (Gomes et al. 2009). The low statistical power of the subject pool is a direct hindrance to the acquisition and its increase could greatly improve the statistical significance of the results. Nevertheless, these results are encouraging in indicating that a block design may be appropriate to test the effects of orientation, which is advantageous in an fMRI experiment, due to the greater sensitivity of such designs relative to event-related designs.

For FR-ER, the overall results of factor block do not exhibit any considerable effect, as results for both response times and error rates exhibit an erratic behaviour, with no foreseeable pattern. This is not surprising; block is not expected to have a significant effect on either variable, given the pseudo-randomization of stimuli. Orientation, on the other hand, was expected to elicit a response similar to the one expected for block design. This is not the case; however, response times results obtained for FR-ER are similar to results obtained for FR-BD, with the additional advantage that he maximum value was indeed registered for orientation 4. For error rates, the pattern obtained is the one expected, with this variable displaying an (approximately) quadratic behaviour.

No significance was found for either factor, when using Repeated Measures ANOVA. However, highly significant results were obtained in a previous study (Gomes et al. 2009), establishing a strong relation between the effect of orientation and response accuracy and speed. The differences in results reported in this current Thesis are most likely consequence of the limited statistical power of the experiment: it seems thus premature to label this paradigm as unfit for this type of study.

Regarding Learning paradigm, the effect of block was negligible for both error rates and response times, as no discernable pattern was found, for either phase. Repeated Measures ANOVA also found no significance to this factor. This was somewhat expected, due to the small effects of block observed in the previous work (Gomes et al., 2009). However, it should be mentioned that for one block, error rates were close to 0.5. Although this is the threshold considered for chance trials, this block occurred in transfer after the subjects had already been submitted to the training phase. It is possible that, probably due to exhaustion, subjects were less focused, thus leading to chance results. Nevertheless, this was an isolated event, and the remaining blocks all had error rates inferior to 0.4, which leads to the conclusion that this particular block may be overlooked without polluting the results.

Performance over time – "Session" – on the other hand, showed a definite effect, especially in response times, with a clear decrease in response times as session progresses. Furthermore, those values hold for transfer phase, suggesting that there is transfer of a learnt "behaviour" to a different orientation. These results are again consistent with the ones from the previous work using a very similar paradigm (Gomes et al. 2009). For error rates the effect of training is less noticeable, but it is present. Still, despite these encouraging results, no statistical significance was found for "Session".

5.2 fMRI pilot experiments

The two imaging sessions included one localiser paradigm, two block design paradigms and one event-related design paradigm of the multiple face orientation experiment. The results validated the localiser paradigm for the identification of the faces, houses and objects brain areas, namely the FFA, PPA and LOC, respectively. In terms of the multiple face orientation paradigms, the pilot results were encouraging. They showed significant quadratic effects of orientation in brain sensitive areas, including the FFA.

This paradigm yielded positive results, as all conditions (faces, houses and objects) elicited detectable responses. Objects elicited responses in LOC, a classic object processing area. Faces also elicited (bilateral) activation in this area. This is not entirely unexpected: despite being a well known area of object processing, a recent study (Epstein et al. 2005) also reported a response to upright faces, especially, on the right hemisphere, which is accordance with data obtained in this Thesis: activation for LOC in the right hemisphere was stronger than the response in the left hemisphere. Additionally, faces also elicited activation in the middle fusiform gyrus (MFG), specifically in the FFA (Epstein et al. 2005). In this instance, activation was unilateral, occurring only on the right, the hemisphere for which FFA is reported to exhibit the strongest activation. However, this paradigm neglected to detect activation in the STS, a region detected in previous studies (Epstein et al. 2005). This difference could be due to the fact that Epstein and colleagues used both upright and inverted faces, while the current Localiser used only upright faces. Additionally, as only one subject was used, this could also be a direct consequence of the poor statistical power of the experiment.

Regarding Houses, the activation detected is consistent with location of the PPA, in both the left and right hemispheres. This is a classic scene-processing area reported by Epstein et al. as responding to houses (Epstein et al. 2005). Furthermore, Epstein and colleagues also reported that activation for upright Houses was stronger on the right hemisphere, a fact that was also verified in this Thesis. Thus, Houses also elicited the expected response; nevertheless, the low statistical power of the experiment should not be ignored, and further experiments, using more subjects might be necessary before definitive conclusions may be drawn.

A quadratic effect was detected in the temporal occipital fusiform cortex, bilaterally but with a predominance of the right hemisphere. The area detected is consistent with the location of the FFA (Epstein et al. 2005), a classic face sensitive area. This result is positive, as it is the accordance with what would be expected for this area. Nevertheless, improvement of the statistical power of the pilot is necessary to obtain more robust conclusions.

Results obtained for FR-BD also indicate the presence of a quadratic effect in LOC, bilaterally. Although this is a classic object-selective region, it has been reported to also respond differentially to upright and inverted faces (Epstein et al. 2005). Furthermore, these results seem to indicate that not only does it responds to faces, but also that the response presents a quadratic trend. Although these results are both interesting and encouraging, it should be noted that the experiment conducted in this Thesis was merely a pilot, with a low statistical power. Further experiments should be conducted, expanding the subject pool, to improve statistical power and further verify these results.

When comparing the results obtained for FR-BD with the results obtained with LOCA (for faces), an overlap of activations is detected. The areas identified by Localiser as being faces areas do indeed register a quadratic modulation of activation in the presence of faces at multiple orientations. This is an interesting result, as it verifies the validity of the results obtained for LOCA, despite the low statistical power of both experiments.

The results obtained for FR-ER pertain only to one run, due to the problems stated above (see Results section). As such, besides being affected by the low statistical power of the pilot, results shown correspond to only 1/3 of the total experiment, thus making them even less robust. Nevertheless, this paradigm yielded significant results: quadratic effects were detected for bilaterally for LOC, as had occurred for FR-BD, which supports the claim that in the presence of faces in multiple orientations, activation in this area does follow a quadratic modulation. Quadratic effects were also detected in a location consistent with the FFA, as would be expected. Naturally, new experiments are necessary to allow for more robust and general conclusions. This entails not only an increasing statistical power but also overcoming the effects of the artifact detected.

Comparing the results obtained for FR-ER with the face areas identified by Localiser, an overlap was detected bilaterally for LOC, but no overlap was detected for FFA. While results for LOC further validate LOCA, the lack of overlapping activation in the area identified as the FFA seems to suggest that there is some incompatibility between the experiments. Although there was no overlap, both paradigms, individually, elicited activation in areas consistent with FFA. In light of the additional limitations of FR-ER (only 1/3 of the results were used), these results can be considered inconclusive, since it is not possible to draw definitive conclusions from this data.

5.3 Conclusions and Future Directions

Despite lacking in statistical power, all the paradigms were able to yield at least partially expected designs. In Learning, for instance, there was no statistically significant effect of session, although there was an overall decrease in response times. Regarding Face Rotation paradigms, their performance in overall tests is considered equivalent. However, in the fMRI pilot, FR-BD detected quadratic activation in more areas associated with face processing. Furthermore, results for this paradigm were in accordance with LOCA results, as overlapping with these results was achieved for identified areas. FR-ER on the other hand, only achieved results consistent with LOCA for 2 of the 3 identified areas. However, these results were analysed using only one third of the experiment (one run), which attests for their robustness. Nevertheless, the current data does not support any definitive conclusions, and results comparing the efficiency of both paradigms in this kind of study are inconclusive. Localiser also failed to elicit activation in STS, a well established face area, although expected scene- object- and (other) face-specific areas were correctly detected by this paradigm.

It is important to stress that all of the behavioural tests displayed poor statistical power, consequence of a limited pool of subjects. Further tests, using more subjects could greatly improve the performance of the paradigms. However, this is not the only point in which there can be improvement. Future developments may also include paradigm optimization or stimuli redesigning. Pre and post-processing scripts could also be improved, and, perhaps be made more effective and generalist. Application of ROI analysis, besides the whole-brain analysis performed here, for the assessment of face orientation effects is also an interesting future development, as it might help to investigate changes in brain activity in pre-defined brain areas, with improved sensitivity.

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References

Amaro, E., and Barker, G. J. (2006). "Study Design in fMRI: Basic Principles." *Brain and Cognition*(60), 220 - 232.

Collins et al. (1995). "Automatic 3-D model-based neuroanatomical segmentation." *Human Brain Mapping*, 3(3), 190-208.

Epstein, R. A., Higgins, J. S., Parker, W., Aguirre, G. K., and Cooperman, S. (2005). "Cortical correlates of face and scene inversion: A comparison." *NeuroPsychologia*(44), 1145-1158.

Friston, K. J. (2005). "Models of Brain Function in Neuroimaging." *Annu. Rev. Psychol.* (56), 57-87.

Gomes, C. A., Mendes, M., and Figueiredo, P. (2009). "Perceptual learning for multiple face orientations." *Perception*, ECVP Abstract Supplement(38), 79.

Grill-Spector, K., and Malach, R. (2004). "The Human Visual Cortex." *Annu. Rev. Neurosci.*(27), 649-677.

Huettel, S. A., Song, A. W., and MacCarthy, G. (2004). *Functional Magnetic Resonance Imaging*, Sinauer, Sunderland, Massachusets U.S.A.

Jacques, C., and Rossion, B. (2007). "Early electrophisiological responses to multiple face orientations correlate with individual discrimination performances in humans." *NeuroImage*(36), 863-876.

Jenkinson, M., Bannister, P., Brady, M., and Smith, S. (2002). "Improved Optimisation for the Robust and Accurate Linear Registration and Motion Correction of Brain Images." *NeuroImage*, 17(2), 825-841.

Jenkinson, M., and Smith, S. M. (2001). "A Global Optimisation Method for Robust Affine Registration of Brain Images." *Medical Image Analysis*, 5(2), 143-156.

Mechelli, A., Price, C. J., Henson, R. N. A., and Friston, K. J. (2003). "Estimating Efficiency a priori: a comparison of blocked and randomized designs." *NeuroImage*(18), 798-805.

Ogawa, S., Lee, T. M., Kay, A. R., and Tank, D. W. (1990). "Brain magnetic resonance imaging with contrast dependent on blood oxygenation." *Proc. Natl. Acad. Sci.*, 87, 9868 - 9872

Peelen, M. V., and Downing, P. E. (2005). "Within-Subject Reproducibility of Category-Specific Visual Activation With Functional MRI." *Human Brain Mapping*(25), 402-408.

Rossion, B., and Gauthier, I. (2002). "How does the brain process upright and inverted faces?" *Behavioral and Cognitive Neuroscience Reviews*, 1(1), 62-74.

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., Luca, M. D., Drobnjak, I., Flitney, D. E., Niazy, R., Saunders, J., Vickers, J., Zhang, Y., Stefano, N. D., Brady, J. M., and Matthews., P. M. (2004). " Advances in functional and structural MR image analysis and implementation as FSL." *NeuroImage*, 23(S1), 208-219.

Webb, A. (2003). *Introduction to Biomedical Imaging*, John Wiley & Sons, Inc., Hoboken, New Jersey.

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., Beckmann, C., Jenkinson, M., and Smith, S. M. (2009). "Bayesian analysis of neuroimaging data in FSL." *NeuroImage*, 45(S), 173-186.