

How Do Familiarity and Expectation Influence the Human Brain Signal?

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Familiarity (i.e., whether an observer has seen an image before) and expectation (i.e., whether an observer can predict which image will follow based on the current image) can influence the processing of visual information in the brain. The electrophysiological signal from the brain can indicate how visual processing changes between familiar and novel as well as between expected and unexpected images. An important question arising from previous research is whether familiarity has an effect on neural activity that is separable from the effect of expectation. In order to address this issue, we adopted an experimental design which manipulated familiarity separately from expectation, and we used magnetoencephalography (MEG) to record brain activity in humans. We found that familiarity, unaffected by expectation, has a genuine influence on the brain signal, such that novel images induced significantly higher amplitude than familiar images. In addition, expectation also had an effect on the neural response: unexpected images were accompanied by significantly higher amplitude than expected images. These outcomes demonstrate that both visual familiarity and expectation influence the human electrophysiological signal, and they do so in similar ways. These findings improve our understanding of how visual processing changes with the amount and type of experience the brain has had with a visual stimulus.

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Our environment is characterised by complex visual features, making visual perception an arduous task. The human visual system has evolved to parse this complicated conglomeration of colours, orientations, and textures and deliver a stable percept of the world. In order to achieve this computationally heavy process, the visual system allots more resources to the processing of some types of information than others. For instance, input can be represented in more detail in visual cortex if it comes from the fovea compared to the periphery of the eye (Engel, Glover, & Wandell, 1997). Attention can also affect how information is prioritised in the brain: input can be weighted more strongly if it is attended compared to unattended (Lu & Doshier, 1998; Yeshurun & Carrasco, 1998; Baldassi & Burr, 2000; Carrasco, Penpeci-Talga, & Eckstein, 2000; Cameron, Tai, & Carrasco, 2002).

Another factor affecting perception is how much experience the visual system has had with a particular object or feature. When an individual observes a specific scene multiple times, the brain learns about this environment. The next time the person encounters this scene, the visual system uses its knowledge about the features characteristic of this scene and does not process it in the same way it would a novel scene (Li, Miller, & Desimone, 1993). Specifically, when an image is presented repeatedly, the brain activity in response to it diminishes (Miller, Li, & Desimone, 1991; Grill-Spector Henson, & Martin, 2006). This shows that the visual system readily adapts to repeated input.

When an observer views an image repeatedly, this renders the image familiar. Li et al. (1993) investigated how the electrophysiological signal measured in macaque inferior temporal (IT) cortex changes as a visual stimulus becomes familiar. The researchers presented streams of images; on multiple occasions, they showed a single image which was initially novel but over time became familiar. They found that, for a subset of neurons, the spike rate gradually diminished as the initially novel stimulus became familiar to the monkey. Then, when a novel stimulus was presented, those same neurons reached a higher spiking rate, signifying that the reduction in activity for familiar stimuli was not simply due to neural fatigue.

Studies in humans also show that familiar images are associated with lower brain activity than novel images. In a functional magnetic resonance imaging (fMRI) study (Rossion, Schiltz, & Crommelinck, 2003), the BOLD response was measured while participants saw familiar and novel faces. In the fusiform face area (FFA) and in the occipital face

area (OFA) of the right hemisphere, which are especially sensitive to facial features, novel stimuli elicited higher activation than familiar stimuli. Even though the BOLD signal in human sensory cortex and the spike rate of monkey IT neurons are two very different measures, in this case they are in agreement and demonstrate that familiar visual stimuli induce lower brain activity than novel images.

The presence of a sequence in visual information can also affect visual processing. If an individual observes images in a specific temporal sequence, then it is possible to predict which image will be presented next. In order to investigate this type of visual expectation, Meyer and Olson (2011) recorded the firing rate of neurons in monkey IT. They presented pairs of images (A and B), one image following the other, so the monkeys learned to expect the second image (B) once they saw the first (A). Sometimes the first image (A) was followed by the trained second one (B), rendering the second image expected. At other times, an image from another pair (C) was shown after A, and in this case the second image (C) was unexpected based on the first one (A). Then, the researchers recorded the neural response to the two images. The firing rate of IT neurons was lower for an expected image than for an unexpected one. Moreover, the response to an expected image was reduced, or truncated, more rapidly than the response to an unexpected one.

Similar results have been acquired in human studies. In an experiment examining expectation with fMRI, participants first heard an auditory cue which predicted the orientation of a visual grating (Kok, Jehee, & de Lange, 2012). Then, a grating was shown, and the BOLD response to that visual stimulus in V1 was analysed. Activity in V1 was lower when the tone correctly predicted the grating orientation, rendering it expected, compared to when the tone induced an incorrect prediction about the grating, making it unexpected. fMRI investigations in humans and electrophysiological experiments in monkeys jointly suggest that expected stimuli are associated with lower activity than unexpected ones, a phenomenon called expectation suppression.

These studies show that both familiarity and expectation can lead to decreased neural activation. This makes sense since familiarity and expectation are related phenomena: conceptually, familiarity is highly relevant for expectation. While observing a visual scene, the brain becomes familiar with the scene's features. This acquired knowledge can be used to predict the upcoming input, which results in expectation (Clark, 2013). Therefore, it seems plausible that people need to

be familiar with something in order to expect it. In terms of brain activity, it is possible that any effect of expectation is actually due to familiarity. Expectation suppression may not be caused by the expectation itself but rather by the familiarity, which is necessary for the expectation to occur. However, the electrophysiological study in monkey IT with expected and unexpected stimuli by Meyer and Olson (2011) suggests that this is not the case. The researchers presented pairs of stimuli, and the leading image could render the trailing one expected or unexpected. Both types of trailing images had been shown during training, so they were familiar to the monkeys. The neural response to expected and unexpected differed even though both types of images were familiar, suggesting that expectation has an effect which cannot be explained by familiarity.

Familiarity has also been investigated with single-cell recordings from IT (Meyer, Walker, Cho, & Olson, 2014). Monkeys first saw single images multiple times and became familiar with them. Later, while neural activity was being recorded, the monkeys saw the images in sequences. Pairs of images were alternated, such that on a single trial the macaques would see image A, then image B, then A, B, A, B. Sometimes these sequences were comprised of familiar images, and other times of novel ones which the monkeys had never seen before. Images were presented very rapidly, a single image lasting for 120 ms and immediately followed by the next image. The recordings showed that the firing rate for familiar images was truncated more quickly than for novel images. In addition, familiar stimuli elicited responses with a larger dynamic range (larger peak-to-trough difference) than novel stimuli.

According to this study (Meyer et al., 2014b), familiarity's influence on the neural response is similar to that of expectation (i.e., truncation of the response) as found by Meyer and Olson (2011). However, based on the experimental design, it is possible that expectation also contributes to this observed effect. In particular, each familiar image was used during 6.5 sessions on average; in contrast, each novel image was only used during one session. Since the monkeys had been exposed to the familiar images extensively but had never seen the novel images before, it is plausible that, when observing a sequence of familiar images, they could predict an upcoming familiar image much more easily than an image in a novel sequence. It is also possible that the same pair of familiar stimuli was observed on multiple occasions, leading to more sequence learning for the familiar images than for the novel ones. In other words, upcoming images were more

predictable in the familiar condition than in the novel condition. Therefore, the way in which familiarity was manipulated also influences expectation, making it possible that the observed effect is not solely caused by familiarity but also by expectation.

The current research project

In order to determine whether familiarity has a genuine effect on the neural signal even when stimuli are not predictable, we conducted a study in which we manipulated familiarity separately from expectation. We used magnetoencephalography (MEG) to record brain activity in humans. Similarly to Meyer et al.'s (2014b) design, participants saw rapidly presented six-image sequences, and the images depicted objects. Subjects were familiarised with images by means of a training session. Then, during the MEG recording session, participants saw the familiar images as well as novel images which they had not seen before. In order to induce expectation, we showed subjects two sets of six images during the training session. For one set, they saw the images in a specific sequence, making it possible to come to expect that sequence (familiar sequenced condition). For the other set, they always saw the images in a shuffled order, so they could not learn to expect a specific sequence (familiar unsequenced condition). During the MEG session, the familiar sequenced images were sometimes presented in the learned order (expected condition) and sometimes in a shuffled order (unexpected condition). The unsequenced familiar images were again presented in shuffled orders during the MEG session (for a diagram depicting the full list of conditions, see Fig. 1.). Thus, familiarity referred to whether the subjects had seen an image during the training session, while expectation referred to whether they could predict the sequence of the upcoming images. With this design, we could isolate the effect of familiarity from that of expectation because we manipulated familiarity separately from expectation. Notably, familiarity still had a significant influence on the neural response even without the influence of expectation.

Method

Participants

Twenty-nine healthy human volunteers (15 female, 14 male, $M_{\text{age}} = 24.17$ years, $SD = 3.80$ years) with normal or corrected-to-normal vision, recruited from the university's

participant pool, completed the experiment and received either monetary compensation or study credits. The study was approved by the local ethics committee (CMO Arnhem-Nijmegen, Radboud University Medical Center) under the general ethics approval (“Imaging Human Cognition”, CMO 2014/288), and the experiment was conducted in compliance with these guidelines. Written informed consent was obtained from each individual.

Procedure

First, participants completed a behavioural training session in which they only saw the familiar images (sequenced and unsequenced). They saw the familiar sequenced images always in the same order, while the familiar unsequenced images were shown in shuffled orders. Importantly, the order for the sequenced images was circular: each of the six images could be presented first, and they all had equal predictive values. Familiar sequenced images comprised 50% of trials, and familiar unsequenced images comprised the other 50%. Participants performed an oddball detection task by pressing the spacebar when they saw an image of a rubber duck. Images of duckies were presented on 10% of trials as one of the six images in the sequence. The duckies were of eight different colours and there were two viewpoints per colour for a total of 16 images of rubber ducks. Multiple images of ducks were used to avoid confounding the oddball stimulus with a particular colour (e.g., yellow) or a specific viewpoint. We chose to include a visual oddball task in order to keep participants’ attention on the visual stimuli even though subjects did not actually have to perform a task on those stimuli.

During the behavioural training session, participants completed 10 blocks of 80 trials each for a total of 800 trials. Each block lasted for 4.9 minutes, so the whole experiment lasted for about one hour. At the end of the behavioural training session, participants’ knowledge of the order of familiar sequenced images was assessed. Participants were shown one of the six sequenced images, and they had to indicate which of the five remaining images was most likely to follow it. This was done for each of the six images in the familiar sequenced set. The assessment took about three minutes.

One or two days later, participants completed the MEG testing session in which they saw familiar and novel images. In contrast with the training session, the familiar sequenced images were sometimes presented in the learned order (expected) and other times in a shuffled order (unexpected). The shuffled

sequences for unexpected trials were chosen in such a way that each image in the sequence was followed by an unpredicted image; in other words, none of the images were followed by the image they predicted. Similarly to the training session, the familiar unsequenced images were shown in shuffled orders. Familiar unsequenced images comprised one third of trials, and familiar sequenced images also comprised one third of trials, half of those being expected and half unexpected (see Fig. 1). Participants also saw novel images which they had not seen before, and these comprised the remaining one third of trials. Different novel images were used for every trial, so each novel image was only shown once during the whole experiment. Similarly to the training session, participants had to perform an oddball task: they had to respond when they saw a rubber duck, and these were presented on 10% of trials (Fig. 1 shows a diagram of the experimental conditions). During the MEG testing session, participants completed 8 blocks of 120 trials each for a total of 960 trials. Each block lasted for 7.4 minutes, so the whole experiment lasted about one hour. At the end of the MEG testing session, participants’ knowledge of the familiar images was assessed. Participants saw 60 images, the twelve familiar ones and 48 randomly selected from the novel images participants had been shown, and subjects had to indicate whether the image was familiar or novel. ‘Familiar’ referred

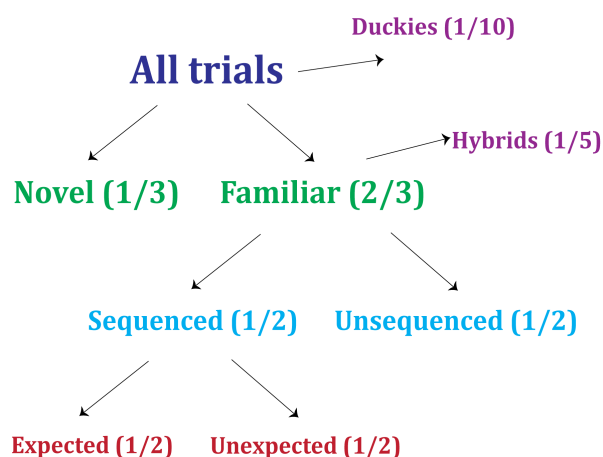


Fig. 1. A diagram of the different conditions included in the experiment. The trials were divided into familiar (2/3 of all trials) and novel (1/3 of all trials). One half of familiar trials were sequenced (1/3 of all trials) and the other half were unsequenced (1/3 of all trials). One half of sequenced trials were expected (1/6 of all trials) and the other half were unexpected (1/6 of all trials). Out of all familiar trials, 20% were hybrids. Out of all trials, ducks were presented in 10% of the cases.

to images seen repeatedly during the behavioural training session as well as during the MEG testing session, while ‘novel’ referred to images seen only once during the MEG testing session.

Trial structure

For the behavioural training session as well as for the MEG testing session, each trial began with a fixation dot. Fixation dots were of the type “bull’s eye”: a small black dot in the middle surrounded by a larger, thin white circle, which was in turn surrounded by a larger, thin black circle. This type of fixation dot has been shown to improve participants’ ability to fixate (Jehee, Brady, & Tong, 2011). For a diagram of the trial structure, see Fig. 2. The fixation dot was presented for a period between 500 and 750 ms; the exact duration was determined randomly per trial. Then an image was shown for 180 ms, immediately followed by another image, also lasting for 180 ms. This was repeated until all six images in the trial were presented. Afterwards, if an oddball was presented during the trial and a response was given, the fixation dot turned green for 500 ms. If the response was incorrect, the fixation dot turned red for 500 ms. A response was considered incorrect on three occasions: 1) if the subject pressed the button during a trial with an oddball stimulus but before the oddball was presented, 2) if the participant pressed the button on a trial where no oddball was presented, or 3) if the subject did not press the button on a trial where an oddball was presented. If no oddball was presented and no response was given, the change in colour of the fixation dot was omitted, and the white-and-black fixation dot remained on the screen for 750 ms. Then, a blank screen was presented for 1250 ms, and participants were encouraged to blink during this period. After this, the next trial began with a fixation dot.

Materials

The stimuli were shown on monitors with a resolution of 1920x1080 pixels using MATLAB (The Mathworks, Inc., Natick, Massachusetts, United States) and the Psychophysics Toolbox extensions (Brainard, 1997). A refresh rate of 100 Hz was used in order to ensure that the presentation of the stimuli lasted for exactly 180 ms. We chose the duration of 180 ms for image presentation based on data from Meyer et al. (2014b) suggesting that this induces a larger response truncation effect in humans than a duration of 120 ms. In both the behavioural and MEG sessions, the images subtended four degrees of visual angle, again following Meyer et al.’s (2014b) paradigm. For the behavioural training session, 24-inch monitors were used. For the MEG session, the screen was 53 cm in width and 41 cm in height, and a PROpixx projector was used to project the images on the screen; the projector had a resolution of 1920x1080 and an aspect ratio of 16:9.

Stimuli

Participants viewed images from the set provided at <http://cvcl.mit.edu/MM/uniqueObjects.html>. A different object was represented in each image, and all objects were shown against a white background. A total of 2377 images were available to be presented, and from those 2377, 2054 were presented for each participant. Familiar images were randomly selected for each pair of participants and manually inspected before presentation in order to avoid any striking pictures. Each pair of participants saw different familiar images, and the familiar sequenced and familiar unsequenced images were counterbalanced within a pair of participants. Specifically, if for participant 1, set 1 comprised the familiar sequenced images and set 2 comprised the familiar unsequenced images, the opposite was true for participant 2: set 2 comprised the familiar sequenced images, and set 1 comprised the familiar unsequenced images.

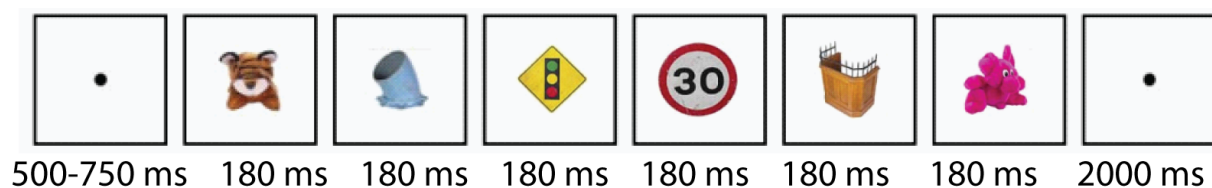


Fig. 2. A diagram of the trial structure. At the beginning of each trial, a fixation dot appeared for a randomly selected time period between 500 and 750 ms. The first image was presented for 180 ms. Immediately after that, the second image was shown for 180 ms. This continued until all six images had been displayed. The last image was followed by a fixation dot present for 2000 ms.

MEG recordings

Brain activity was recorded using a 275-channel MEG system with axial gradiometers (VSM/CTF Systems, Coquitlam, BC, Canada) in a magnetically shielded room. During the experiment, head position was monitored online and corrected if necessary (Stolk, Todorovic, Schoffelen, & Oostenveld, 2013). This method uses three coils: one placed on the nasion, one in an earplug in the left ear, and one in an earplug in the right ear. To aid in the removal of eye- and heart-related artifacts, horizontal and vertical electrooculograms (EOG) as well as an electrocardiogram (ECG) were recorded. A reference electrode was placed on the left mastoid. The sampling rate for all signals was 1200 Hz. A projector outside the magnetically shielded room projected the visual stimuli onto a screen in front of the participant via mirrors. Participants gave their behavioural responses via an MEG-compatible button box. Participants' eye movements and blinks were also monitored by an eye-tracker system (EyeLink, SR Research Ltd., Mississauga, Ontario, Canada).

MRI Recordings

Anatomical magnetic resonance imaging (MRI) scans were acquired for the purpose of source localization analysis. Anatomical images were collected for each participant or retrieved from a database when available. The images were acquired using a 3T MRI system (Siemens, Erlangen, Germany). These recordings were not used for the current work.

MEG data analysis

The MEG data were preprocessed offline using the FieldTrip software (Oostenveld, Fries, Maris, & Schoffelen, 2010) (www.fieldtriptoolbox.org). Trials with high variance were manually inspected and removed if they contained excessive and irregular artifacts. Independent component analysis (ICA) was applied to identify regular artifacts such as heartbeat and eye blinks. The independent components for each participant were then correlated to the horizontal and vertical EOG signals and to the ECG signal. In this way, it was possible to identify which components most likely corresponded to the heartbeat and eye blinks. Furthermore, the data were baseline-corrected on the interval starting at 200 ms before stimulus onset until stimulus onset (0 ms).

A low-pass filter at 30 Hz was applied to the data. Trials where oddball stimuli were presented and/or a response was given were removed from analysis. This was done because oddballs and responses elicited neural activity unrelated to the research question. A planar transformation and event-related field (ERF) analysis were applied to the MEG data.

Statistical analysis

For the behavioural results, mean reaction time and accuracy were first calculated within participant per condition. Paired-samples *t*-tests (p -value = .05, two-tailed) were applied to the data of all participants within the two relevant conditions for a comparison.

In order to statistically test the MEG results and control for multiple comparisons, we applied cluster-based permutation tests (Maris & Oostenveld, 2007), as implemented by FieldTrip (Oostenveld et al., 2010). The tests were carried out on the time period between 0 and 1200 ms, 0 ms being the onset of the first stimulus, over all sensors, and 1000 permutations were used per contrast. For each sensor over multiple time points, the MEG signal was compared between two conditions, yielding a *t*-value. A sensor could potentially contribute to a cluster at a certain time point if its corresponding p -value was lower than .05 (two-tailed). Temporally adjacent time points with such p -values were grouped into positive and negative clusters. Cluster-level statistics were calculated by summing the *t*-values within a cluster, and a cluster was considered significant if its p -value was smaller than .05. The standard error of the mean was computed within participants, as described by Cousineau (2005) and with the correction suggested by Morey (2008).

Results

Behavioural results

The participants' task was to press a button whenever they saw an oddball stimulus, in this case an image of a rubber duck. Participants correctly identified almost all oddballs and refrained from responding when no oddball was presented ($M = 99.35\%$, $SD = 0.35\%$). Participants' accuracy was not significantly influenced by familiarity ($t(28) = -1.20$, $p = .24$), sequence ($t(28) = 1.17$, $p = .25$), or expectation ($t(28) = 1.04$, $p = .31$). This high accuracy was not driven by the fact that participants did not respond regardless of trial type. On oddball trials when they had to press a

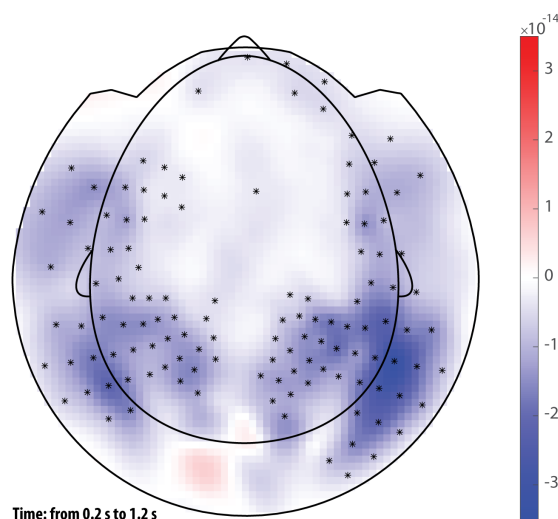


Fig. 3. Topography of the difference between the familiar unsequenced and novel conditions. Black asterisks mark sensors that contribute to the significant cluster for at least half of the time period from 200 ms to 1200 ms.

button, their accuracy remained high ($M = 94.90\%$, $SD = 2.88\%$). Participants' accuracy on oddball trials was not significantly affected by familiarity ($t(28) = -0.81$, $p = .42$), sequence ($t(28) = 0.07$, $p = .95$), or expectation ($t(28) = 0.57$, $p = .57$). Furthermore, participants' reaction times were not significantly affected by condition either. Familiarity did not affect reaction times ($t(27) = -1.74$, $p = .09$) and neither did sequence ($t(27) = -0.30$, $p = .77$) nor expectation ($t(27) = -0.04$, $p = .97$). Reaction time data were analysed for 28 out of the 29 participants because an error in data acquisition rendered accuracy and reaction times from the first participant unusable.

At the end of the behavioural training session, participants' knowledge of the order of the familiar sequenced images was assessed. On average, when participants were shown an image and had to report which image followed it, they selected the correct image 25% of the time ($SD = 19.7\%$), which was not significantly different from chance level, i.e., 20% ($t(27) = 1.29$, $p = .21$). This suggests that subjects were mostly unaware of the sequence, which is in agreement with their informal verbal reports.

At the end of the MEG session, participants' knowledge of the familiar images was assessed. On average, when participants had to report whether an image was familiar or novel, they did so correctly with a mean accuracy of 91.9% ($SD = 5.8\%$). Apparently, subjects were aware which images were familiar and which were novel.

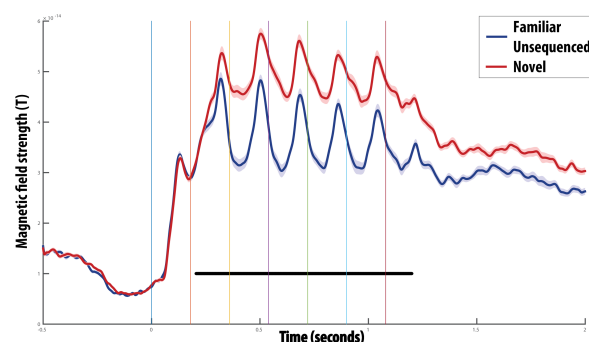


Fig. 4. Activity over time for the familiar unsequenced condition (blue) and the novel condition (red). Activity is averaged over the sensors highlighted in Figure 3. The shaded areas are error bars illustrating the within-subject SEM for the unsequenced familiar (light blue) and the novel (light red) conditions. The horizontal black bar at the bottom shows that at least one of the selected sensors contributes to the significant cluster at this time point. The coloured vertical lines denote the onset of each image (1-6), and the last one denotes the offset of the last image.

MEG results

To look at the difference between familiar and novel items without any influence of the expectation manipulation, we tested the difference between the familiar unsequenced vs. novel conditions since participants did not learn a sequence for the images in the unsequenced condition. A significant difference emerged for the cluster shown in Figure 3 from approximately 200 ms until 1200 ms ($p = .001$). The black asterisks in the figure denote sensors that contribute to the significant cluster for at least half of the time period from 200 ms to 1200 ms (Fig. 4). Clearly, there is a significant difference in the amplitude of brain activity between familiar and novel items. A visual inspection of the signal demonstrates that the dynamic range (peak-to-trough difference) for familiar images is larger than for novel ones. Also, there appears to be a sharper reduction, i.e., truncation, of the signal for familiar than for novel images.

To look at the difference between expected and unexpected items when familiarity was held constant, we tested the difference between the expected vs. unexpected conditions. A significant difference emerged for the cluster shown in Figure 5 from approximately 500 ms until 900 ms ($p = .005$). The black asterisks in the figure denote sensors that

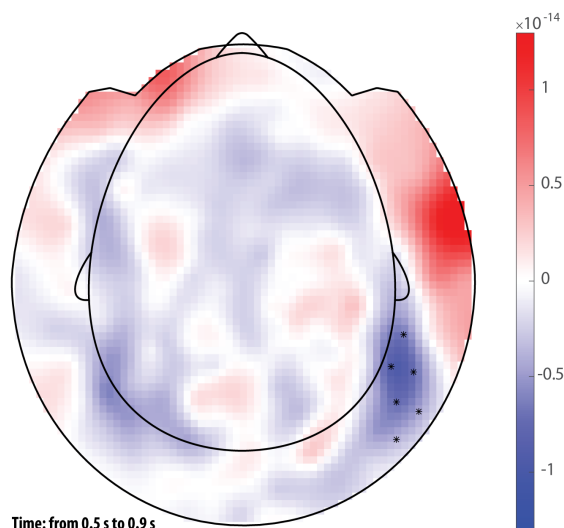


Fig. 5. Topography of the difference between the expected and unexpected conditions. Black asterisks mark sensors that contribute to the significant cluster for at least half of the time period from 500 ms to 900 ms.

contribute to the significant cluster for at least half of the time period from 500 ms to 900 ms (Fig. 6). Evidently, there is a significant difference in the amplitude of neural activity between expected and unexpected items. A visual inspection of the signal does not reveal a clear difference in dynamic range or truncation between the two types of responses.

Moreover, we were interested in the expected vs. unsequenced comparison as well as the unexpected vs. unsequenced comparison, since the former could illustrate the effect of a confirmed expectation and the latter could demonstrate the effect of a violated expectation. However, neither contrast yielded a significant difference.

Discussion

Our findings demonstrate that familiarity and expectation both affect brain activity. Notably, the effects of familiarity and expectation on the electrophysiological signal are similar. Both lead to a significant reduction in the amplitude of the signal, and in both cases this decrease in activity is strongest in posterior, right-lateralised areas. A visual inspection of the timecourse for the familiar-novel comparison reveals a truncated response with a higher dynamic range for familiar stimuli compared to novel ones. This effect is not so clearly present in the expected-unexpected comparison.

We qualitatively observe a larger dynamic range

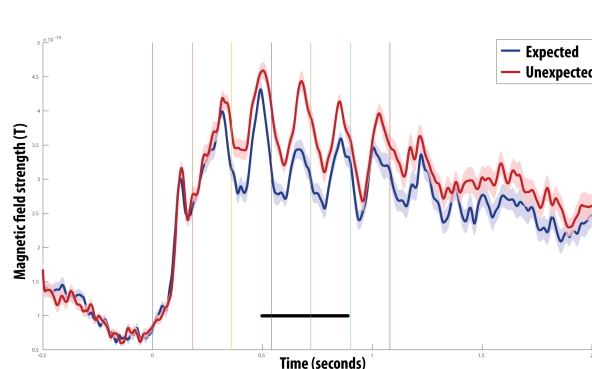


Fig. 6. Activity over time for the expected condition (blue) and the unexpected condition (red). Activity is averaged over the sensors highlighted in Figure 5. The shaded areas are error bars illustrating the within-subject SEM for the expected (light blue) and the unexpected (light red) conditions. The horizontal black bar at the bottom shows that at least one of the selected sensors contributes to the significant cluster at this time point. The coloured vertical lines denote the onset of each image (1-6), and the last one denotes the offset of the last image.

and more truncation of the signal for familiar than novel stimuli, which is in agreement with Meyer et al.'s (2014b) findings. The topography maps for the differences in neural activity between familiar and novel and between expected and unexpected point to a posterior, right-lateralised brain area. It is possible that this area reflects the ventral visual stream and specifically inferior temporal (IT) cortex, which would be in accordance with the fact that Meyer et al. (2014b) recorded neural firing from monkey IT. The amplitude effect we found for familiar compared to novel items has not been reported by Meyer et al. (2014b). Perhaps the measures used by Meyer et al. (2014b), spike rate and local field potentials, did not reveal this difference in amplitude, while the MEG signal did. Our finding fits with other studies showing that familiarity reduces neural activity (Li et al., 1993; Rossion et al., 2003).

In our experimental design, familiarity was manipulated separately from expectation, allowing us to distinguish between the effects of these two factors. The results suggest that familiarity, defined as having seen an image in the past, has a genuine effect on brain activity even when the stimuli occur in an unpredictable sequence. Moreover, expectation, defined as being able to predict the upcoming image, influences the neural response even when familiarity is held constant. This is noteworthy because Meyer et al. (2014b) show how the electrophysiological signal differs between familiar and novel images

presented with a rapid presentation design, but in their design, the way familiarity was manipulated also influenced expectation. Therefore, the neural effect they observed may have partially occurred because monkeys were able to predict the upcoming stimulus better when the images were familiar than when they were novel. In our design, which also featured rapidly presented image sequences of six objects, the familiarity manipulation was unaffected by expectation. We isolated the effect of familiarity by comparing the brain activity for familiar unsequenced images and for novel images. This comparison did not include any influence from expectation because familiar unsequenced images always appeared in unpredictable orders and because each novel image was only shown once, so it was not possible to form expectations about novel items. The comparison between familiar unsequenced and novel images still showed a significant difference, indicating that familiarity has a genuine effect on the amplitude of the signal and on the qualitatively observed truncation and dynamic range of the response. Furthermore, the expectation manipulation was in addition to the familiarity one: participants saw familiar sequenced images, which could be expected (shown in the learned sequence) or unexpected (shown in a shuffled order). The comparison between expected and unexpected images also showed a significant difference in terms of amplitude. These results demonstrate the separable effects of familiarity and expectation.

Interestingly, the difference between familiar and novel items becomes significant around 200 ms, while the difference between expected and unexpected images attains significance later, around 500 ms. This may be caused by a distinction between the two types of experimental manipulations. With respect to the familiar vs. novel comparison, participants know whether the trial will be comprised of familiar or novel images once they see the first image. Regarding the expected vs. unexpected comparison, however, the first image is uninformative; based on the second image, the visual system can tell whether the current trial is expected or unexpected. Since the presentation of one image lasts for 180 ms, this difference between the manipulations could explain why the expectation effect becomes significant 180 ms later than the familiarity effect. In our data, however, the expectation effect attains significance approximately 300 ms later than the familiarity effect. This further delay could be due to noise, or it may occur because the visual system needs to accumulate more information before detecting a violation of expectations than before detecting

novel input, resulting in a delayed latency for the expectation effect compared to the familiarity effect.

It is intriguing that neither the expected-unsequenced comparison nor the unexpected-unsequenced comparison yielded a significant difference. Perhaps this is the case because the difference in neural processing between a confirmed expectation (expected) and a lack of expectation (unsequenced) was not substantial enough in our dataset to produce a significant result. Likewise, the difference between a violated expectation (unexpected) and a lack of expectation (unsequenced) was not prominent enough to bring about a significant outcome. Apparently, in our dataset, the difference in neural processing between a confirmed expectation (expected) and a violated one (unexpected) was larger than in the previous two cases and was substantial enough to induce a significant result.

Importantly, in our experiment we did not have a complete orthogonal manipulation of familiarity and expectation. Such a design would have required a novel, expected condition which we did not have. This is because it is difficult to build an expectation for which image is going to come next without being familiar with the images. Usually, familiarity is necessary for expectation, and this is also the case in our operationalization of these two concepts. Future studies could examine how to manipulate expectation without familiarity. Perhaps this can be done if expectation is based on an abstract rule: an image of class A (e.g., animal) is followed by an image of class B (e.g., fruit). Participants would then expect the type of image coming next even if they are not familiar with the exact image, i.e., even if they have not seen this specific exemplar from the general category that is expected. This would manipulate expectation on a more conceptual level than the low-level sequence-based expectation we implemented in our experiment because participants' expectations would refer to categories of objects instead of specific items.

It is of interest that we observed an effect of expectation although participants were not consciously aware of having learned a sequence for the familiar sequenced images. When asked, participants said that they did not notice any specific order for the images. The behavioural assessment of sequence knowledge also showed that participants' performance was very low when they were shown an image and had to report which image should follow. Nevertheless, the neural response distinguished between expected and unexpected conditions. It appears that the observed difference represents

low-level sequence learning which may occur only in sensory cortical areas and may not reach widespread recurrent processing, thus not entering conscious awareness (Lamme, 2006). Moreover, this effect of expectation was induced by only a one-hour training session one or two days before the MEG recording session, which is very little time compared to the extensive training monkeys underwent (Meyer et al., 2014b). The fact that participants were unaware of the image sequence and the short training period make it even more remarkable that we found a significant difference in neural activity between expected and unexpected trials.

In our study, expectation operated on a stimulus-to-stimulus basis. In the learned sequence, image A predicted image B, image B predicted image C, image C predicted image D, etc. Importantly, the sixth image, F, predicted the first image, A, meaning that the sequence was circular. This ensured that image A did not carry the predictive value for the whole sequence; rather, each image predicted the next one. When the image order was shuffled, each image was followed by an unexpected image; therefore, the stimulus-to-stimulus expectation was violated by every image in the sequence. In accordance with this, the significant difference in the electrophysiological signal between expected and unexpected trials was sustained over the presentation of multiple images, suggesting that each of those images was unexpected based on the previous one. If the prediction value had been carried by a single image only, when the expectation was violated, we would have expected to see a significant difference only for the image after that one. In this case, expectation would have operated on a trial-to-trial basis: as soon as the brain discovers that the images in this trial will appear in a shuffled order, expectations about following images are not employed. However, the fact that we found a sustained difference over multiple stimuli suggests that expectation operated on a stimulus-to-stimulus basis in our experiment. Meyer, Ramachandran, and Olson (2014) revealed a similar outcome when they presented monkeys with sequences of three images. The authors found that the expectation for the current image was based on the immediately preceding image and not on the first image in the sequence. Perhaps rapid presentation designs such as theirs and ours preclude the categorization of a trial as expected or unexpected, thus preventing trial-to-trial expectation, but rather permit low-level expectation effects on a stimulus-to-stimulus basis.

We aimed to determine the neural effects of familiarity and expectation by strictly defining these concepts in terms of experimental manipulations.

Obviously, being familiar with visual input can be much more nuanced than simply having seen an image during a training session, and expecting a visual feature can take many other forms besides predicting which image will be presented next. In reality, these concepts are much richer than how they are defined by our experimental manipulations, so the division between familiarity and expectation may not be so clear-cut. In our everyday environments, we usually become familiar with images because they appear more often, which means that we also expect to see them more often. In this sense, familiarity and expectation are necessarily intertwined; perhaps familiarity can even be construed as a type of expectation. A noteworthy difference remains between the two, however: familiarity refers to the fact that the system has knowledge of certain past visual input, while expectation implies that the system is making predictions about upcoming visual information. This focus on past or future input may indeed be a true distinction between familiarity and expectation, or it may only be a semantic difference. It remains unclear whether this distinction is actually implemented in neural processes or whether a single neural mechanism underlies both familiarity and expectation.

Conclusion

This study aimed to determine whether the effects of familiarity and expectation can be dissociated and, specifically, whether familiarity can influence brain activity even when stimuli cannot be predicted. We found that familiarity has a genuine effect on the amplitude of the electrophysiological signal in the human brain. Moreover, expectation also influences the amplitude of the neural response when familiarity is held constant. These findings give rise to numerous possibilities for future investigations. An important direction is to explore the corresponding brain activity when participants have expectations about upcoming images without being familiar with the stimuli. Another intriguing option is to investigate how the neural signal changes as the contingencies between stimuli change, so that images are not simply expected or unexpected but rather they can be expected to different degrees. Alternatively, the familiarity with the visual input can be graded, so stimuli can be familiar to different extents. These research trajectories can enhance our understanding of how familiarity and expectation influence the brain signal, so that ultimately we can discover whether these two concepts refer to the same or to distinct neural phenomena.

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