

available at www.sciencedirect.comwww.elsevier.com/locate/brainres

**BRAIN
RESEARCH**

Research Report

Widespread fMRI activity differences between perceptual states in visual rivalry are correlated with differences in observer biases

M. Raemaekers^{a,*}, M.E. van der Schaaf^a, R. van Ee^b, R.J.A. van Wezel^a

^aDepartment of Functional Neurobiology, Helmholtz Institute, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

^bDepartment of Physics, Helmholtz Institute, Utrecht University, The Netherlands

ARTICLE INFO
Article history:

Accepted 18 November 2008

Available online 3 December 2008

Keywords:

fMRI

Bistable

Voluntary control

Structure from motion

ABSTRACT

When observing bistable stimuli, the percept can change in the absence of changes in the stimulus itself. When intermittently presenting a bistable stimulus, the number of perceptual alternations can increase or decrease, depending on the duration of the period that the stimulus is removed from screen between stimulus presentations (off-period). Longer off-periods lead to stabilization of the percept, while short off-periods produce perceptual alternations. Here we compare fMRI brain activation across percept repetitions and alternations when observing an intermittently presented ambiguously rotating structure from motion sphere. In the first experimental session, subjects were requested to voluntarily control the percept into either a repeating or an alternating perceptual regime at a single off-period. In a consecutive session, subjects observed the sphere uninstructed, and reported alternations and repetitions. The behavioral data showed that there were marked individual biases for observing the sphere as either repeating or alternating. The fMRI data showed activation differences between alternating and repeating perceptual regimes in an extensive network that included parietal cortex, dorsal premotor area, dorsolateral prefrontal cortex, supplementary motor area, insula, and cerebellum. However, these activation differences could all be explained by intersubject differences in the bias for one of the two perceptual regimes. The stronger the bias was for a particular perceptual regime, the less activation and vice versa. We conclude that widespread activation differences between perceptual regimes can be accounted for by differences in the perceptual bias for one of the two regimes.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Bistable figures, such as the Necker cube, instigate two perceptual interpretations. When presented continuously for a prolonged period of time, the two interpretations alternate spontaneously. Here we focus on a specific aspect of

perceptual bistability: the normal course of perceptual alternations is radically disrupted when the ambiguous stimulus is presented with interrupting blanks in which the stimulus is removed from sight. When the blanks are longer than about 800 ms, the percept of the reappearing stimulus tends to be the same as before the 'off' period. This priming effect (Cole and

* Corresponding author. Fax: +31 30 2505443.

E-mail address: m.a.h.l.l.raemaekers@uu.nl (M. Raemaekers).

Schneider 2007) can stabilize the percept for periods of up to several minutes (Leopold et al., 2002; Maier et al., 2003; Orbach et al., 1963). By contrast, off-periods shorter than about 200 ms give rise to a perceptual regime where the percept alternates upon each new stimulus reappearance (Orbach et al., 1966; Noest et al., 2007). The neural mechanisms behind alternating and repeating choice sequences are as yet poorly understood. In this study we investigate the neural correlates of these regimes using functional Magnetic Resonance Imaging (fMRI).

As a bistable stimulus we use an ambiguous structure-from-motion (SFM) stimulus (Wallach and O'Connell 1953; Treue et al., 1991) consisting of a sphere that can be perceived to rotate about its vertical axis with its front surface moving either to the left or to the right (Fig. 1). The idea behind our experiment is that a repetition sequence gives rise to more neuronal adaptation than an alternation sequence in brain areas that represent the conscious percept of the bistable stimulus. Differences in adaptation state induce BOLD signal changes that can be detected with fMRI (Krekelberg et al., 2006). In other words, a repetition sequence ought to give rise to less activation in brain areas representing the conscious percept than an alternation sequence because of the adaptation that takes place during repetition.

To compare fMRI signals during perceptual alternation and repetition regimes, it is imperative that the visual input during both regime types is identical. This also implies that the ambiguous stimulus for both repeating and alternating perceptual regimes must be intermittently presented at a single fixed 'off'-period. In this experiment, we therefore used an 'off'-period that was in the range where both sequences can occur (Kornmeier et al., 2007; Noest et al., 2007). Each sequence tends to last for several ON/OFF cycles (Klink et al., 2008).

For good statistical power of the fMRI design, this 'off'-period should induce roughly an equal proportion of percept repetitions and alternations. Furthermore, as a result of the slowness of the blood oxygenation level dependent response and low frequency scanner drifts as well as physiological noise, the contrast-to-noise ratio aspects of the fMRI experiment will strongly depend on the duration of the perceptual regimes (Bandettini and Cox 2000). When regimes switch too quickly, the slowness of the hemodynamic response reduces the contrast between regimes. When regimes switch too slowly, low frequency scanner fluctuations start to interfere with the contrast activation between regimes. By requesting subjects to voluntarily favor either a repeating or an alternating choice sequence for durations that are optimal for fMRI, the power of the fMRI design can be further enhanced.

Requesting subjects to control their perceptual regime could possibly introduce effects that are not present during uncontrolled bistable perception. It is known that subjects differ in their bias for perceiving the stimulus as either alternating or repeating (Leopold et al., 2002; Sterzer and Rees 2007; Klink et al., 2008). These subjective preferences may confound activation differences between alternating and stabilized perceptual regimes through differences in the amount of effort needed for maintaining the perceptual regimes. Therefore, the current experiment takes the subject's regime bias into account when comparing voluntarily controlled alternating and repeating perceptual regimes. To study

activation differences between controlled and uncontrolled perception during rivalry, in a consecutive second session, subjects were asked to report the rotation direction of an intermittently presented ambiguous stimulus without being requested to maintain a particular perceptual regime.

Other factors may influence activation differences between alternating and repeating perceptual regimes as well. During a number of trials within a series of stimuli, subjects switch between alternation and repetition perceptual regimes. When there is a bias for one particular regime over the other (Leopold et al., 2002; Sterzer and Rees 2007; Klink et al., 2008), the least frequent regime will have an increased proportion of percepts that represent regime switches. These trials could involve the interruption of a motor program, as subjects have to switch between a motor sequence of pressing the same button, and a motor sequence where they have to alternate between two buttons. In addition, these trials also have a strong resemblance to visual oddballs. Both aspects are known to have a strong influence on fMRI signals in extensive parts of the brain (Liddle et al., 2001; Ardekani et al., 2002; Stevens et al., 2000). Percepts that switch the regime are therefore separately modeled and analyzed in our experiment.

In this study we investigate the neural correlates of alternating and repeating choice sequences using fMRI, when observing an intermittently presented ambiguously rotating sphere. In the first part of the experiment, subjects are requested to maintain a particular perceptual regime. In the second part, subjects are asked to report their percept with each new presentation of the stimulus without instructions to repeat or alternate.

2. Results

2.1. Psychophysics prior to scanning

The experiment started with a psychophysical session to determine the off-period where subjects were best able to control their percept of the ambiguously rotating sphere (Fig. 1). Subjects were asked to report the rotation direction of the front surface of the sphere (at 20 different off-periods), and instructed to 1:) repeat the percept, 2:) alternate the percept, and 3:) to report the rotation direction without instruction. Effects of off-period and instruction on the number of alternations/repetitions were analyzed using a repeated measures ANOVA (duration off-period*instruction). Results showed a main effect of the off-period on the number of perceptual alternations ($F_{(19,456)}=6.00$; $p<0.001$) (not shown), in accordance with previous studies (Noest et al., 2007; Klink et al., 2008). The number of perceptual alternations increased with shorter off-periods, which could be explained for 93% by a combination of a linear ($F_{(1,12)}=8.64$; $p=0.012$) and a quadratic trend ($F_{(1,12)}=5.23$; $p=0.041$). In addition there was a main effect of condition ($F_{(2,456)}=10.72$; $p<0.001$). When subjects were asked to influence their percept into an alternating or repeating choice sequence, they were able to do so, and the number of alternations increased (linearly) from 'repeat instructions' to 'non-instructed' to 'alternate instructions' ($F_{(1,12)}=13.09$; $p=0.004$). The duration of the off-period with the strongest voluntary control ((% correct repetitions + %

correct alternations)/2) that was calibrated for each subject in the psychophysical experiment was on average 286 ms (SD=65 ms), ranging between 155–366 ms. The individually calibrated off-period was used for the entire experiment in the scanner.

2.2. Psychophysics during scanning

In the first session in the scanner, subjects were instructed to repeat or alternate the percept of the SFM stimulus, presented intermittently with the off-period that was individually calibrated during the psychophysical session. Subjects were indeed able to influence their perceptual regime according to the instructions, producing more alternations during alternations instructions and more repetitions during repetitions instructions (paired $t_{(15)}=6.30$; $p<0.001$) (Fig. 2). However, in spite of the individual calibration of the off-period, there were still individual biases for either the alternating or repeating regime. Subjects exhibited on average a (non-significant) bias for a repeating perceptual regime (mean difference between the percentage of repetitions and alternations=14%, $t_{(15)}=1.54$; $p=0.15$), and the total percentage of repetitions in SFM trials ranged between 26% and 90% (Fig. 2). In the second session in the scanner, subjects did not receive instructions to repeat or alternate the percept. In this second session, there also was on average a small bias for a repeating perceptual regime (mean difference between the percentage of repetitions and alternations=14%, $t_{(11)}=1.77$; $p=0.11$), which was positively correlated with the regime bias during the first (instructed) session ($r=0.67$; $p=0.017$) (Fig. 3).

The bias for a repeating percept was calculated for every subject as the difference between the proportion of repeating

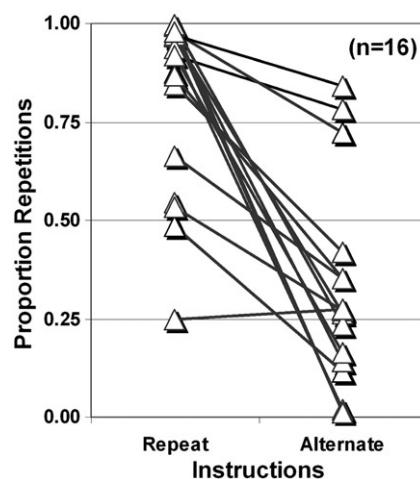


Fig. 2– For all subjects ($n=16$), the proportion of perceptual repetitions for the alternate and repeat instructions while performing the first experimental session of the experiment in the scanner.

trials and alternating trials, relative to all SFM trials. This bias was used as a covariate in the fMRI-analysis.

2.3. fMRI

During the first session, subjects were instructed to perceive the intermittently presented ambiguous SFM stimulus as either repeating or alternating. To control for activation differences between alternations and repetitions as a result of differences in the motor response, we used separate reference conditions for alternation and repetition trials.

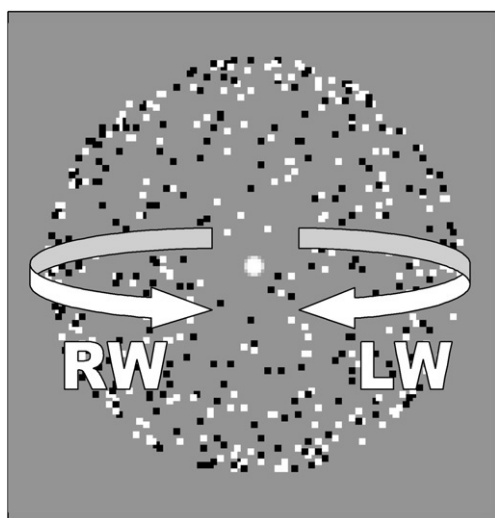


Fig. 1– The SFM stimulus creating the percept of an ambiguously rotating sphere. Arrows indicate the two perceived rotation directions of the front surface of the sphere: leftwards (LW) or rightwards. Black and white dots on a grey background were used to keep the luminance of the screen constant during the ‘on’ and ‘off’ periods of the stimulus, to avoid BOLD saturation in motion sensitive areas as a result of stimulus flicker.

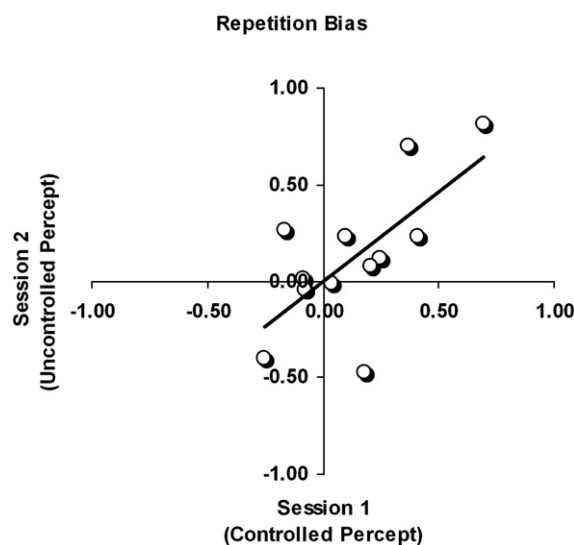


Fig. 3– Relationship between the individual bias for a repetition regime during the first (controlled) session (% correct and incorrect repetitions–% correct and incorrect alternations) and bias for a repetition regime during the second (uncontrolled) session (% repetitions–% alternations) ($n=12$).

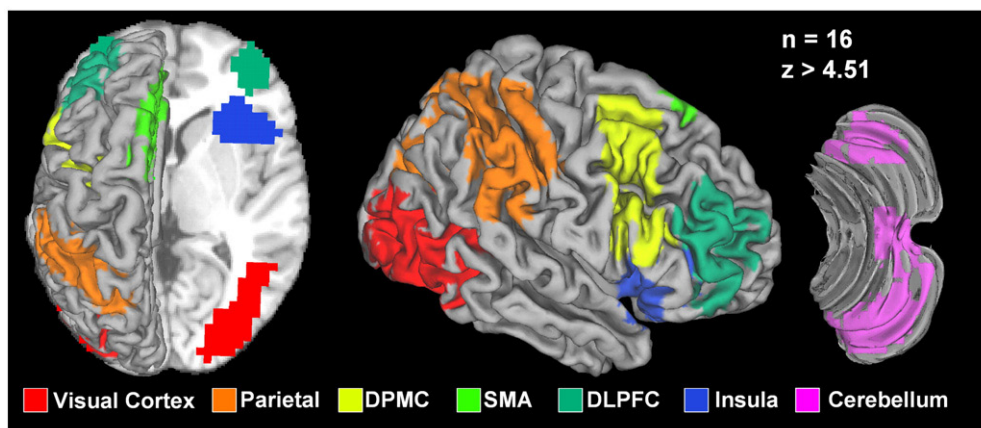


Fig. 4– Group-wise activation during the first session for the contrast between correct SFM trials (both alternations and repetitions) vs. static trials (trials without SFM), superimposed on a surface reconstruction of the single subject T1 weighted scan that is used in SPM5 (Bonferroni corrected). Different colors correspond to the 7 different ROI's that activated voxels are attributed to. Both the repetition and alternation condition involved an extensive network of brain areas. The average levels of activation within the ROI's were used for further analysis.

Incorrect and correct trials were modeled separately (i.e. alternations during repetition instruction and repetitions during alternation instructions). When comparing the two reference conditions, there was more activation during alternating button presses than during repeating button presses in the primary motor cortex and cerebellum (not shown), demonstrating that the use of separate reference conditions was indeed necessary.

To detect brain areas that were involved in this task, a group-wise voxel-based comparison was done between correct alternation/repetition trials and reference trials (trials where there was no motion in the dots of the sphere, and subjects had to press a predefined button with each new presentation) of the first session. This comparison revealed an extensive neuronal network and all activated voxels were attributed to seven bilateral ROI's including visual cortex, parietal cortex, Dorsal Premotor Cortex (DPMC), Supplementary Motor Area (SMA), anterior part of the insula, Dorsolateral Prefrontal Cortex (DLPFC), and cerebellum (Fig. 4). Further details of the ROI's are displayed in Table 1. The average activation for correct alternations and repetitions was calculated for each subject and each ROI. The group results for activation during correct alternations and correct repetitions within the ROI's are depicted in Fig. 5. There was considerably more activation during alternations than during repetitions in a large part of this widespread network.

A multivariate (7 ROI's) repeated measures (2 regimes) MANOVA was used to detect activation differences between correct alternations and repetitions. The bias for a repetition regime (% correct and incorrect repetitions — % correct and incorrect alternations; can range between -1 and 1 and is opposite to the bias for an alternation regime) was added as a covariate, as we explained that this bias may confound activation differences between correct alternations and repetitions. In spite of more activation during correct alternations than correct repetitions (Fig. 5), this test revealed no significant main effect of regime ($F_{(7,8)}=.836$; $p=.587$), indicating

that there were no activation differences as a result of perceiving an alternating or stabilized perceptual sequence. However, there was a significant interaction effect between the (covariate) bias to repeat and regime ($F_{(7,8)}=8.11$; $p=.004$) which indicates that although there were activation differences between correct alternations and repetitions, they could be explained by differences in a subject's bias for one of the two perceptual regimes. The higher activation during alternations in some ROI's thus arises as a result of the (non-significant) bias of the group for a repeating perceptual regime.

This interaction effect was significant in all the ROI's except for the visual cortex. The relationship between the bias to repeat and the contrast activation between alternations and repetitions during the first session is depicted in Fig. 6 for the ROI's with a significant interaction effect. Note that all the regression lines pass nearly through the origin, indicating that

Table 1 – ROI details

Area	x	y	z	Max. Z	Nr voxels
Left visual cortex	-32	-84	8	28.09	611
Right visual cortex	35	-82	6	12.24	749
Left parietal	-31	-52	54	7.37	366
Right parietal	24	-76	44	12.10	714
Left DPMC	-44	-2	44	5.03	170
Right DPMC	44	2	48	9.05	398
SMA	-1	14	44	11.59	533
Left DLPFC	-40	51	12	5.81	265
Right DLPFC	37	48	27	6.59	396
Left insula	-36	23	0	10.20	189
Right insula	45	19	-2	10.96	278
Left cerebellum	-41	-56	-18	5.61	137
Right cerebellum	41	-56	-18	5.40	75

Coordinates of the peak activation and number of voxels of the ROI's that are depicted in Fig. 4.

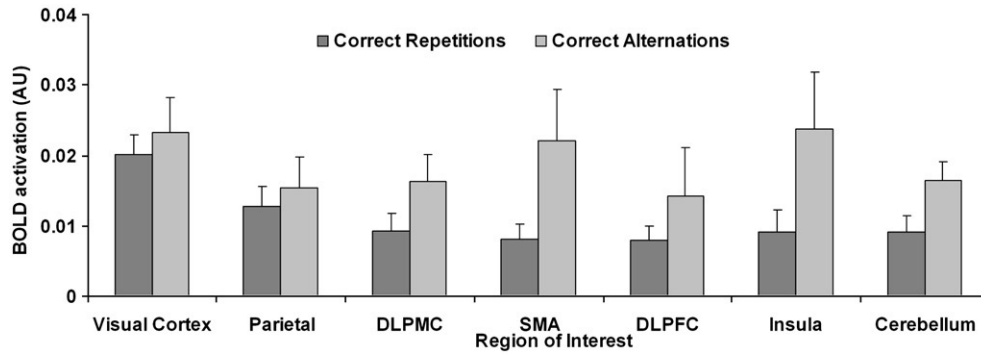


Fig. 5 – Mean BOLD activation (in arbitrary units) of ROI's (Fig. 4) during correct alternation and correct repetition trials of the first session. Bars indicate standard error of the mean activation. There were activation differences between alternating and repeating perceptual regimes in the entire network, except for visual cortex.

after correction for regime bias, there were hardly any activation differences between alternations and repetitions. The visual cortex is not shown, as it demonstrated no significant interaction effect between regime and the bias to repeat ($F_{(1,14)} = 1.41; p = .26$).

It is theoretically possible that differences in the contrast activation between alternations and repetitions are somehow caused by intersubject differences in the individually calibrated off-period. This would render the currently observed results trivial, as the observed differences could be related to

low-level differences in the perception of the stimulus. To check this possibility, we correlated the individually calibrated off-period and the contrast activation between alternations and repetitions in the 6 ROI's. In none of these 6 ROI's there was however a significant correlation (maximum $r = .19; p = .47$), indicating that it is unlikely that the individual differences in the calibrated off-period confounded the results.

A principle component analysis revealed that 69% of the variance in this contrast activation in these 6 ROI's could be

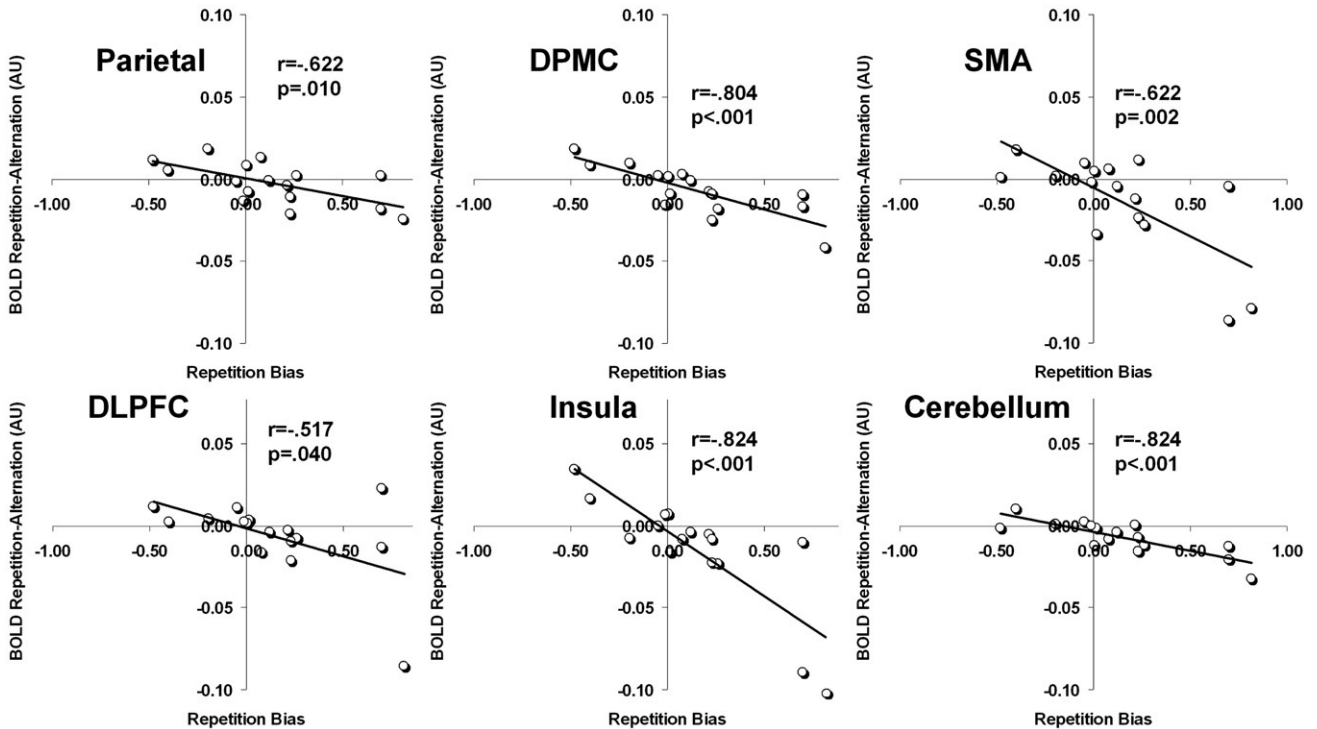


Fig. 6 – For the first part of the experiment (where subjects had to voluntarily control their percept), the relationship between the individual bias for a repetition regime (% correct and incorrect repetitions-% correct and incorrect alternations (Fig. 2), and differential BOLD activation between correct repetitions and correct alternations ($n = 16$). The stronger the subject's bias for a perceptual regime, the lower the ROI activation was. When the observer bias is low or absent, there are no activation differences between repetitions and alternations.

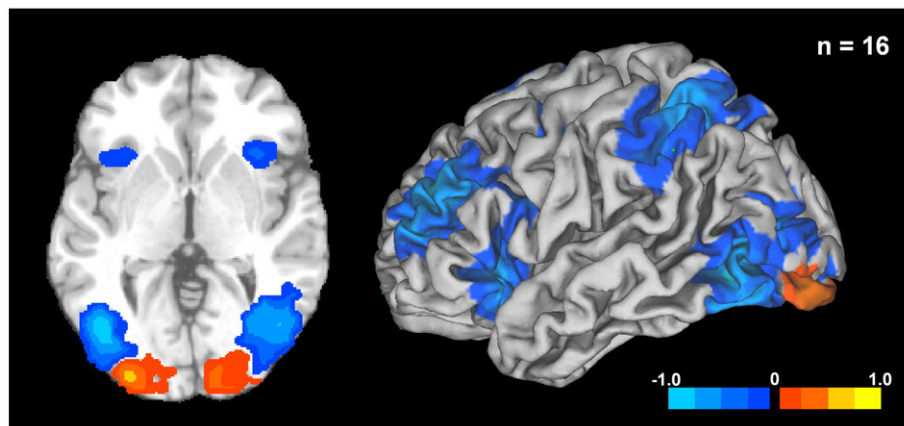


Fig. 7 – For the first part of the experiment (where subjects had to voluntarily control their percept), the correlation between the individual bias for a repetition regime, and contrast activation between correct repetitions and correct alternations within the ROI's on a surface reconstruction of the CH template (correlations are unthresholded). There was a (non-significant) positive correlation in early visual cortex, while from late visual cortex onwards, there was a negative correlation.

explained by a single component. This component was positive and had nearly identical values for all ROI's (mean=.83, SD=.08), indicating that the bias for a repeating regime had a similar effect in all these 6 ROI's. To closer examine the effects of regime bias on brain activation, we did a post hoc analyses and calculated a voxel-based correlation between the bias to repeat and the contrast activation between repetitions and alternations within the ROI's. This analysis was done to check for possible large variations of the effect of regime bias within the ROI's. This analysis revealed that the voxel-based correlation was negative in all ROI's, similar to what was observed when taking the ROI's as a whole, except for the visual cortex (Fig. 7). After Bonferroni correction for the number of voxels within the ROI's, ($p < 0.05$; $r > 0.76$), there was a negative correlation in dorsal/lateral visual cortex as in the other ROI's (peak values; $r = -.84$ for the left hemisphere; $x\ y\ z = 38\ -66\ -14$, $r = -.84$ for the right hemisphere; $x\ y\ z = 30\ -72\ -14$). The positive correlations in posterior/central visual cortex were not significant (peak values, $r = .64$ for the left hemisphere, $x\ y\ z = -30\ -95\ -6$, $r = .66$ for the right hemisphere $x\ y\ z = 30\ -72\ -14$). The border between these areas, where the correlations were approximately 0, was located approximately within Brodmann area 18.

To examine if the same regime bias also influenced uncontrolled perception of the ambiguous stimulus, brain activation differences between repetitions and alternations during the second session were calculated in the seven predefined ROI's (Fig. 4). These brain activation differences were correlated (one sided) with the repetition bias as calculated for the first (controlled) session and with the repetition bias as calculated for the (uncontrolled) second session (Table 2). Note that the power of the fMRI data of the second session is considerably lower due to a lower number of scans, suboptimal regime durations, and a lower number of subjects. However, still the correlations with regime bias were positive and significant in most ROI's (Fig. 4). Although the correlations with the regime bias during the second session

were on average the highest, they were not significantly different with the correlations with regime bias during the first session (maximum $Z = 0.93$; $p = 0.35$).

By defining ROI's from voxels that were activated during both correct alternations and repetitions, differences between correct alternations and repetitions outside the ROI's would have remained unnoticed. To inspect if there were differences outside the defined ROI's, we also did a voxel-wise group comparison between the regimes during the first and second session. For the first session, activation differences between correct alternations and repetitions were assessed after correcting for the bias to repeat in every voxel using a linear regression. The same was done for the second, but now for the activation differences alternations and repetitions, and corrected for the repetition bias of second session. Both analyses

Table 2 – Biases during session 2

	n	Repetition bias session 1 (controlled percept)		Repetition bias session 2 (uncontrolled percept)	
		Pearson r	P	Pearson r	P
Visual cortex	12	0.42	0.09	0.50*	0.05
Parietal	12	0.49*	0.05	0.67*	0.01
DPMC	12	0.54*	0.03	0.60*	0.02
SMA	12	0.58*	0.02	0.76*	0.00
DLPFC	12	0.54*	0.04	0.59*	0.02
Insula	12	0.46*	0.07	0.56*	0.03
Cerebellum	12	0.28	0.18	0.61*	0.02

Correlation between the activation difference between repetitions and alternations during the second session of the experiment in the seven ROI's, and the bias to repeat during experiment 1 and experiment 2 respectively. The data indicate that regime bias also plays a role during uninstructed perception of the intermittently presented ambiguous sphere.

* $p < 0.05$ (one-sided).

revealed no voxels that showed a significant difference between the regimes.

Trials that represented switches between regimes, and trials where subjects did not follow instructions (i.e. alternate during repetition instruction and repeat during alternate instruction) were modeled separately as they could confound activation differences between repetitions and alternations. The average number of trials that represented switches between perceptual regimes was low and varied highly over subjects (see Table 3). As a result, the statistical power of the factors that represented the switches (factor 7 to 10) was too low in most of the subjects for doing a group-wise analysis. The same was true for the number of incorrect trials of session 1, making factors 3 and 4 also unusable for group-wise analyses.

3. Discussion

In this study we set out to investigate the neural correlates of perceptual stabilization and alternation regimes using fMRI. As activation differences between conditions can arise as a result of differential observer bias for one or the other regime, we used the bias to perceive the repeating regime as a covariate in our fMRI data analysis. During the first part of the experiment, we observed activation differences between correct alternations and repetitions in a widespread network that encompassed dorsal and ventral visual cortex, parietal areas, DPMC, SMA, DLPFC, insula, and the cerebellum. However, these activation differences were clearly correlated with individual subject biases (Fig. 6) for perceiving one of the two perceptual regimes; the stronger a bias for a perceptual regime, the lower the activation in this widespread neuronal network. The same bias was also present during uninstructed

observation of the stimulus during the second part of the experiment.

After careful correction, we did not observe any differences in brain activation between percept alternations and repetitions and thus found no evidence for a stabilization signal. Percept stabilization has been previously linked to higher order visual processing such as pattern completion (Maloney et al., 2005) and perceptual memory (Leopold et al., 2002; Maier et al., 2003). However, higher order visual processing and widespread activation are not necessarily needed according to a recently proposed low-level model that provided computational evidence that even a single neural stage of rapidly competing and slowly adapting percept representations can induce the choice sequences that are observed with different ON/OFF cycles of an ambiguous stimulus (Noest et al., 2007). The absence of activation differences between repetitions and alternations (after correction for subjective regime bias), suggests that differences between perceptual repetition and alternation regimes are subtle. This is not in line with widespread activation differences and higher order visual processing.

The subjective biases for a particular perceptual regime correlated with brain activation in an extensive network of brain areas. This correlation is unspecific for task instructions and therefore probably relates to general aspects of attention when observing either of the two perceptual regimes. The combination of areas where we found this correlation shows a strong resemblance to the cognitive control network (CCN) which includes the DLPFC, anterior insula, anterior cingulate cortex/SMA, DPMC, posterior parietal cortex (Cole and Schneider 2007; Corbetta and Shulman 2002; Pastor et al., 2004) and possibly also the cerebellum (Tomasi et al., 2007; Salmi et al., 2007). The CCN is found during many fMRI tasks that involve functions such as working memory, attention, or response preparation and initiation. The different areas of the CCN

Table 3 – Number of trials per type

Subject	Correct ALT	Correct REP	Incor. ALT	Incor. REP	Switch ALT-REP during ALT	Switch REP-ALT during ALT	Switch ALT-REP during REP	Switch ALT-REP during REP	ALT Exp 2	REP Exp 2
1	137	228	41	83	29	36	5	11	178	121
2	142	147	58	58	42	43	38	42	166	133
3	136	217	8	54	49	46	31	29	–	–
4	167	269	2	31	43	44	7	7	88	211
5	280	282	1	1	2	2	1	1	–	–
6	234	93	105	11	19	22	41	45	188	111
7	179	118	104	46	28	32	30	33	–	–
8	37	252	15	209	24	16	7	10	–	–
9	58	272	4	184	22	21	4	5	98	201
10	200	282	1	52	13	19	2	1	134	165
11	235	275	3	41	4	4	4	4	115	184
12	23	272	3	216	22	22	5	7	45	254
13	186	39	187	54	22	23	27	32	195	104
14	274	260	12	1	5	4	6	8	198	101
15	280	277	4	1	2	2	2	2	159	140
16	212	250	11	17	27	28	13	12	118	181

Number of trials that constitute the different factors of the GLM for the first and second experiment. Although, the number of trials of each type differed substantially between subjects, there were still at least 23 trials for estimation of the BOLD response for correct repetitions and alternations during the first experiment, and a minimum of 45 trials for repetitions and alternations during the second experiment (see columns Correct REP, Correct ALT, REP Exp2, and ALT Exp2).

show a strong functional connectivity, something that we also observed in this study, which suggests that these areas operate as a tightly coupled network. Our results may indicate that activation differences between alternations and repetitions reflect subjective differences in the effort that is needed to maintain a particular perceptual regime.

The fact that we observed similar differences between repetitions and alternations when subjects were not instructed to maintain a particular perceptual regime raises doubt on the explanation by cognitive control. The finding of brain activation biases during uninstructed perception of the stimulus is in line with an observation that was made by Sterzer and Rees (2007), who recently reported a correlation in a fronto-parietal network between activation during successfully maintaining a percept, and the subjective bias of subjects for experiencing percept stabilization. Sterzer et al. did not instruct their subjects to maintain a particular perceptual regime. Our observation in the present experiment is similar, with the additional demonstration that for our data this correlation is most likely related to a bias for either experiencing percept repetitions or alternations, instead of specifically percept repetitions. When there is a bias for one of the perceptual regimes, than this perceptual regime could have an increased amount of perceptual adaptation and attract less attention. With more adaptation and less attention, the most frequent regime will have less activation. Although some of these effects may have been removed by separately analyzing switch trials (Liddle et al., 2001; Ardekani et al., 2002; Stevens et al., 2000), adaptation effects may extend beyond only switch trials. Note that this adaptation does not refer to adaptation of the perceived motion direction of the sphere, but to adaptation of the perceptual regime instead. Alternatively, when subjects perceive the bistable stimulus uninstructed, this does not guarantee that there are no effects of effort, as there is no way to disprove that some form of control may have occurred.

In any event, the widespread activation differences that we observed are unrelated to the conscious percept of the ambiguous stimulus. Widespread activity changes have previously been reported for sustained rivalry under prolonged observation in both fMRI studies (Lumer et al., 1998; Lumer and Rees 1999; Sterzer et al., 2002) and in MEG studies (Tononi et al., 1998; Srinivasan et al., 1999). These widespread activity changes were linked to higher order and conscious visual processing. Concerning the fMRI studies it is unclear however, whether observer bias could have influenced the reported results. It is therefore an interesting question whether under normal sustained rivalry conditions observer biases also plays a role. In another fMRI study, also using sustained rivalry conditions, Brouwer and van Ee (2007) found that activation biases in V3A, V4D, V7, MT+, and parietal areas, but not in early visual cortex, allowed for the prediction of conscious perceptual states. If bias also plays a role under sustained rivalry, it is interesting to see to what extent these results can be related to the effect of observer bias that we found in the current study, which was present in late visual cortex (including MT+) but not in early visual cortex. Concerning the MEG studies there is a report (Kamphuisen et al., 2008) concluding that the claims about widespread synchronized networks to consciously perceive visual rivalry are unconvincing. While these authors

also found stimulus entrained activity across the entire scalp, a MEG sensor phase analysis revealed that the spatially extended nature of the activity could have been produced by a limited set of occipital sources.

Although eye movements could have confounded our results in that subjects would make more eye movements when they find it more difficult to follow instructions, or during either the most or least frequent regime, we believe that this is not very likely. It is known that subjects can voluntarily control the percept of a bistable stimulus without making eye movements (Brouwer and van Ee 2006; Brouwer and van Ee 2007). Furthermore, in a psychophysical experiment that was similar to this experiment, but with eye movement recordings, Klink et al. (2008) found no differences in gaze position or saccade directions between blocks without instruction, blocks with instructions to alternate, and blocks with instructions to repeat. Subjects are thus equally well able to fixate with different task instructions. In addition, the correlation between regime bias and brain activation that we found was present in late visual cortex, but not early visual cortex. It is difficult to explain how eye movements would account for this discrepancy, as they would most probably affect early visual cortex as well.

In conclusion, we found that widespread activation differences between perceptual alternations and repetitions are correlated with the perceptual bias for one of the two perceptual regimes. This bias could be linked to the amount of effort a subject needs to maintain a particular regime, but also to differences in adaptation of the regimes. This finding is in line with the notion that low-level mechanisms can account for stabilized and repeating perceptual regimes.

4. Experimental procedures

4.1. Scanning protocol

Scanning was performed on a Philips Achieva 3 T scanner (Philips Medical Systems, Best, the Netherlands) with a Quasar Dual gradient set. For functional scans, a navigated 3D-PRESTO pulse sequence was used (Ramsey et al., 1998; van Gelderen et al., 1995). The acquisition parameters were: TR=21.75 ms (time between 2 subsequent RF pulses); effective TE=32.4 ms; FOV(anterior–posterior, inferior–superior, right–left)=224*256*128 mm; flip-angle=10°; matrix: 56*64*32 slices; voxel size 4 mm isotropic; 8 channel head coil; SENSE factors=2.0 (left–right) and 1.8 (anterior–posterior). A new volume was acquired every 500.3 ms, and encompassed the entire brain. Immediately after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high flip-angle (27°, FA27) for the image coregistration routine (see below). A T1 weighted structural image of the whole brain was acquired at the end of the functional runs.

4.2. Subjects

16 subjects (9 male, 7 female) recruited from the Utrecht University participated in the experiment. All subjects gave informed consent for participation (approved by the Human Ethics Committee of the University Medical Center Utrecht).

All were right handed according to the Edinburgh Handedness inventory (Oldfield 1971).

4.3. Task design

For stimulus presentation we used a PC laptop, a rear projection screen, and a video-projector system that operated at 85 Hz. The stimuli were programmed in C++ software (Bjarne Stroustrup, Bell Laboratories, USA). Responses were recorded using an MRI-compatible air pressure buttonbox.

The first part of the experiment consisted of structure from motion (SFM) trials and static trials. During the second part, there were only SFM trials. Each SFM trial started with a 1000 ms presentation of a rotating transparent sphere that can be perceived as rotating in two opposite directions (Fig. 1). Subjects viewed this stimulus with only their dominant eye. The non-dominant eye was occluded by a patch. The sphere contained moving dots that followed an imaginary circle in the horizontal plane. There were 250 white and 250 black dots that were randomly distributed on the sphere and with an equal amount of black and white dots moving in each direction on a grey background. The sphere had a diameter of 2.20° of visual angle and had a rotational speed of 60°/s. Single dots, including a central red fixation dot, had an angular size of 0.08°.

After the first 1000 ms of the trial ('on' period), there was a brief period during which only the fixation dot was visible ('off' period). The duration of the off-period depended on the subject: T-off was chosen such that the subject had the strongest voluntary control over the perceived rotation direction of the sphere (see further below under 'calibrating the off-time'). With each new presentation of the sphere, the dots were randomly redistributed. Static trials were used for the reference conditions, and included a stationary 'sphere' (technically a flat circle, as there is no SFM) with otherwise the same characteristics. The average luminance of the entire screen was constant for the entire duration of the trial.

The first part of the fMRI experiment consisted of 20 trial blocks that started with a brief instruction. Instructions for blocks of SFM trials could be either to 'Alternate' or to 'Repeat' the perceived rotation direction upon the presentation of each sphere, relative to the perceived rotation direction in the previous trial. Subjects were asked to report the perceived motion direction of the sphere's front surface upon each sphere presentation with a button press. Subjects could occasionally also perceive the stimulus as either two convex surfaces or two concave surfaces that are sliding on top of each other (Hol et al., 2003; Chen and He 2004). Nevertheless, they still perceived one surface sliding in front of the other, meaning that our instruction to report the direction of the front surface was unambiguous. A new block automatically occurred every 60 scans.

There were two separate static reference conditions. Instructions for the blocks with a stationary sphere could be either 'Rest Alternate' or 'Rest Repeat', which indicated that subjects had to respectively alternate or repeat their motor response with the presentation of each new stationary sphere. We used a separate reference condition for alternating and repeating SFM trials, to control for subtle differences in motor output. In total there were 15 Alternate blocks (total of 300

trials), 15 Repeat blocks (total of 300 trials), 7 Rest Repeat block (total of 140 trials), and 8 Rest Alternate blocks (total of 160 trials). Blocks were presented in a pseudo randomized order over 3 scanning runs of 900 scans each. The first session of the experiment had a total duration of 22.5 min. Directly after the first session, there was an additional second session that consisted of a single scanning run of 900 scans (7.5 min). This session was performed by 12 out of 16 subjects (the second session was added at a later stage during research, when four subjects already participated). The second session consisted of a single block of 360 trials without instructions, but trials were furthermore identical to the SFM trials of the first session. Subjects had to report the rotation direction of each new stimulus without instructions to repeat or alternate.

4.4. Analysis of fMRI data

All preprocessing steps were done using SPM2 (<http://www.fil.ion.ucl.ac.uk/spm/>). After realignment, the functional scans were coregistered to the FA27 volume, using the first functional volume as a source. The structural scan was also coregistered to the FA27-scan, thereby providing spatial alignment between the structural scan and the functional volumes. Normalization parameters were estimated using the MNI T1-standard brain as template, and the coregistered T1 volume as a source. All functional scans were then normalized and resliced to a 4×4×4 mm resolution. A 3D-gaussian filter (8 mm full width at half max) was applied to all fMRI volumes.

The functional data was subjected to a multiple regression analyses using IDL (Research Systems Inc., Boulder, USA). The design matrix for the first session contained twelve factors that represented:

- 1: Trial with an alternating percept during alternation instructions (correct trial)
- 2: Trial with a repeating percept during repetition instructions (correct trial)
- 3: Trial with an alternating percept during repetition instructions (incorrect trial)
- 4: Trial with a repeating percept during alternation instructions (incorrect trial)
- 5: Alternate during a stationary sphere (static trial)
- 6: Repeat during a stationary sphere (static trial)
- 7: Switch trial from alternation regime to repetition regime during alternation instructions
- 8: Switch trial from repetition regime to alternation regime during alternation instructions
- 9: Switch trial from alternation regime to repetition regime during repetition instructions
- 10: Switch trial from repetition regime to alternation regime during repetition instructions
- 11: First trial of each block
- 12: Instructions at the start of each block

The factors together represented a full factorial design which allows to disentangle the effects of trial type (alternations/repetitions), success or failure to follow instructions (thereby removing error detection as confounder), and effects of switching regimes. The number of trials that a factor represents depends on the ability of subjects to follow task

instructions. This can result in low statistical power of some factors, especially incorrect trial and switch trials, when individual performance is near perfect. However, by modeling these trials separately, they cannot confound the activation found for other trial types. The number of trials that constitute the factors for each subject are displayed in Table 3. All trials were represented as 1000 ms events that were convolved with a predefined hemodynamic response function (canonical HRF of SPM2) (Friston et al., 1995). In addition, the design matrix contained factors for low frequency noise i.e. the mean signal intensity of each scan, and cosine functions forming a high pass filter with a cut-off at 10^{-2} Hz.

The regression analysis for the second session was similar, but now there were only factors for alternations, repetitions, switch trials from alternations to repetitions, switch trials from repetitions to alternations, and for the first trial of the session.

A voxel-wise one sample t-test was done for the contrast of the regressor-coefficients of correct trials (factor 1 and 2) vs. static trials (factor 5 and 6) of the first session, and using the pooled standard deviation. Results were Bonferroni corrected, and a three dimensional watershed algorithm and anatomical landmarks were used to discriminate the regions of interest (ROI's) that were involved in correct task performance. Further group-wise analyses were done using the average regressor-coefficients within the ROI's for all task factors of the first and second session. The activation levels for alternation and repetition trials (factors 1 to 4 and factors 7 to 10) were all determined in reference to their corresponding static condition (factor 5 and 6). Activation differences between repetitions and alternations during the second session were controlled for activations differences between the static conditions (factor 5 and 6) of the first session. The Computerized Anatomical Reconstruction and Editing Tool Kit (CARET) was used for visualization of fMRI results (Van Essen et al., 2001).

4.5. Calibrating the 'off-period'

Prior to the fMRI experiment, for each subject the off-period was calibrated at which the subject exhibited the best ability to follow instructions to alternate or to repeat the percept of the SFM stimulus with each new presentation. The purpose of this calibration was twofold. Firstly, the efficiency of the fMRI design increases with an increasing ability of subjects to follow instructions. Better performance improves statistical power as a result of a higher number of correct trials, and optimizes the length of perceptual regimes for fMRI. Secondly, the best performance is obtained at the off-period at which the bias of the subjects to repeat or alternate the percept is minimized. A minimal bias results in a minimal difference in the amount of effort that a subject needs for either alternating or repeating the percept.

Subjects were seated in a dark room behind a 21 in. CRT monitor (85 Hz). Head movements were constrained using a chin-rest. The psychophysics experiment used an intermittently presented SFM stimulus that was identical to the one that was used in the scanner. Similar to the task in the scanner, subjects had to report the perceptual rotation direction of the stimulus with each new presentation.

The first part of the psychophysics experiment served to familiarize subjects with the stimulus and its possible perceptual regimes. Subjects had to report the rotation direction of the sphere without exerting voluntary control over the rotation direction. Between blocks of 15 stimuli, the off-period was randomly varied between 95 ms and 659 ms in 20 time steps. The second part of the psychophysics experiment used the same setup, but now subjects were given instructions at the start of each block to alternate or to repeat the rotation direction of the sphere. Subsequently, the off-period was determined as that which the subject had on average the best ability to follow instructions ((% correct repetitions + % correct alternations)/2). This was the off-period that was used during the entire fMRI experiment. The entire psychophysics experiment took approximately 1 h. The results of the psychophysical experiment were analyzed with a 3 (different instructions) by 20 (different off-periods) repeated measures ANOVA.

REFERENCES

- Ardekani, B.A., Choi, S.J., Hossein-Zadeh, G.A., Porjesz, B., Tanabe, J.L., Lim, K.O., Bilder, R., Helpert, J.A., Begleiter, H., 2002. Functional magnetic resonance imaging of brain activity in the visual oddball task. *Brain Res. Cogn. Brain Res.* 14, 347–356.
- Bandettini, P.A., Cox, R.W., 2000. Event-related fMRI contrast when using constant interstimulus interval: theory and experiment. *Magn. Reson. Med.* 43, 540–548.
- Brouwer, G.J., van Ee, R., 2006. Endogenous influences on perceptual bistability depend on exogenous stimulus characteristics. *Vision Res.* 46, 3393–3402.
- Brouwer, G.J., van Ee, R., 2007. Visual cortex allows prediction of perceptual states during ambiguous structure-from-motion. *J. Neurosci.* 27, 1015–1023.
- Chen, X., He, S., 2004. Local factors determine the stabilization of monocular ambiguous and binocular rivalry stimuli. *Curr. Biol.* 14, 1013–1017.
- Cole, M.W., Schneider, W., 2007. The cognitive control network: integrated cortical regions with dissociable functions. *Neuroimage* 37, 343–360.
- Corbetta, M., Shulman, G.L., 2002. Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* 3, 201–215.
- Friston, K.J., Frith, C.D., Turner, R., Frackowiak, R.S., 1995. Characterizing evoked hemodynamics with fMRI. *Neuroimage* 2, 157–165.
- Hol, K., Koene, A., van Ee, R., 2003. Attention-biased multi-stable surface perception in three-dimensional structure-from-motion. *J. Vis.* 3, 486–498.
- Kamphuisen, A., Bauer, M., van Ee, R., 2008. No evidence for widespread synchronized networks in binocular rivalry: MEG frequency tagging entrains primarily early visual cortex. *J. Vis.* 8, 4–8.
- Klink, P.C., van Ee, R., Brouwer, G.J., Noest, A.J., van Wezel, R.J., 2008. Early interactions between neuronal adaptation and voluntary control determine perceptual choices in bistable vision. *J. Vis.* 8 (5), 1–18. 30-5-2008.
- Kornmeier, J., Ehm, W., Bigalke, H., Bach, M., 2007. Discontinuous presentation of ambiguous figures: how interstimulus-interval durations affect reversal dynamics and ERPs. *Psychophysiology* 44, 552–560.
- Krekelberg, B., Boynton, G.M., van Wezel, R.J., 2006. Adaptation: from single cells to BOLD signals. *Trends Neurosci.* 29, 250–256.

- Leopold, D.A., Wilke, M., Maier, A., Logothetis, N.K., 2002. Stable perception of visually ambiguous patterns. *Nat. Neurosci.* 5, 605–609.
- Liddle, P.F., Kiehl, K.A., Smith, A.M., 2001. Event-related fMRI study of response inhibition. *Hum. Brain Mapp.* 12, 100–109.
- Lumer, E.D., Rees, G., 1999. Covariation of activity in visual and prefrontal cortex associated with subjective visual perception. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1669–1673.
- Lumer, E.D., Friston, K.J., Rees, G., 1998. Neural correlates of perceptual rivalry in the human brain. *Science* 280, 1930–1934.
- Maier, A., Wilke, M., Logothetis, N.K., Leopold, D.A., 2003. Perception of temporally interleaved ambiguous patterns. *Curr. Biol.* 13, 1076–1085.
- Maloney, L.T., Dal Martello, M.F., Sahn, C., Spillmann, L., 2005. Past trials influence perception of ambiguous motion quartets through pattern completion. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3164–3169.
- Noest, A.J., van Ee, R., Nijs, M.M., van Wezel, R.J., 2007. Percept-choice sequences driven by interrupted ambiguous stimuli: a low-level neural model. *J. Vis.* 7, 10.
- Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113.
- Orbach, J., Ehrlich, D., Vainstein, E., 1963. Reversibility of the necker cube: III. Effects of interpolation on reversal rate of the cube presented repetitively. *Percept. Mot. Skills* 17, 571–582.
- Orbach, J., Zucker, E., Olson, R., 1966. Reversibility of the necker cube: VII. Reversal rate as a function of figure-on and figure-off durations. *Percept. Mot. Skills* 22, 615–618.
- Pastor, M.A., Day, B.L., Macaluso, E., Friston, K.J., Frackowiak, R.S., 2004. The functional neuroanatomy of temporal discrimination. *J. Neurosci.* 24, 2585–2591.
- Ramsey, N.F., van den Brink, J.S., van Muiswinkel, A.M., Folkers, P.J., Moonen, C.T., Jansma, J.M., Kahn, R.S., 1998. Phase navigator correction in 3D fMRI improves detection of brain activation: quantitative assessment with a graded motor activation procedure. *Neuroimage* 8, 240–248.
- Salmi, J., Rinne, T., Degerman, A., Salonen, O., Alho, K., 2007. Orienting and maintenance of spatial attention in audition and vision: multimodal and modality-specific brain activations. *Brain Struct. Funct.* 212, 181–194.
- Srinivasan, R., Russell, D.P., Edelman, G.M., Tononi, G., 1999. Increased synchronization of neuromagnetic responses during conscious perception. *J. Neurosci.* 19, 5435–5448.
- Sterzer, P., Rees, G., 2008. A neural basis for percept stabilization in binocular rivalry. *J. Cogn. Neurosci.* 20, 389–399.
- Sterzer, P., Russ, M.O., Preibisch, C., Kleinschmidt, A., 2002. Neural correlates of spontaneous direction reversals in ambiguous apparent visual motion. *Neuroimage* 15, 908–916.
- Stevens, A.A., Skudlarski, P., Gatenby, J.C., Gore, J.C., 2000. Event-related fMRI of auditory and visual oddball tasks. *Magn. Reson. Imaging.* 18, 495–502.
- Tomasi, D., Chang, L., Caparelli, E.C., Ernst, T., 2007. Different activation patterns for working memory load and visual attention load. *Brain Res.* 1132, 158–165.
- Tononi, G., Srinivasan, R., Russell, D.P., Edelman, G.M., 1998. Investigating neural correlates of conscious perception by frequency-tagged neuromagnetic responses. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3198–3203.
- Treue, S., Husain, M., Andersen, R.A., 1991. Human perception of structure from motion. *Vision Res.* 31, 59–75.
- Van Essen, D.C., Drury, H.A., Dickson, J., Harwell, J., Hanlon, D., Anderson, C.H., 2001. An integrated software suite for surface-based analyses of cerebral cortex. *J. Am. Med. Inform. Assoc.* 8, 443–459.
- van Gelderen, P., Ramsey, N.F., Liu, G., Duyn, J.H., Frank, J.A., Weinberger, D.R., Moonen, C.T., 1995. Three-dimensional functional magnetic resonance imaging of human brain on a clinical 1.5-T scanner. *Proc. Natl. Acad. Sci. U. S. A.* 92, 6906–6910.
- Wallach, H., O'Connell, D.N., 1953. The kinetic depth effect. *J. Exp. Psychol.* 45, 205–217.