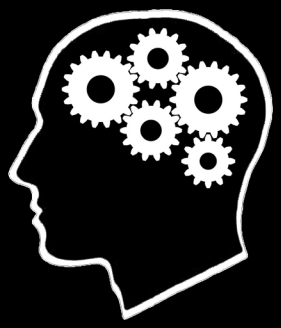
Proceedings of the Master's Programme

COGNITIVE NEUROSCIENCE



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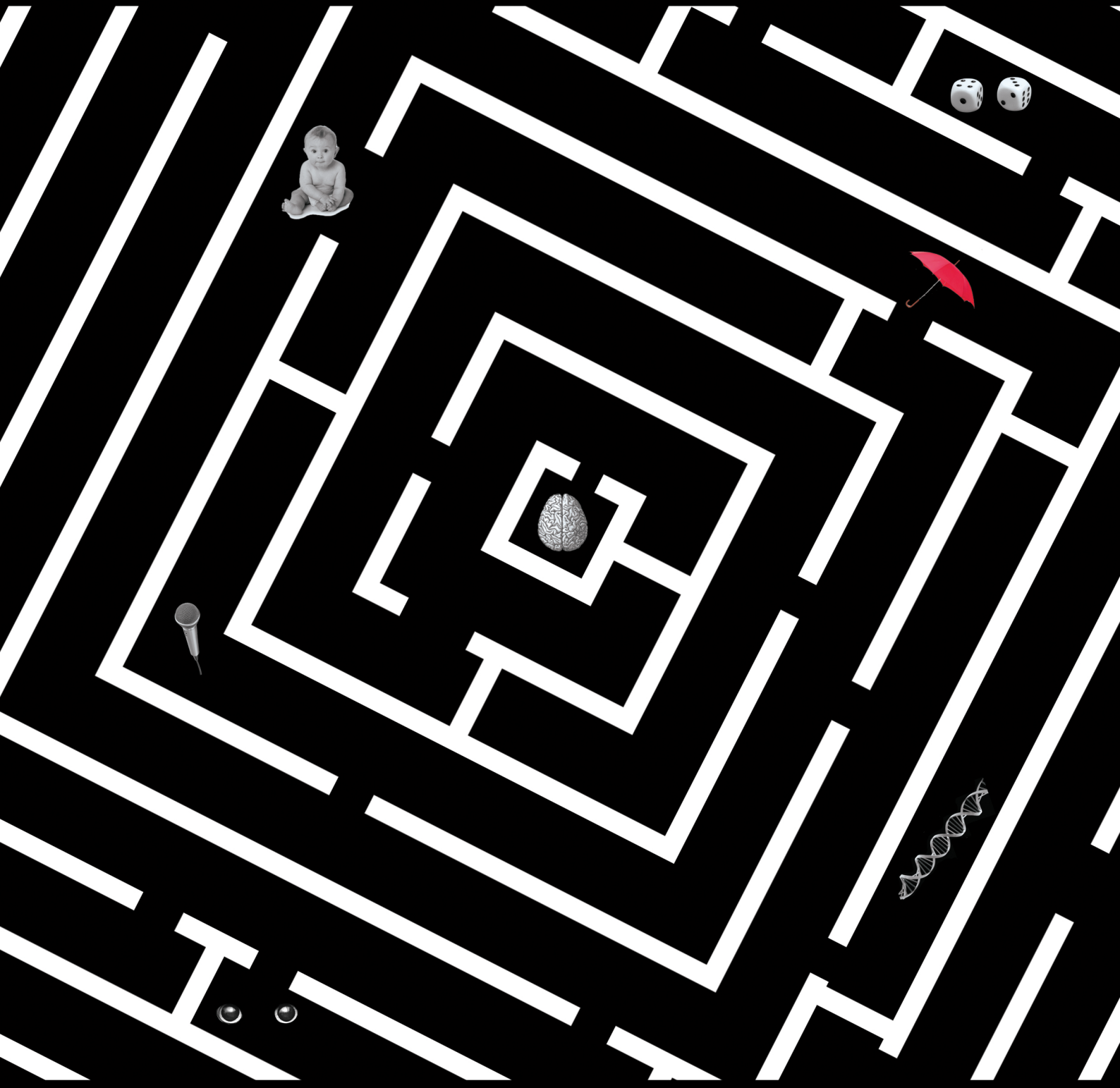

Donders Institute
for Brain, Cognition and Behaviour

Radboud University Nijmegen

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Proceedings of the Master's Programme Cognitive Neuroscience of the Radboud University Nijmegen

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From the Editors-in-Chief of the CNS Journal

Dear Reader,

It is with great pleasure that we introduce to you this issue of the *Proceedings of the Master's Programme Cognitive Neuroscience*. This is particularly noteworthy as it coincides with the 10th anniversary of the inauguration of our programme, and reflects this auspicious occasion by including a number of special editorials to commemorate this milestone.

That is, in addition to our usual complement of high quality research articles – covering such diverse topics as visual perception, auditory processing, memory, action observation, pathological gambling, and gene/environment interactions. We are also pleased to present to you multiple perspectives on the programme and the institute in our editorial space. First, Professor Ruud Meulenbroek and Dr. Arno Koning, our tireless programme coordinators, discuss the founding of the MSc course, and its subsequent growth and evolution. Next, we hear from the director of the Max Planck Institute and the founding director of the Donders Centre for Cognitive Neuroimaging, Professor Peter Hagoort, on the topic of the Donders, the programme, and our role as scientists in the broader world. Dr. Nanda Rommelse continues this discussion with a special focus on what she, as an upcoming and highly successful young academic, sees as the future of the programme and our research. Finally, we take a retrospective look at one of the first graduates of our programme – Dr. Jasper Poort – as he discusses his experiences during the MSc and reflects on his research and time spent here at Radboud University's Donders Institute.

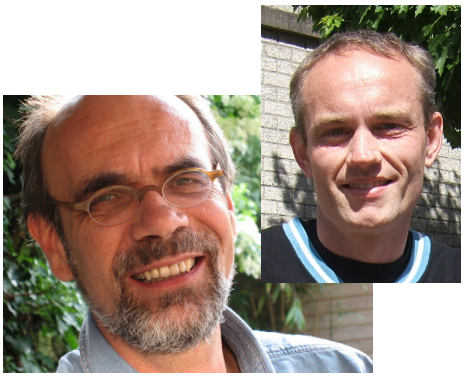
The close relationship between the programme and the journal is evident from both its content, and its sustained attainment of excellence. The journal team is always comprised of students currently in the Master's programme, and reflects in its own 8-year history the dedication of many years of enthusiastic students to a quality publication.

Concomitant with the evolution of the programme, we have also seen great changes in the journal itself – including a striking aesthetic redesign and the expansion to two issues per year. Even with this increased capacity, however, we find ourselves in the unenviable position of having to select from an ever widening pool of high quality research articles. It will be a very exciting and challenging time for the junior members of our journal team as the programme continues to grow in both size and quality – and we are confident of both their, and the programme's, continued success.

On a personal note, this issue marks the end of our tenure as Editors-in-Chief, and also the first time the journal has been composed with one Editor-in-Chief at large, pursuing an internship abroad. The experience of collaborating to produce the previous issue, as well as the one you now hold, has been complex, challenging, and ultimately a highly rewarding experience. In these latter aspects, it is not unlike the MSc programme itself – where the intellectual demands placed on us as students inspire us to new heights of achievement. It is this spirit that has permitted the journal team, time and time again, to rise to the tasks set before them. Needless to say, we are exceedingly proud of the *Proceedings* journal, and likewise our membership in the CNS programme. Throughout the Masters, we have benefited from world-class interactions and opportunities with faculty, facilities and fellow candidates. It goes without saying that it has been a continual pleasure to work on both the journal and in the programme, and we trust this will be reflected in your own enjoyment of this very special edition.

Jana Krutwig & Jeffrey Martin
Editors-in-Chief

From the CNS Management



Dear Reader,

The two-year multidisciplinary international Research Master's programme in Cognitive Neuroscience currently celebrates its tenth anniversary. The special issue of the Proceedings of the Master's programme Cognitive Neuroscience that you are currently reading marks this milestone and with this editorial we aim to give a brief historical overview of both the educational programme and the Journal.

The programme

The Cognitive Neuroscience Research Master programme at the Radboud University started in 2003 after a team of senior researchers and department heads of the subdisciplines contributing to this field took the stance that properly preparing Masters students for challenging PhD research projects in cognitive neuroscience required a high-quality, two-year MSc training. Institutes involved were the then called Nijmegen Institute for Cognition and Information, the Centre for Language Studies, the Max Planck Institute for Psycholinguistics, the Science Faculty and the University Medical Center Nijmegen. Members of the steering group of those early days were Wietske Vonk, Peter Hagoort, Charles de Weert, Herbert Schriefers, Stan Gielen, Harold Bekkering and Ruud Meulenbroek. The programme was initially organized around three tracks, i.e., Psycholinguistics, Perception and Action, and Neurocognition. The programme started with 9 students but the number of students steadily increased (see Table 1) to a current influx of 50 students per year, of whom 43% obtained their bachelor at the Radboud University, 25% at another Dutch university, and 32% at a foreign university.

Table 1. Number of local, national and international students between 2003-2012

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Radboud University	5	10	11	9	13	11	8	24	13	20	124
Other Dutch Universities	2	4	7	10	7	8	3	15	8	11	75
Foreign students	2	7	7	4	8	6	12	6	23	18	93
Total	9	21	25	23	28	25	23	45	44	49	292

Overall, the programme attracts a relatively large number of non-European students, i.e. 8% (see Table 2), and has a good international reputation.

Table 2. Number of EU and non-EU students between 2003-2012

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
European	8	20	23	22	27	22	21	43	38	46	270
Non-European	1	1	2	1	1	3	2	2	6	3	22
Total	9	21	25	23	28	25	23	45	44	49	292

In 2010 the student numbers almost doubled, probably due to the visibility of the newly initiated Donders Institute for Brain, Cognition and Behaviour and the organization of its Masters and PhD training programmes into the Donders Graduate School for Cognitive Neuroscience (DGCN; Fig. 1). The Research Master programme was restructured into four tracks, one-to-one related to the four research lines of the Donders

Institute, namely (i) Language and Communication, (ii) Perception, Action and Control, (iii) Learning, Memory and Plasticity and (iv) Brain Networks and Neuronal Communication. In addition to the greater visibility of the Master's programme in the context of the DGCN, another factor played an important role in the rise of the student numbers. More specifically, for the past four years (2010-2013) the CNS programme has been voted the best among similar Master's programmes in the Netherlands. Moreover, with the fourth consecutive win in April 2013, a so-called quality seal was given, indicating that the CNS programme belongs to the best Master's programmes in the Netherlands. With the acceptance rate continuing to be around 50% each year, and the number of accepted students having increased, it is clear that the number of excellent student who apply has also increased. Alongside these numbers is the fact that when asked why students apply to the CNS programme, the answer is often 'because it has been voted the best programme'.

Fig. 1 Recognition of the Graduate School for Cognitive Neuroscience by the Dutch Minister of Education Ronald Plasterk in 2009.

The Journal

The Proceedings of the Master's programme Cognitive Neuroscience, or 'the Journal' as it is colloquially referred to, is an integral part of the programme. Inspired by students from Stanford University (U.S.A.), in March 2006 the first issue of the first volume of the then called Nijmegen CNS journal was published both on-line and in hard-copy format. From its conception, the journal has been run entirely by students and is only advised by a senior researcher. This of course created the need for editorial choices, decisions on revisions, acceptance and rejection, layout and editing decisions, PR and website maintenance. Students could apply for different positions and thus be part of the Journal team. Ever since the first team, there has not been a shortage of team members. In fact, the size of the team, and with that certainly the professionality of the Journal, has increased tremendously. Since 2011 two issues per year are published and that same year a trip to the headquarters of Nature in Oxford (UK) was a great success. Since 2012, a launch party is organized for each new issue. For these events, a more general topic/theme is chosen and a speaker invited. For example, the famous neuroscientist blogger Neuroskeptic recently gave a presentation about the theme 'neuroscience and the general public'. The Proceedings of the Master's programme Cognitive Neuroscience continues to evolve, and thereby remains an important part of the educational training of young top scientists.

The future

The brain is a hot topic. General audience books on cognitive neuroscience top the bestsellers lists and new discoveries are liked and shared on all social media. To guarantee that the experiments that lead to these discoveries are done correctly, and that their results are interpreted properly, there is a need for top young scientists with a strong theoretical background, and high-quality hands-on training, in cognitive neuroscience. The Radboud University has long since acknowledged the importance of brain research by making cognitive neuroscience one of its major research spearheads and the Donders Graduate School for Cognitive Neuroscience, with its Master's and PhD studies, provides this training. The Journal has played an important role in the programme and continues to do so.

Prof. Ruud Meulenbroek
Director of the CNS Programme

Dr. Arno Koning
Coordinator of the CNS Programme

From the Founding Director of the Donders Centre for Cognitive Neuroimaging

Dear Reader,

This is a very special issue of the journal. It is celebrating the tenth anniversary of the MSc Cognitive Neuroscience. I had the privilege to experience the inception and growth of the masters program from the very beginning. The ambition was to build it up from scratch. That is, we decided to develop a curriculum that consisted of a whole series of new courses instead of selecting from existing ones. Only in this way a coherent program could be designed. A lot of effort and creativity went into this first program, which has seen major improvements ever since it started ten years ago. In addition, new ideas were launched, one of which is this journal run by the students. The journal has been a tremendous success, and is one of the flagships of our masters program. Modern day science is built as a complex social structure that one has to learn to understand in order to be successful as a scientist. Running the journal is an excellent way of experiencing one of the key components of modern day science, which is peer communication. As scientists we compete for resources and recognition. At the same time this can be done fairly and correctly only if we realize that we are part of a scientific community. Peer reviews, helpful comments by your colleague experts, the realization that your support will be paid back at some time in the future; these are all vital aspects of a scientific life. It is not always sufficiently appreciated and not always clearly told by senior scientists that, next to intellectual quality and courage, there is one overriding characteristic that will promote your scientific career: generosity. Being generous to your colleagues, being loyal to the institutions you work for might sometimes look like altruistic behaviour and self-sacrifice. But this is a misperception. In the end they pay off. If you want to see the mathematical proof that this is true, you should read Martin Nowak's fascinating book Super cooperators: beyond the survival of the fittest. Why cooperation, not competition, is the key to life.

This masters program is special. From the very beginning quality of the students and quality of the program were seen as key. Financially it would have been very attractive to allow more student to enroll in the program than were in fact selected. However, we believed that in the end the program would profit most from selecting only the very best students. They not only provide quality, they also demand quality. Both are a major source of motivation for teaching staff and supervisors to get the best out of themselves. The focus on quality has turned out to be the right choice. In successive years our masters program has been chosen to be the best of its sort within the Netherlands, and this year has acquired the highest quality predicate that is available for a masters program. Ruud Meulenbroek and Arno Koning are to be congratulated for steering students and teachers in the right direction. As students you should feel proud to be part of this masters program. All of us who are involved in teaching and making the MSc Cognitive Neuroscience a success are entitled to join in the feelings of pride.

What will the next ten years bring us? One of the current trends in science politics is that translation possibilities of basic research to domains of application and guarantees for economic return on investment begin to dominate the political agenda. This is based on a serious misconception about the acquisition of new insights and knowledge. Politics and management are by necessity based on the knowledge of today. Science is about the knowledge of tomorrow. If we could anticipate today what we shall only know

tomorrow, it would be wise to invest in an apartment in Stockholm. One Nobel Prize after the other could be picked up. However, the magic of science is that there is no known algorithm for discoveries. Freedom for basic research is the best guarantee that new insights will emerge. These new insights are the best guarantee that successful applications will follow, although we cannot predict what they will be. André Geim, Nobel Prize winner for Physics from our university, discovered graphene out of curiosity and a genuine interest in discovering basic properties of matter and materials. This discovery has opened up a world of new applications with enormous potential for economic spinoff. Geim and no one else had been able to foresee this.

Neuroscience is one of the most fascinating areas of research, with vast unchartered territories and hence a lot to discover. What our masters program should continue to do in the coming ten years is to stimulate the curiosity of our students, teach them the skills needed to give their curiosity a vehicle, and show them that perseverance is a necessary characteristic to move forward with this vehicle of skills. Whatever politicians and managers tell us, if we succeed in doing just that in the next ten years our masters program will remain equally successful. Let me end with the wish that the students of the future will be as great in running this journal as the previous generation of editors. You have done a marvelous job. The personal impact factor of this journal is commensurate with that of the top journals in our field. Congratulations.

Prof. Peter Hagoort
Founding Director of the Donders Centre for Cognitive Neuroimaging

From an Aspiring Principal Investigator: Future outlook

Dear reader,

It is a great honor to contribute to this special edition of the Proceedings of the Master's Program on the occasion of the 10th anniversary of the Cognitive Neuroscience Master. I would like to take this opportunity to congratulate both staff members and Master students of the Donders Institute with achieving this milestone. The CNS Master of the Donders Institute is nationally and internationally renowned for its high quality, both in terms of the content of the program as well as the scientific quality and integrity of the teachers and students. Over the past 10 years a program of high esteem has been developed, in which bright young talents develop their skills to become the new interdisciplinary-working scientists of the future.

Being a young researcher myself, I was asked to give my perspective on the future directions of the Master's program. Through my research I aim to detect the risk factors underlying child psychiatric disorders such as autism, ADHD and aggression. The greatest challenge I encounter in this type of research is the translation from fundamental research to clinical practice. Parents sometimes ask me: 'What may we expect from participating in this study? Will you discover the reason why my child has ADHD? What are the odds for his/her children to develop ADHD as well? Can this be prevented?' Unfortunately, my answers to these questions haven't changed in the last 10 years: 'I'm sorry, no; currently we won't be able to detect the individual factors that have contributed to your child's disorder. We know from studies that at a group level certain factors increase the risk of developing ADHD, but this knowledge is as yet not applicable at an individual level'. How unsatisfying!

However, I strongly believe that major progress will be made in this field of translational neuroscience in the next 10 years. The rapid development of cognitive neuroscience in child psychiatrics together with the refinement of sophisticated mathematical techniques able to combine the multitude of relevant parameters from different sources (such as genetic, imaging, cognitive and phenotypic data) makes the classification (i.e. diagnosis) based on risk factors at an individual level within hand reach. Hopefully, my answer to parents will then be: 'Your child will undergo several tests. We'll make a genetic profile of known ADHD risk genes, and then we will do several types of MRI scans to detect ADHD typical abnormalities in structure and function of his/her brain. We will also examine cognitive functions often affected in ADHD patients. Based on all these tests, we'll be able to calculate the probability that he/she suffers from a specific subtype of ADHD, which will help us determine the most appropriate treatment.' Such major progress can only be made when people from multiple disciplines (such as psychologists/psychiatrists, molecular biologists, statisticians/mathematicians, and neuroscientists) will be collaborating in order to approach this goal. I'm confident the Donders Institute with its CNS Master will be in the frontline of these developments, having the expertise and motivation required for this type of research.

Nanda Rommelse
aPI Donders Institute

From an Alumnus of the CNS Master's Programme

Dear Reader,

The research master Cognitive Neuroscience exists this year for 10 years, which is an event that deserves to be celebrated. For this occasion I was invited to look back on my experience as one of the first students in the programme and in particular reflect on the second year of the master in which I did my research internship, the results of which were published in the first issue of this very journal.
When I entered the programme it was unique in its kind. On the one hand, it was highly specialized in teaching students to study how the brain enables complex cognitive processes. On the other hand, it was highly multidisciplinary in teaching all the various backgrounds and approaches that are needed to crack this problem, with special emphasis on modern neuroimaging techniques.

In the courses scientists from different domains would teach their own topics of expertise to small groups of students. We would never just follow the textbooks but we would actively explore the boundaries of knowledge and ongoing debates, which stimulated a desire to move beyond that what is already known.

One feature that sets the research master apart from other master programmes is the opportunity for long term practical experience within a research group. This 'mini-PhD' gives you the first-person perspective of what it is like to be a researcher. For my internship I decided to work on a project under supervision of Jan-Mathijs Schoffelen in the "neuronal coherence" research group, led by principal investigator Pascal Fries at the Donders Centre for Cognitive Neuroimaging, which is closely affiliated with the programme.

Every cognitive task depends on the coordinated activity of a number of specialized cortical areas, and we studied the fundamental question how groups of neurons in different parts of the brain interact. The traditional idea is that neurons communicate via a rate code and that a neuron transmits information to neurons in other areas by modulating the number of action potentials it emits. However, in addition to this, brain cells also make use of a temporal code: groups of neurons within a cortical area display rhythmically synchronized activity, which can enhance the impact of their output. The efficiency of interactions between areas can be further enhanced when these excitability fluctuations are also synchronized across areas. Such coherent activity can ensure that output from one area is timed to arrive in the other area when it is maximally excitable.

We studied the role of neural synchrony during a task in which visual information needed to be transformed into a specific motor command. To this end I learned to simultaneously record cortical brain activity with magnetoencephalography (MEG) and muscle activity with electromyography (EMG). We asked test subjects to perform the following task while they were in the MEG scanner: first they fixated a small cueing stimulus consisting of an arrow pointing either to the left or to the right. The cueing stimulus was then replaced by a white fixation dot, and the subjects had to extend both their wrists against two levers and keep the force measured within a specified range during the baseline period. Once subjects were exerting constant force on both sides, a moving visual stimulus was presented during the stimulus period. The subjects' task was to react as fast as possible to a speed change of the stimulus occurring at an unpredictable time after stimulus onset. Subjects had to respond by increasing the extension of the left or right wrist, dependent on the direction of the cue.

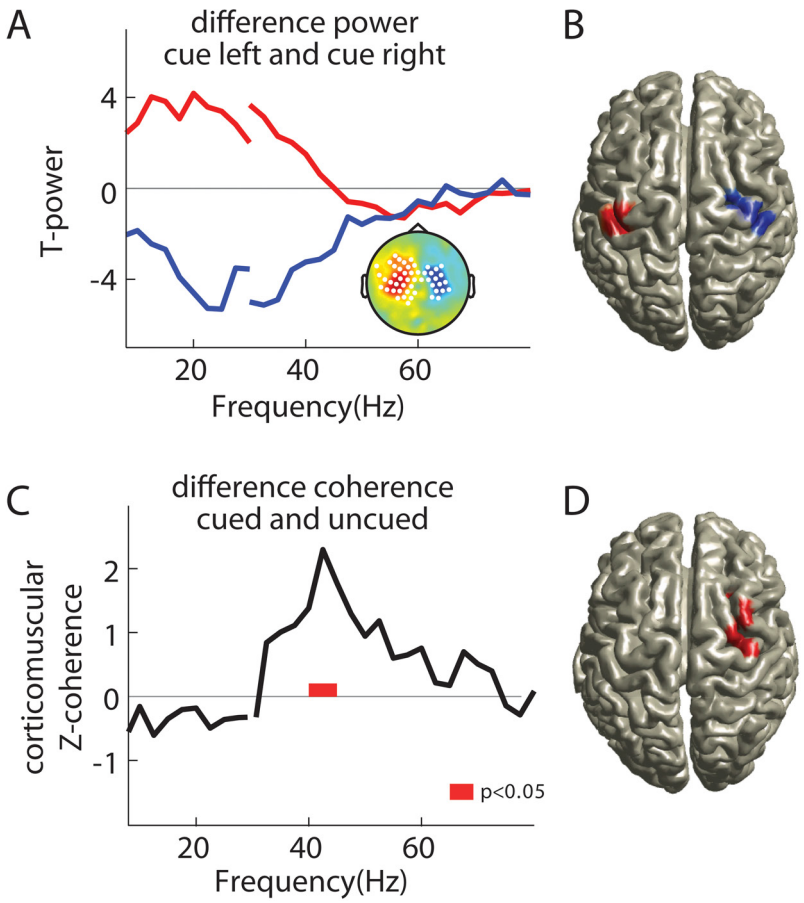


Fig. 1 Reduced beta-band power and increased gamma-band corticomuscular coherence signifies selective movement preparation. A. Reduction of beta-band (20 Hz) activity in the hemisphere contralateral to the cued side. T-power quantifying the power difference between cue left and cue right conditions averaged across significant clusters of MEG sensors (topography shown in the inset). The red line shows the left hemisphere sensors, the blue line right hemisphere sensors. B. Source analysis shows that the effect in the beta-band is localized to contralateral motor regions. C. Increased corticomuscular coherence in the gamma band (40 Hz) between muscle activity and contralateral MEG sensors (selected on the basis of a separate localizer experiment) when driving the cued hand. D. Source analysis localizes the effect to motor regions contralateral to the cued hand. Figure adapted from Schoffelen, Poort, Oostenveld, and Fries, Journal of Neuroscience, 2011.

An important skill I learned during my internship and that I have profited from during all my subsequent research was to analyze complex neurophysiological datasets. In fact, the Donders Centre is the place where the FieldTrip toolbox, a software package for analysis of electrophysiological data, is developed. This toolbox is now widely used in the neuroscience community (see fieldtrip.fcdonders.nl for tutorials and other information) and continues to be expanded.

We first investigated the oscillatory power within cortical areas during the stimulus period when the subjects were preparing to respond. We found that the effect of the response cue on local synchrony was a decrease in beta-band activity (20 Hz) in motor areas. This effect was strongest for the hemisphere controlling movements of the cued hand (Figure 1A and B). The advantage of simultaneously recording the muscle activity of both hands was that we could control for the possible effect of differential muscle activity in the different conditions by comparing the power for trials with identical muscle activity. Beta-band activity has been linked to inhibition of motor commands and a reduction of beta therefore reflects a removal of inhibition to allow the movement to be made.

Next, we focused on the long-range coupling between groups of neurons in motor cortex and spinal motorneurons that drive the hand movement. In a separate experiment, in which subjects were only extending the left or right hand, we localized the MEG sensors overlaying cortical motor regions. We then compared

the corticomuscular coherence between cortical and muscle activity in conditions in which the hemisphere was driving the cued and when it was driving the uncued hand. Our data showed that the corticomuscular coherence was significantly increased in the gamma-band range (40 Hz) when it was driving the cued motor response (see Figure 1C and D), suggesting that coherence can indeed be used to flexibly couple distant nodes of a network dependent on behavioural requirements. Before our study was published in the Journal of Neuroscience (Schoffelen et al., 2011), the initial results from my internship were first described in the Proceedings of the Master's programme Cognitive Neuroscience, which continues to provide a unique platform for the latest exciting research done by students of the programme, and in turn offers them valuable experience with the process of scientific publishing.

After completing the master Cognitive Neuroscience I joined the research group of Pieter Roelfsema at the Netherlands Institute for Neuroscience in Amsterdam as a PhD student where I studied the role of different visual cortical areas in attention using single- and multi-neuron recording techniques. The education I received during my master's studies and my experience of all stages of the research process during my internship have been important factors in receiving this opportunity and they also proved to be a very good preparation. In 2012 I defended my thesis and I am now part of the lab of Tom Mrsic-Flogel at University College London as a postdoctoral researcher where I continue to study activity of cell populations in visual cortex during behaviour using 2-photon imaging.

My experience with the Master's programme has taught me to appreciate and make use of all tools available in order to come a little bit closer to understanding how our brain enables us to perceive, think and act. I want to congratulate and thank all those who initially started the programme and who have been involved in it since then with so much enthusiasm to make it a success.

Dr. Jasper Poort
Alumnus of the CNS Master's Programme

Location and Orientation Updating of Visual Lines During Passive Linear Motion

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When navigating through the environment, it is important to know the location and spatial extent of objects, and remember this information when they are no longer in view. This ability, commonly referred to as 'spatial updating', is typically studied using a localization test of remembered single point targets after intervening movements. In this study, we tested spatial updating performance for more complex objects, such as 2D visual lines, and compared the results to the updating of single point targets. Subjects (n = 12) were seated with their head immobilized on a vestibular sled that oscillated laterally over 30cm, in complete darkness. At one turning point a line was flashed in the transverse plane, at different orientations and locations in the plane, while subjects' gaze was fixed on a central fixation point. At the next motion reversal of the sled a target dot (probe) was presented. Using a 2AFC approach, subjects reported the location of a target probe (left vs. right) relative to the remembered location of the line. Target probes were presented at different depths along the line and different lateral distances from the line. Based on the reported locations of the probes we assessed the spatial update of the line in terms of a midpoint shift and a general orientation bias. Results indicate depth-dependent midpoint shifts across subjects, and small (~2 deg), but significant errors in the orientation updates. We further show that these results can be best explained by an updating model which assumes underestimation of translation amplitude.

Keywords: spatial updating, object pointers, visual stability, passive motion, vector modelling

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1. Introduction

Knowing the location and spatial extent of objects relative to our own body is important for situational awareness as well as all kind of actions like goal directed movements. When the location of objects changes due to self-motion or object-motion, we need to incorporate the movement made and update the objects' spatial locations to achieve visual stability. This process has been termed 'spatial updating' and is especially useful when objects are barely visible or even disappear from view.

A correct update starts with an internal representation of the initial object locations. This representation can exist in different reference frames, i.e. relative to which coordinate system the object is coded. Possible reference frames are eye, head, gaze, body etc. Previous literature showed that in posterior parietal cortex (PPC) object locations are accurately encoded in a body-centered reference frame (Colby, 1998), gaze-centered reference frame (Ward, MacEvoy, & Epstein, 2010), or even a mixture of multiple reference frames (Beurze, Toni, Pisella, & Medendorp, 2010; Pouget, Deneve, & Duhamel, 2002a).

The updating process from initial to final object locations is typically studied using a localization test of remembered single point targets after intervening active movements. Wurtz (2008) and Sommer and Wurtz (2006) for example showed that for saccadic eye movements an efference copy of the outgoing motor command serves the purpose of coding motion. But the environment contains much more complex objects than single point targets. Therefore the present study investigates updating of two dimensional visual lines. This brings along an additional task for the brain, namely updating the orientation of the two dimensional object. Several behavioural studies looked at orientation updating of objects during reaching and grasping movements, and showed that orientation is coded in a gaze-centered reference frame for both intervening saccades and active body movements (Henriques, Klier, Smith, Lowy, & Crawford, 1998; Selen & Medendorp, 2011; Selen & Medendorp, 2011; Van Pelt & Medendorp, 2007; Van Pelt & Medendorp, 2008).

Bays and Husain (2007) and Klier and Angelaki (2008) recently suggested an active mechanism that spatially updates the orientation of a line as object pointers instead of the whole object. This means that the retinal image of the object is coded as a set of points and the spatial updating mechanism acts upon each point independently. However, our perception of a visual scene is markedly different than the retinal image. It depends on highly complex cognitive processing, involving exploratory eye movements, directing of attention, and integrating visual input with stored representations and prior knowledge about the world.

Various lines of research have shown that the brain does not build complete internal maps of the external world. For example, studies on change blindness (Cavanaugh & Wurtz, 2004) have shown that subjects are surprisingly poor at detecting changes between two subsequent images. Internal maps are therefore thought to be relatively sparse, leading to the idea that we use the external world as a memory buffer and make extensive use of attentive processes to track particular aspects of the environment.

Koch and colleagues argued that an image of the external world is filtered through feature selective filters and then combined into a saliency map (Itti & Koch, 2000; Walther & Koch, 2006). This is a map in which the salience strength of a particular feature in the image is presented as an activity measure. Attention is then focused on the feature which shows the highest activity in the saliency map. Bisley, Ipata, Krishna, and Gee (2009) termed these maps priority maps, including top-down influences besides bottom-up influences (for a review see Bisley and Goldberg, 2010).

How stable these maps are, and what information is being tracked can be challenged by introducing self-motion. Take, for example, the saccades we make to explore the scene. Each saccade shifts the image of the world on the retina and imparts a considerable delay (~30 ms) until the new image of the updated visual world can enter the brain. Although these saccades are made about 3 times per second, we perceive our visual world to be stable. This suggests that a centrally synthesized spatiotopic representation is constructed, either as a stable internal map of absolute space, or as a dynamic retinotopic map. Recent evidence points to the latter. Sommer and Wurtz (2006) and Medendorp, Goltz, Vilis, and Crawford (2003) for example showed that PPC dynamically updates spatial goals for action in a gaze-centered reference frame.

Cavanagh, Hunt, Afraz, and Rolfs (2010) suggested a different interpretation of these dynamic changes. In their view, visual stability is based on the updating of attention pointers only (Ahw, Armstrong, & Moore) and not on object pointers. Importantly, they argue that feature information, like shape, colour etc., can be dealt

with independently and is not spatially updated across movement. Melcher (2010) and Mayo and Sommer (2010) disagreed and showed that areas involving visual reference field updating also encode stimulus properties and that at least some tuning is maintained during the updating process.

All the above studies examined visual stability during saccades, but saccades are a special case. In the real world, we not only make saccades to shift gaze, but also generate head and body movements. The goal of the present study is to study how passive body movements interact with behaviourally relevant scene information. It should be noted that this is different from previous literature using either active body motion or saccadic eye movements. Using passive motion poses interesting constraints for the brain because an efference copy of the motor commands is no longer for use, e.g. when seated in a moving car.

Instead, extra-retinal information is needed to encode the motion trajectory. The traditional view, introduced by Helmholtz (1868), is that perfect spatial updating is achieved by a simple subtraction model, in which the extra-retinal signal for motion is subtracted from the retinal image shifts. So far, the only studies known for using passive body translation during a spatial updating task are Li and Angelaki (2005) and Clemens, Selen, Koppen and Medendorp (2012). However, Li and Angelaki (2005) based their paradigm on spatially updating targets along the vergence direction, whereas our paradigm targets along the version direction. Clemens, Selen, Koppen and Medendorp (2012) have shown that large errors are made in updating single point targets during passive motion. Modelling results showed that this could be explained by a gaze-centered updating model assuming a large underestimation of translation.

In the present study we test how self-motion updating deals with more complex object information (lines), rather than single point targets. We hypothesize that two dimensional visual lines contain more relevant information for the brain to be used in spatial updating, and therefore we would expect smaller errors than in Clemens, Selen, Koppen and Medendorp (2012). Subjects were asked to report the location of a target dot relative to an updated reference line. Based on the results, we make a comparison to the errors in self-motion updating of single point targets. In addition to this we will fit some vector models to examine whether the internal representation of the line is updated as a set of (attention) points (Bays & Husain, 2007; Cavanagh, Hunt, Afraz, & Rolfs, 2010; Klier & Angelaki, 2008) or whether a more complex, holistic representation (like orientation) is used in the updating.

2. Materials and Methods

2.1 Participants

Twelve naive participants (4 male, 8 female), aged between 17 and 25 years old, provided written informed consent to take part in the experiment. All participants had normal or corrected to normal vision and were free of any vestibular or neurological disorders. One subject was excluded from the analysis because she felt nauseous due to the sinusoidal sled motion.

2.2 Setup

Subjects were seated on a vestibular sled in a completely darkened room. Their interaural axis was aligned with the motion direction of the sled. The sled, powered by a linear motor (TB15N, Technotion, Almelo, Netherlands) and controlled by a Kollmorgen S700 drive (Danaher, Washington DC, USA), was used to translate participants laterally on a 800 mm track. Participants' body and head movement were restrained using a five-point seat belt and sled mounted headphones. Subjects viewed a world-fixed CRT screen (420 mm x 320 mm, refresh rate = 60 Hz) pointing to the ceiling, placed 1 m in front of the vestibular sled and mounted on top of a height adjustable table. The midpoint of the screen was aligned with the subjects' cyclopean eye using a LED laser such that the downward gaze angle was ± 4.5 degrees. A fixation point (4 mm x 4 mm), a flashed line (4 mm x 26 mm) and probe stimulus (4 mm x 4 mm) were presented on the screen with a timing resolution better than 17ms.

2.3 Experimental paradigm

Figure 1A shows the experimental paradigm of one trial. Each run of 25 trials started with the onset of the fixation point, to be fixated for the entire run. Next, sled motion was ramped to a steady 300 mm sinusoidal translation of 0.625 Hz in one cycle. At the rightmost position of the sled (150 mm, second panel Figure 1A), i.e. motion reverse, a reference line was flashed for 3 frames (50ms). When the sled reached the other extreme of the sled (-150 mm, fourth panel figure 1A), subjects' updating performance was tested by flashing a probe stimulus, also for three frames. Participants had to

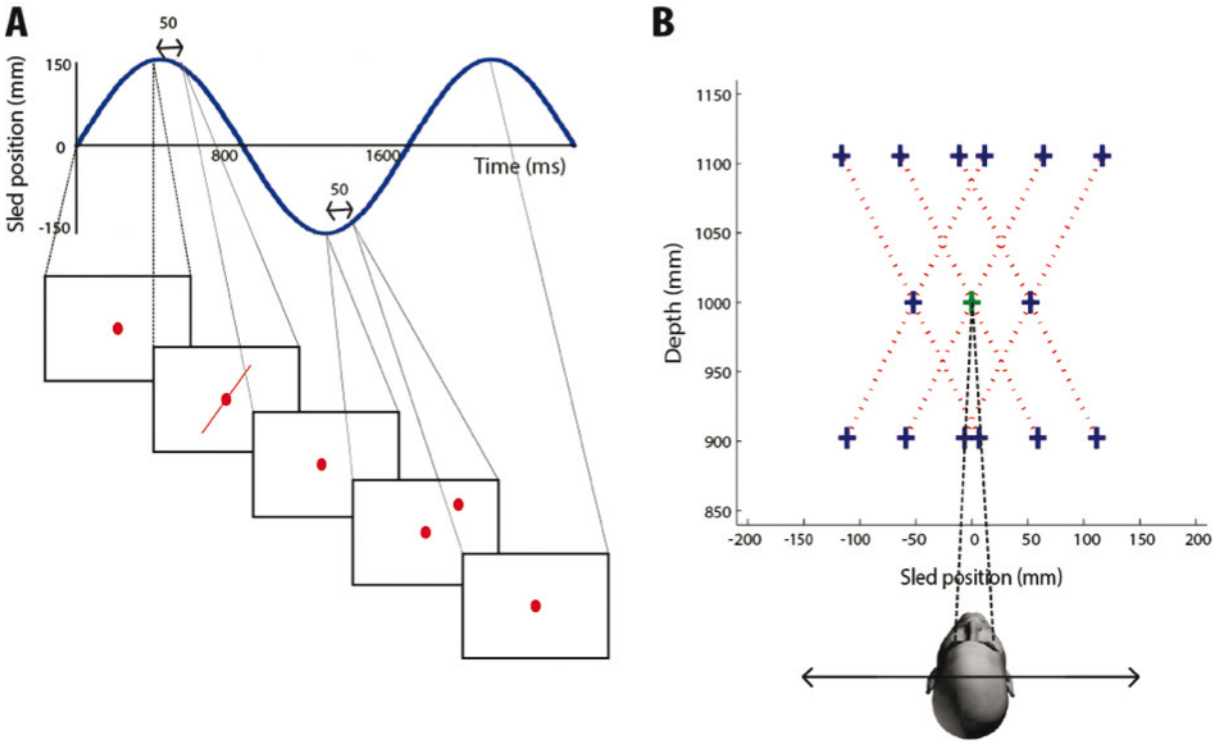

Fig. 1A. Experimental paradigm: subjects fixated at a fixation point during the whole experiment. Each trial started when the right-most sled position was reached (150 mm). A line was flashed for 50 ms from which the location had to be remembered during translation to the left-most sled position (-150 mm). Consequently, a probe was flashed for 50 ms to test subjects' spatial updating performance (left or right relative to line location). **B.** Experimental setup: subjects' updating performance was tested for 6 different lines, using 16 conditions indicated by the blue crosses. Note that the two crosses at a depth of 1000 mm are tested for both the clockwise and counter-clockwise rotated line. Lines are rotated either 30° counter-clockwise (CC) or 30° clockwise (C). The midpoint of the line can lie 52 mm left of the FP (L), at FP (M), or 52 mm right of the FP (R).The two lines rotated around x = 0 are reconstructed from two conditions only because the centre of the line (y = 1000 mm) corresponds to the fixation point.

indicate with a joystick whether the location of this probe stimulus was left or right relative to the flashed line location (2AFC task). An adaptive algorithm (Kontsevich & Tyler, 1999), based on an entropy measure, varied the location of the probe relative to the line, mapping the precision and accuracy of spatial updating. After response recording, the next trial was started when the sled position reached its position of 150 mm again. When a run was completed, sled motion stopped and the lights were turned on. A 30 s break was introduced to prevent darkness adaptation. The total experiment consisted of two equivalent sessions of 32 runs, i.e. 800 trials, tested on two different days to control for consistency of responses. Ten out of twelve participants also took part in the second session.

Subjects were tested in 16 conditions of 300 mm sinusoidal translation, each comprising a unique combination of line orientation and location (blue crosses, figure 1B). Note that the two crosses at a depth of 1000 mm are tested for both the clockwise and counter-clockwise rotated line. In the results section the orientation is denoted as CC (30° counter-clockwise rotation) or C (30° clockwise rotation) and the location as L (midpoint of line is 52 mm left of FP), M (midpoint of line is at FP) or R (midpoint of line is 52 mm right of FP). Each condition contained about 100 trials which were randomized within the experiment.

2.4 Data Analysis

Data analyses were performed offline using Matlab R2012a (Mathworks Inc, Natick, USA). For each condition, that is combination of orientation and position, the performance was calculated as the probability of a rightward choice for the probe location relative to the line location. A cumulative Gaussian was fitted to the psychometric data using the maximum likelihood method (Wichmann & Hill, 2001a):

$$P(x) = \lambda + (1 - 2\lambda)\frac{1}{\sigma\sqrt{2\pi}}\int_{-\infty}^{x} \exp^{\frac{-(y-\mu)^2}{2\sigma^2}} \quad (1)$$

in which P represents the probability of rightward responses and x the x-location of the line in space. The mean of the Gaussian μ, is the subjects' perception of the line location, whereas the width of the curve σ, inversely related to the precision, serves as a measure for the variability in responses. In our experiment, the lapse rate λ, accounting for stimulus-independent errors, was set to zero. Fits were performed using the 'psignifit' Matlab toolbox (Wichmann & Hill, 2001a).

The performance of subjects was quantified in a bias, standard deviation and orientation error measure. The bias was calculated by subtracting the original line location from the updated line location, indicated by μ, the 50% rightward choices point (positive bias meaning a bias to the right). The standard deviation σ of a subject could be derived from the psychometric fit, whereas the orientation error was computed as the difference in angle between the actual line orientation and the updated line orientation via a regression analysis.

A 2D vectorial analysis of all 16 conditions independently was performed to show whether the subjects represented the line in a gaze-centered or body-centered reference frame. We compared the Root Mean Squared Error (RMSE) of this analysis to another 2D vectorial analysis which addressed line updating as an object instead of a set of points. The point vector analysis quantified the interaction between the initial location of a particular point of the line, the translational motion, and the probe responses, whereas the 2D object vector analysis quantified the interaction between the initial location of the whole line, initial line orientation, and the probe responses (see results). Statistical tests were performed at the 0.05 alpha-level.

3. Results

For each subject, we pooled the data of the two sessions because there was no significant difference in performance between the two sessions. At each updated location of the line, the percentage of right responses is fitted with a psychometric curve (equation 1) to investigate the updating performance. Figure 2 displays the results for a single subject in the near condition, i.e. updated locations in front of the fixation point. The intersection between the 50% right response probability (dashed horizontal line) and the psychometric fit is the subjects' updated reference line location. The difference between this location and the actual reference line location, indicated by vertically dashed lines, represents the subjects' bias. The slopes of the fits are inversely related to the standard deviation of responses. As the steepness of the slope increases, the standard deviation of responding at that particular location

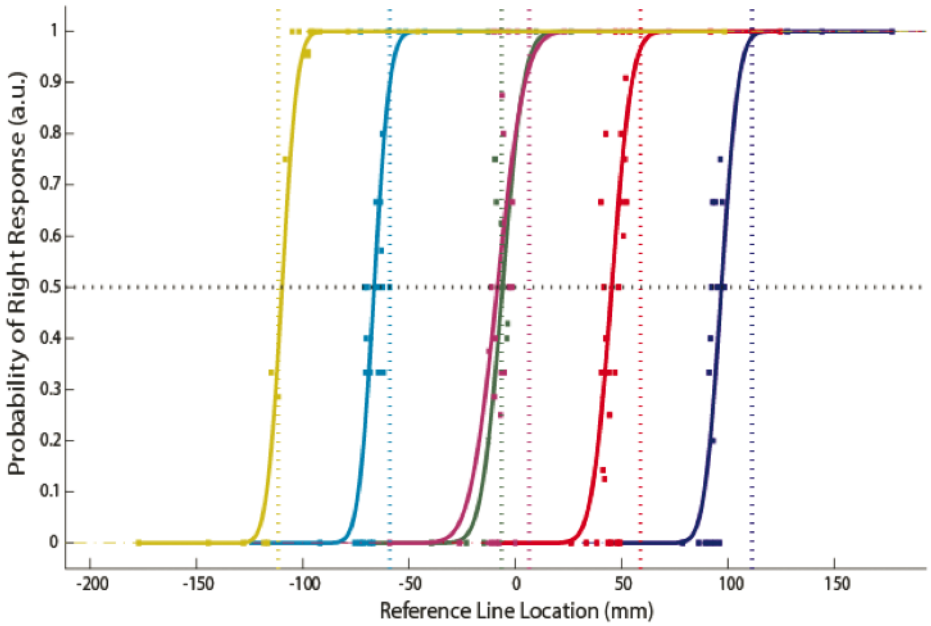

Fig. 2 Single subject's responses in the near condition plotted as a probability of right responses versus the location of the reference line. A cumulative Gaussian fit (see Materials and Methods) through the responses indicates the updated location, i.e. the 50% right response point intersection. The difference between the actual and updated location, indicated by the dashed vertical lines, is called the bias. The slopes of the psychometric fits are an indication of the variance in responses.

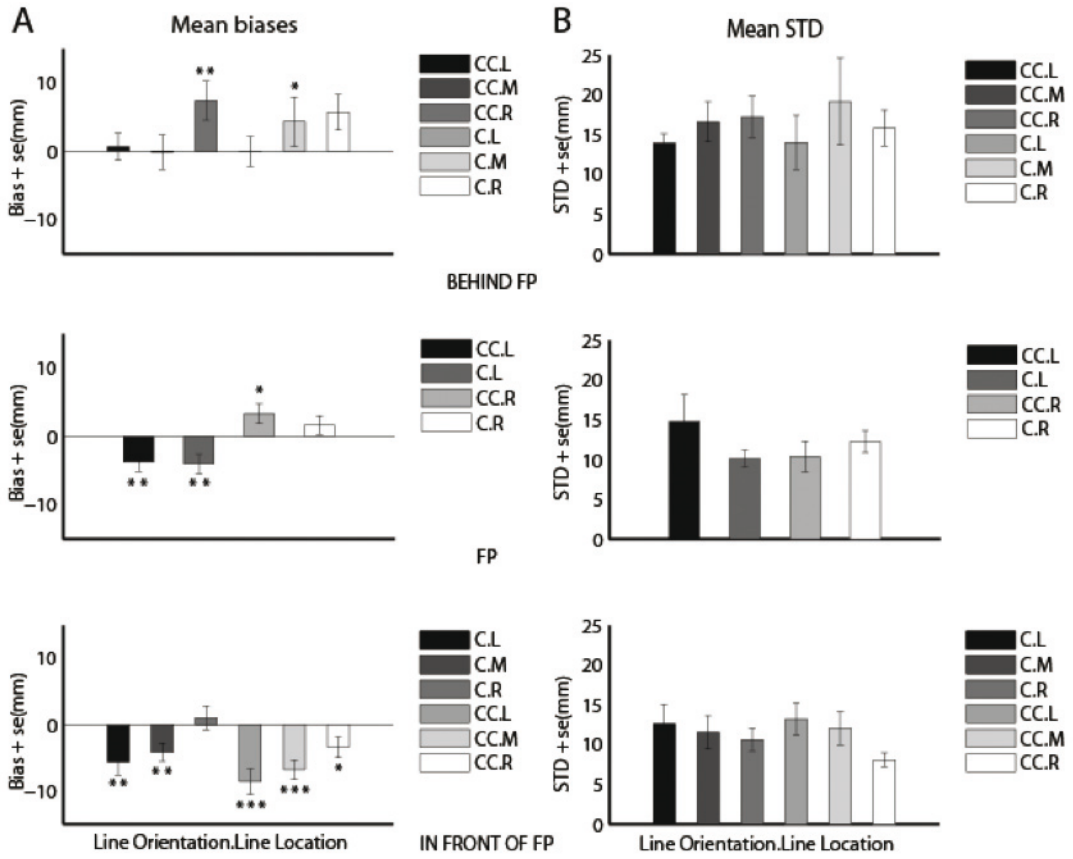

Fig. 3A. Mean biases including the standard error over all subjects ordered by their depth location from the fixation point (FP) for different line orientations (CC = 30° counterclockwise rotation, C = 30° clockwise rotation) and lateral locations (L = 52 mm left of FP, M = at FP, R = 52mm right of FP, see figure 1B). With a t-test it is tested whether the mean biases are significantly different from zero (* = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$). Biases appear to depend on the depth location from FP. **B.** Mean standard deviation (STD) including the standard error over all subjects plotted in the same way as the mean biases. Standard deviations appear to increase when moving away from the FP in depth.

becomes less.

3.1 Bias analysis

Figure 3A shows the mean biases (N = 11) for probe conditions in front (bottom panel), on the same depth plane (mid panel) or behind (top panel) the fixation point (FP). Each bar indicates a different reference line orientation (CC = 30° counter clockwise rotation, C = 30° clockwise rotation), in combination with a lateral location relative to the fixation point FP (L = 52 mm left of FP, M = at FP, R = 52 mm right of FP, see figure 1B). Negative biases indicate that subjects perceive the probe location left of its actual location. Note that five out of six probe locations in front of FP have a negative bias, which is significantly different from zero (t-test, $p < 0.05$). At the fixation plane, probe locations left of FP show significant negative biases (t-test, $p < 0.01$), whereas locations right of FP show positive biases of which one is significantly different from zero (t-test, $p < 0.05$). Two out of five probe locations behind FP show significant positive biases (t-test, $p < 0.05$). These results are in line with a gaze-centered updating model, which suggests that biases in front of and behind FP have opposite signs, i.e. depth dependent error patterns.

3.2 Standard deviation analysis

The standard deviation of a psychometric curve is an indication of how well the cumulative Gaussian fits the response data, i.e. how precisely the final updated location can be judged from the 50% right response line. It is known from previous literature that subjects response variability increases when testing further away in depth (McCann, Hayhoe, & Geisler, 2011). However, fixating at the FP might introduce a foveal bias (Osaka, 1977; O'Regan, 1984; Van der Heijden, Van der Geest, De Leeuw, Krikke, and Müsseler, 1999), which suggests that the standard deviation at the fixation plane is lower than at the two other depth locations. The mean standard deviations over all subjects (N = 11) are depicted in figure 3B in the same depth order as the mean biases. Only the standard deviations in the CC.R

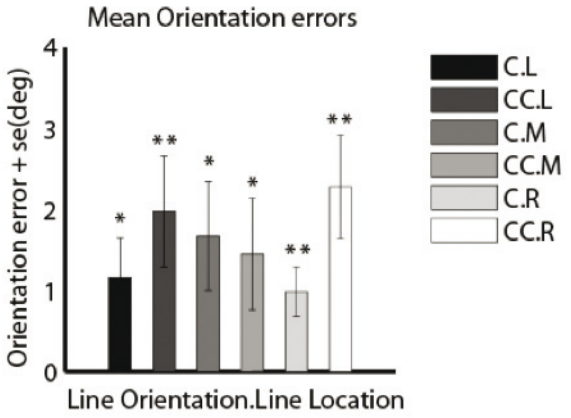

Fig. 4 Orientation angle differences between the actual line orientation and updated line orientation. Mean orientation errors in degrees, including the standard error over all subjects, for the different combinations of line orientation (CC = 30° counterclockwise rotation, C = 30° clockwise rotation) and line location (L = 52 mm left of FP, M = at FP, R = 52 mm right of FP). With a t-test it is tested whether the mean orientation errors are significantly different from zero (* = $p < 0.05$ and ** = $p < 0.01$).

and the C.R condition are significantly larger behind FP than in front of FP (t-test, $p < 0.05$). The CC.R condition behind FP is also significantly larger than at the FP plane (t-test, $p < 0.05$), but all the other conditions in front of and behind FP are not.

3.3 Orientation error

So far we only described the results of the psychometric analysis, but we haven't considered what this tells us about the representation of the line as a set of points or as an orientation measure. Therefore we also investigated the difference in orientation between the actual line orientation and the updated line orientation, constructed by a regression analysis through the three individual three probe biases corresponding to the line. Figure 4 shows the mean orientation errors over all subjects (N = 11), listed as a combination of line orientation and line location. Note that the mean orientation error for all lines presented is significantly larger than zero (t-test, $p < 0.05$). This means that at least a part of our data can be explained as an orientation shift of the line.

3.4 Vector analysis

3.4.1 Point-updating

In order to quantify the findings above, we performed a vector analysis in Cartesian coordinates and examined how much variance is explained. Object pointer theory suggests that lines can be seen as a set of independent points and biases can only occur due to a translation shift of these points. Based on this theory, we fitted both a gaze-centered and a body-centered *point-updating* model to the data. They investigate how the Cartesian vectors of the initial reference location ($\overrightarrow{R_i}$), the final reference location ($\overrightarrow{R_f}$), and the response location ($\overrightarrow{L}$) of a particular point of the line are related in a gaze-centered or body-centered reference frame (Figure 5). Figure 5A shows a top view of one trial in world-coordinates. In the gaze-centered updating model (Figure 5B), the two different views of the 2D setup (initial vs. final) are mapped on to each other, with the two fixation vectors ($\overrightarrow{F}$) overlapping. Note that the updated location can only be seen in the final position, whereas the reference point is present in both representations (actual flash and spatially updated across translation). The two vectors orthogonal to body translation are overlapping in the body-centered updating model (Figure 5C). In both models we can specify the following relationship:

in which $\overrightarrow{L} - \overrightarrow{R_i}$ represents the updated translation vector, $\overrightarrow{R_f} - \overrightarrow{R_i}$ the perfectly updated

$$\vec{L} - \vec{R}_l = \alpha(\vec{R}_f - \vec{R}_l) + C \qquad (2)$$

translation vector, fit parameter α the updating gain, and C a remaining constant, which is the magnitude of the unexplained vector.

If we assume the constant to be zero, subjects are perfect in updating the location of the reference line when $\overrightarrow{L}$ would be equivalent to $\overrightarrow{R_f}$, and hence α would be 1. If subjects do not account for the passive translation of the sled, $\overrightarrow{L}$ would be equivalent to $\overrightarrow{R_i}$, and thus α would be 0. We fitted equation 2 to our data and calculated the fit parameter α and the remaining constant. Table 1 shows for each subject the updating gain, the constant, and the Root Mean Squared Error (RMSE) in both models. A non-zero constant might suggest that other mechanisms can account for the variability in the data. Averaged across subjects, the mean updating gain in gaze-centered coordinates was 0.88 ± 0.10 (SD), which is significantly different from 1 ($F(1) = 16.9, p < 0.001$). Within subjects, seven out of eleven subjects show a significant difference between the fitted values of α and 1 (t-test, $p < 0.05$) The updating gain in body-centered coordinates was 1.00 ± 0.01(SD), which is not significantly different from 1 ($F(1) = 1.3$, $p = 0.26$). The RMSE of the gaze-centered point-updating model was 3.87 ± 0.71 mm, whereas the RMSE of the body-centered point-updating model was 5.18 ± 0.49 mm, which is significantly higher

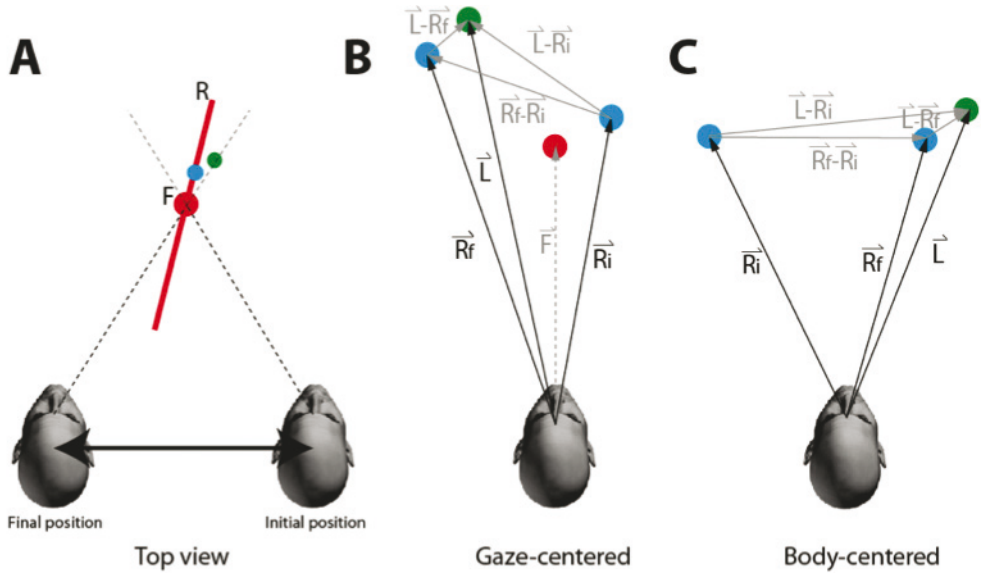

Fig. 5A. Top view of one trial characterized by a reference line (R) and a fixation point (F), to be fixated during both the initial position (150 mm) and the final position (-150 mm). Updating performance is calculated as a bias between a point on the reference line (blue dot) and the updated location of that point (L, green dot). **B.** Initial view and final view of the reference point (blue dot) and updated location (green dot) collapsed onto each other in gaze-centered coordinates. Note that the two views of the fixation point are now on top of each other. From the vector analysis we can see that $\vec{L} - \vec{R_f}$ is the bias, $\vec{L} - \vec{R_i}$ the updated translation vector and $\vec{R_f} - \vec{R_i}$ the actual translation vector. **C.** Same vector analysis as in B, but now in a body-centered reference frame. In this analysis the two body vectors (orthogonal to translation direction) are collapsed onto each other.

Table 1. Fit parameters of the gaze-centered and body-centered point-updating model.

	Gaze-centered updating model			**Body-centered updating model**		
	α	**C (mm)**	**RMSE (mm)**	**α**	**C(mm)**	**RMSE (mm)**
S1	0.72*	3.54	4.86	1.01	0.00	6.06
S2	0.97	-2.53	4.11	0.99	0.00	5.32
S3	0.75*	-0.69	4.38	1.00	0.00	4.70
S4	1.02	-3.30	5.01	0.99	0.00	5.75
S5	0.98	-3.09	4.15	0.99	0.00	5.61
S6	0.84*	0.08	3.15	1.00	0.00	4.69
S7	0.97	-1.45	4.14	1.00	0.00	4.89
S8	0.88*	-0.84	3.12	1.00	0.00	4.74
S9	0.81*	1.70	3.39	1.01	0.00	5.05
S10	0.83*	-2.99	3.19	0.99	0.00	5.49
S11	0.87*	-0.13	3.09	1.00	0.00	4.68
Mean ± SD	0.876 ± 0.099*	-0.88 ± 2.14	3.87 ± 0.71	1.00 ± 0.01	0.00	5.18 ± 0.49

Best fit values of α, and **C** refer to the updating gain, and the remaining constant. RMSE refers to the Root Mean Squared Error of the model. * Indicates that the updating gain value is significantly different from 1 (p<0.05).

($F(1) = 25.0$, $p < 0.001$). Both the updating gain fits as well as the RMSE suggest a gaze-centered updating model assuming an underestimation of translation as a source for the biases in perception of the updated reference line.

3.4.2 Line updating

Although the updating gain in our gaze-centered point-updating model is significantly smaller than one, the RMSE of the model is still high if we compare this to the magnitude of the biases. This might be an indication that a line has more relevant information than a single point target, which the brain can use to make a correct spatial update. Therefore we fitted a gaze-centered line updating model that takes an orientation shift of the initially flashed line $\overrightarrow{L_i}$ into account (Figure 6). From this figure we can specify the following line relationship:

$$\overrightarrow{L_{upd}} - \overrightarrow{L_i} = \overline{\overline{R}}\overrightarrow{L_i} + C$$
$$\overline{\overline{R}} = \begin{bmatrix} \cos\alpha\frac{T}{d} & -\sin\alpha\frac{T}{d} \\ \sin\alpha\frac{T}{d} & \cos\alpha\frac{T}{d} \end{bmatrix} \quad (3)$$

in which $\overrightarrow{L_{upd}} - \overrightarrow{L_i}$ represents the updated line across translation, and $\overline{\overline{R}}\overrightarrow{L_i}$ an orientation shift of the initially flashed line. The translation contribution is computed as a translation gain α in the 2D rotation matrix R. If we once again assume the remaining constant to be zero, a perfect update of $\overrightarrow{L_i}$ will be achieved when the updating gain α is 1. Table 2 shows for each subject the individual values of α, including the root mean squared error (RMSE). Averaged across subjects, the updating gain of the gaze-centered line-updating model was 0.92 ± 0.06 with a RMSE of 6.62 ± 3.18 mm, which is significantly different from 1 ($F(1) = 20.5$, $p < 0.001$), suggesting that an orientation shift of the initial reference line is another explanation of the perceived biases. Within subjects the same seven subjects show significant parameter values (t-test, $p < 0.05$).

3.4.3 Comparing the different gaze-centered models

Because both the gaze-centered point-updating and line-updating model estimate one free parameter (see equation 2 and 3), the degrees of freedom of both models will be the same. This means that we can compare the Root Mean Squared Errors directly without any model-based adjustments. Figure 7 plots

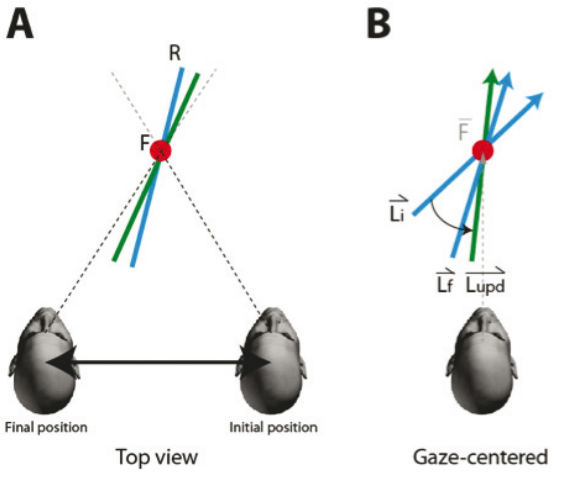

Fig. 6A. Top view of one trial characterized by a reference line (R) and a fixation point (F), to be fixated during both the initial position (150 mm) and the final position (-150mm). Updating performance is calculated as an orientation bias between the initial reference line (blue line) and the updated line (green line). **B.** Initial view and final view of the reference line (blue lines) and updated line (green line) collapsed onto each other in gaze-centered coordinates.

Table 2. Fit parameters of the gaze-centered line-updating model

Gaze-centered updating model		
	α	RMSE (mm)
S1	0.82*	13.40
S2	0.98	4.73
S3	0.84*	6.87
S4	1.00	6.95
S5	0.98	6.31
S6	0.90*	4.46
S7	0.98	3.73
S8	0.92*	6.04
S9	0.88*	11.79
S10	0.89*	4.82
S11	0.91*	3.76
Mean ± SD	0.916 ± 0.06	6.62 ± 3.18

Best fit values of α refers to the orientation updating gain. RMSE refers to the Root Mean Squared Error of the model. * Indicates that the updating gain value is significantly different from 1 ($p<0.05$).

the RMSE of the point-updating model against the line-updating model. The red unity line indicates that both models explain the variance in the data equally well. The data shows that the point-updating model explains the variance in the data significantly ($F(1) = 7.82$, $p < 0.05$) better than the line-updating model, suggesting point-updating as the mechanism the brain uses to spatially update objects.

4. Discussion

This study investigated how accurately subjects spatially update an internal representation of a line during combined eye and body motion. Subjects reported the location of a probe relative to an updated reference line during 300 mm lateral translation. We showed that subjects appear to have depth-dependent biases; left of the actual line in front of FP, and right of the actual line behind FP.

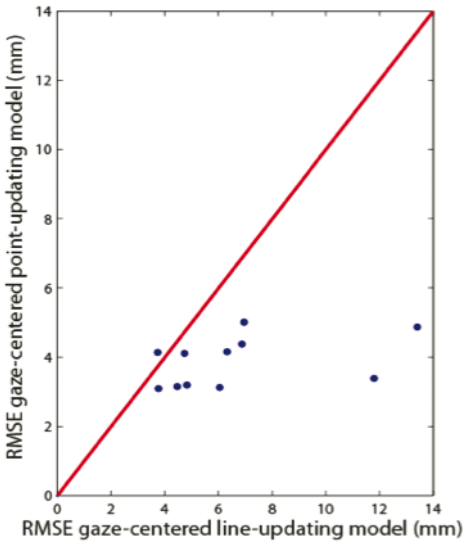

Fig. 7 RMSE of the point-updating model plotted against the RMSE of the line-updating model for each individual subject. Red unity line indicates that both models explain variance equally well.

The updating process could be best described by a gaze-centered point-updating model. This is in line with recent behavioural studies that suggest that the spatial update of an object for reaching movements takes place in a gaze-centered reference frame for both intervening eye and active body movements (Henriques, Klier, Smith, Lowy, & Crawford, 1998; Selen & Medendorp, 2011; Van Pelt & Medendorp, 2007; Van Pelt & Medendorp, 2008).

The reported updating gain of 0.876 suggests an underestimation of translation amplitude as a source for biases in perception. This underestimation is caused by the extra-retinal signals coding the motion trajectory. It is however unknown whether this extra-retinal signal is of vestibular origin (Dokka, MacNeilage, DeAngelis, and Angelaki, 2011; otoliths, which detect the heads' linear acceleration (Angelaki, 1992; Angelaki, 1998), based on visually derived eye position signals (optokinetic reflex (OKR); a visual subsystem for motion detection based on optic flow; Lappe, Bremmer, & van den Berg, 1999; Murakami & Cavanagh, 1998), or derived from smooth eye pursuit signals (Nadler, Nawrot, Angelaki, & DeAngelis, 2009). The brain faces the problem of combining all the different sensory signals into a correct representation of motion. Solving this problem is complex because during translation objects' depth and direction of movement are ambiguous from optic flow, as in motion parallax (Rogers & Graham, 1979; Rogers & Rogers, 1992). Nadler, Angelaki, & DeAngelis (2008) for example showed that during leftward translations subjects' retinal input of an object in front of the fixation plane is the same as during rightward translations for objects behind fixation plane.

In theory, the vestibular signal would be the most likely explanation, but Angelaki (1998) showed that otolith responses are veridical for frequencies higher than 0.5 Hz. Since we used a sinusoidal translation frequency of 0.67 Hz, this suggests that vestibular input only is not the cause of the underestimation. However, some subjects reported that they saw the world-fixed FP moving along with them. This perception effect is extensively described by Dyde and Harris (2008). They show that world-fixed targets need to be moved in the direction of self-motion in order to be perceived as world-fixed. When subjects are seated in a dark room and when they are translated passively, this perception effect is even enhanced. Both factors are present in this study, so both errors in perception of self-motion and visual motion of the world-fixed FP can lead to underestimation of translation.

The error patterns of Selen and Medendorp (2011) and Van Pelt and Medendorp (2007) show an updating gain greater than 1, i.e. an overestimation of translation. However, both studies used active eye and whole-body translations in their paradigm, suggesting mechanisms like an efference copy of the motor command to be involved as well. So far, the only study known for using passive body translation during a spatial updating task, found a perfect spatial update of saccade amplitude to remembered locations (Li & Angelaki, 2005). However, their paradigm is based on spatially updating targets along the vergence direction, whereas our paradigm targets along the version direction and therefore we cannot compare the results.

In our lab we used the same experimental paradigm to address the updating of single point targets as Clemens, Selen, Koppen and Medendorp (2012). The updating gain of their experiment show an even larger underestimation of translation, i.e. $\alpha = 0.2$. This is in agreement with our original hypothesis that the updating of single point targets shows

larger updating errors than the updating of visual lines. A possible explanation for the big difference in updating gain with our study is that subjects are asked to define whether the probe location was left or right of the reference point target. The question is how subjects define this distinction in an accelerating environment. Do they simply make an internal division of the field orthogonal to motion direction or to gaze direction or a completely different structure? In our experiment this decision is not ambiguous because the line clearly divides the plane, but in single point target studies like Clemens et al. the decision is ambiguous. This might be an indication why subjects perform so badly in this spatial updating task. Another explanation might be that the single point targets are attracted towards the fixation point, more commonly known as a foveal bias.

Besides point updating, we also fitted a line-updating model to explain the biases as an underestimation of orientation angle. We hypothesized that a line would carry more information for spatial updating than single point targets, but we found the opposite; RMSE's were larger for the line-updating model. A possible explanation is that we only included an orientation gain in order to be able to compare the two models (both have same degrees of freedom), but it is more realistic to fit both an orientation and translation gain because 2D objects can be shifted and rotated. Selen and Medendorp (2011) investigated error contributions of orientation and translation seperately, and showed that they both contributed to the updating error. In addition they showed that the errors were correlated in a way that argued for seperate spatial representations in the brain. Although these observations were based on grasp and reach errors respectively, it poses interesting insights for this experiment. Orientation of the line is coded more ventral than line location (Colby, Duhamel, & Goldberg, 1993), which implies that the two seperate visual streams (what – where; Mishkin and Ungerleider, 1982) need to be integrated to come to a veridical update. In our experiment, the perceived biases can be constructed at each level, i.e. the separate representations, the integration process, integration of motion etc.

Finally, we quantified between subjects differences and flaws of the experiment. From eleven participants, four show perfect updating in both the gaze-centered and the body-centered reference frame. Therefore we analysed the data of the separate sessions on darkness or vestibular adaptation by comparing perceptual biases calculated from the first 13 trials of each run versus the last 13 trials of the same run. For all subjects, there was no significant difference between the two of them. In the future it would also be interesting to look at the response strategies of these subjects, using for example Recurrence Quantification Analysis (RQA).

A flaw of the experimental paradigm is that we do not know to which point on the reference line we should compare the calculated updated location. In this experiment we assumed it to be the location of the line which lies on the same depth as the presented probe locations. However, it could also be the location of the line which is closest to the calculated updated location, more commonly known as the orthogonal equivalent. Calculating the updating gains of the vector analysis, when comparing it to the orthogonal location of the reference line, did not show a significant difference. This does still not prove that the location we assumed is the actual location the brain uses for creating the update. A solution would be to test a few subjects in the same experimental paradigm but randomly flashing a reference line or a reference dot somewhere on that line using the same probe location. Next, the bias of the reference line trials is calculated. This bias is then compared to the biases of the different dot trials. The bias of the dot trials that corresponds to the bias of the line trial is most likely the location of the line to which the calculated updated location is compared. This due to the fact that the biases correspond. However, it might also be compared to a more holistic representation like orientation as we discussed above.

Taken together, the results of the experiment lead to the conclusion that the brain uses a gaze-centered reference frame to update internal line representations. Whether the line is updated as a set of independent point targets or as a whole line requires further analysis.

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Congruency With Prior Knowledge Affects Memory Formation

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Information that is congruent with prior knowledge has been shown to be remembered better and consolidated faster than incongruent information, which has been attributed to the influence of activated schemata on memory processing. Here, we employed a manipulation of subjective congruency of to-be-learned information with prior knowledge over three levels, to probe the neural correlates of memory encoding in an associative memory study using functional magnetic resonance imaging. Behaviourally, we found better memory performance for increasingly congruent associations, but more confident retrieval of increasingly incongruent associations. Our imaging data show that medial prefrontal cortex (mPFC) contributed more to successful encoding as subjective congruency of new information with prior knowledge increased, while the left medial temporal lobe (MTL) exhibited an inverse relationship with congruency, i.e. left MTL contributed more to successful encoding the more incongruent information was with prior knowledge. This became evident both in brain activation and functional connectivity patterns of mPFC and MTL. Our results support predictions made by a framework integrating schema and memory research, which proposes that two distinct neural mechanisms interact in memory processing, with their interaction governed by the extent to which processed information is congruent with activated schemata. Our findings corroborate the notion that encoding of congruent information is mediated by the mPFC, which integrates a memory into a pre-existing schema, while encoding of incongruent information relies on the MTL, which acts to capture novel experiences. Thus, this study provides new evidence in favour of a novel account of schema theory, extending previous results on the effect of activated schemata on the neural correlates of memory formation.

Keywords: schema, congruency, memory encoding, medial temporal lobe, medial prefrontal cortex

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1. Introduction

Efficient memory formation plays a pivotal role in adaptive behaviour, providing the ability to transfer knowledge gained at present to events remote in time and space. That prior knowledge can facilitate the encoding of related new information has long been reported behaviourally (Bartlett, 1932; Anderson, 1981; Ericsson, 1985; Bransford, Brown, & Cocking, 2000; de Witt, Knight, Hicks, & Ball, 2012). This has been attributed to changes in memory processing due to the activation of schemata (van Kesteren, Ruiter, Fernández, & Henson, 2012a), i.e. frameworks of acquired knowledge, implemented within a network of connected neurons, in which memory traces of associated information have been stored that, when activated, can alter the manner in which information is processed. When perceived information is related to a schema, this schema gets activated and gains the capacity to affect information processing, which encompasses the processes of memory encoding, consolidation, and retrieval. However, how this retention benefit for schema-consistent information arises remains an open question, which requires further investigation of the underlying neural correlates for construction of a mechanistic account.

The medial temporal lobe (MTL) has been recognized to be crucial for declarative memory encoding (Scoville & Milner, 1957; Squire, Stark, & Clark, 2004). It is commonly assumed to bind distributed cortical representations of an experience's various features, thus forming a new memory trace whose retrieval depends on the integrity of hippocampal-cortical connections (Squire, 1992; Frankland & Bontempi, 2005). Over time, cortico-cortical connections are assumed to be strengthened during systems consolidation, enabling the retrieval of remote memories without hippocampal contribution (Squire & Alvarez, 1995; Frankland & Bontempi 2005; but see also Nadel & Moscovitch 1997; Nadel, Samsonovich, Ryan, & Moscovitch, 2000). The temporally restricted involvement of the hippocampus in memory retrieval becomes strikingly evident in the temporally graded retrograde amnesia caused by bilateral MTL lesions (Scoville & Milner, 1957; Bayley, Hopkins, & Squire, 2006). For consolidated memories, medial prefrontal cortex (mPFC) has been shown to contribute increasingly to retrieval (Frankland & Bontempi 2005; Takashima et al., 2006; Gais et al., 2007; Takehara-Nishiuchi & McNaughton, 2008), and it has been suggested to take over MTL's pointer function, which is crucial for retrieving all associations comprising an experience, with consolidation (Frankland & Bontempi, 2006). An alternative account connects mPFC involvement during remote retrieval to the detection of a cue's relation to neocortically stored associations, i.e. its congruency with prior knowledge, without assigning a causal role in retrieval to mPFC (van Kesteren et al., 2012a).

Recent studies in rodents and humans have begun to probe the neural underpinnings of schema-effects, delineating specific features in the processing of schema-congruent information. Tse and colleagues (2007) have shown in rodents that memory for information congruent with a spatial schema rapidly became independent of the hippocampus. In humans, it has been shown that retrieval of schema-congruent information is associated with increased mPFC activity as well as functional connectivity of mPFC to relevant sensory representation areas (van Kesteren, Rijpkema, Ruiter, & Fernández, 2010b). Thus, these two studies demonstrated a relation between activated schemata and rapid shifting of retrieval contribution away from hippocampus and towards mPFC. Wang & Morris (2010) conceptualized this as schema-mediated acceleration of systems consolidation, attributing a causal role to activated schemata in the unusually fast assimilation of schema-congruent new information into neocortical networks.

However, schemata also affect memory already at initial acquisition: During encoding of schema-congruent information, increased brain activity in mPFC has been observed in rodents (Tse et al., 2011) and humans (van Kesteren, Rijpkema, Ruiter, Morris, & Fernández, 2012b), and encoding of schema-congruent information has been shown to require weaker mPFC-hippocampus coupling, indicating facilitated assimilation of schema-congruent information into neocortical networks (van Kesteren, Fernández, Norris, & Hermans, 2010a).

To integrate these results demonstrating effects of activated schemata on memory processing, van Kesteren and colleagues (2012a) proposed a framework termed 'schema-linked interactions between medial prefrontal and medial temporal regions' (SLIMM). It posits that two distinct encoding mechanisms, closely linked to mPFC and MTL, interact during the encoding of new information, with the contribution of each mechanism governed by the extent to which to-be-learned information is congruent with activated schemata. According to this model, if new information is congruent with an activated schema, mPFC facilitates the

selective integration of this information into neocortical networks. To prevent twofold encoding of the information, mPFC is hypothesized to inhibit further encoding via MTL-dependent processes. If, however, to-be-learned information is not congruent with any activated schema, its encoding is suggested to depend on the MTL, which is in line with the role attributed to the MTL in automatically capturing any consciously apprehended information (Moscovitch, 2008). Intermediately congruent information is hypothesized to activate both encoding mechanisms to some extent, leading to suboptimal memory encoding due to diminished efficiency of either system.

Previous studies suggest that new information's congruency with prior knowledge affects the way the information is processed. In this study, we tested predictions made by SLIMM about contribution of mPFC and MTL to encoding of differentially schema-congruent associative information. To this end, we employed a manipulation of subjective schema-congruency of paired associations learned during functional magnetic resonance imaging (fMRI).

2. Material and Methods

2.1 General procedure

We conducted an associative memory experiment, in which participants learned paired associations of 185 pairs of colour photographs in the MR scanner on day 1, and were tested on their memory for these associations on day 2 (Fig. 1). On day 1, participants were taken into the MR scanner, where we first ran a functional localizer experiment that took about 10 minutes. Participants were then given a short practice session for the encoding task, to ensure that they were familiar with the procedure. Subsequently, we ran the study phase of the memory experiment, during which participants encoded the paired associations. After the encoding task, which took about 25 minutes, an anatomical scan was acquired, which took 7 minutes. Participants were then taken out of the MR scanner and sent home. They returned on day 2 at about the same time for a memory test, which was administered outside the MR scanner.

2.2 Participants

Thirty-two native Dutch right-handed students participated in this experiment. All participants were healthy and had normal or corrected-to-normal vision. They gave written informed consent and were paid for their participation. One participant was excluded from all analyses due to technical failure during the encoding task on day 1, three participants were excluded due to technical failure during the retrieval test on day 2. Two further participants were excluded, because their behavioural performance did not yield a minimum of 9 trials for all categories included into contrasts of interest in the fMRI data analyses. Therefore, the data of these six participants were discarded and all analyses were performed on data of the remaining 26 participants (four male, age 18 - 27 years, mean 21.5 years). On average, participants reported to have slept 7.6 hours the night in between study days (range 6.5 - 9 hours).

2.3 Stimuli

Participants performed an associative memory task, where they learned paired associations of 185 pairs of colour photographs, of which one picture depicted an isolated object and the other a scene. We used both indoor- and outdoor-scenes which were easily recognizable and commonly associated with particular objects (e.g. classroom, tennis court). Pairs were predefined, such that object-scene associations would cover the whole range of the congruency scale. Pairings were unique, i.e. each picture was shown in only one pair. 10% of the pairs were constructed to be very incongruent, while another 10% were constructed to be very congruent (e.g. tennis court - soup ladle and classroom - chalk). To counterbalance the design across participants, for half of the participants, the objects that were paired very congruently before were re-paired within their subset to give very incongruent combinations, while the objects that were paired very incongruently for the first half of participants were re-paired within their subset to give very congruent combinations. Thus, 20% of the objects and scenes were shown in either a very congruent or a very incongruent pairing, counterbalanced across participants, to control for biases resulting from our construction of the pairs.

Prior to conducting the associative memory experiment in the MR scanner, we performed behavioural pilots in a separate group of participants (N=26). In these, we verified that the congruency of the constructed pairs fit the intended distribution across the congruency scale. During piloting we also confirmed that the number of trials was appropriate to attain behavioural performance above chance level.

2.4 Associative memory experiment

The experiment was executed on two consecutive days (Fig. 1). On day 1, participants learned paired associations between 185 objects and scenes while brain activation was measured using fMRI. Participants lay in the MR scanner supine and viewed the screen through a mirror mounted on the head coil; they gave responses on a button box using their right index and middle finger. We used Presentation 14.9 (NeuroBehavioural Systems Inc., Albany, CA, USA) to present the stimuli. Participants were instructed to remember the pairs for a test 24 hours later, but were not told what aspects they would be tested on. During the encoding task, the object- and the scene-picture making up a pair were presented simultaneously and next to each other on a grey background for 3.5 seconds, followed by an intertrial interval of 2-6 seconds duration (jittered in steps of 1 second), during which a black fixation cross on grey background was shown. All items (objects and scenes) were presented on two screen locations left and right off centre. The side of the object and scene on screen (left or right) was randomized across trials. Additionally, 12 baseline periods of 10 seconds length were interspersed evenly, during which a black fixation cross on grey background was shown. Due to technical problems, all durations were scaled by a factor of 1.5 for one participant. Because her results did not deviate, we included her data in the analysis.

During pair presentation, participants had to judge the congruency of the pair by moving a red bar acting as cursor on a visual analogue scale (which was in fact a discrete scale with 33 parts that were not discernible for the participants), labelled on one end as 'does not fit', in the middle as 'possible' and on the other end as 'fits very well' (Fig. 1). The orientation of the scale ('well <> not well' or 'not well <> well') was counterbalanced across participants.

On day 2, approximately 24 hours later (mean 24 hours, standard deviation (*SD*) 1 hour), participants returned for the memory test (Fig. 1). On a computer screen, they were shown 300 object pictures, the 185 from day 1 as well as 115 new ones that served as lures, and had to decide whether they had seen them the previous day by moving a red bar on a visual analogue scale (which was in fact a discrete scale with 33 parts that were not discernible for the participants), labelled on one end as 'surely new', in the middle as 'don't know', and on the other end as 'surely old'. For items judged as old, participants had to indicate which scene was associated to it, choosing from three one-word descriptions. We used verbal descriptions instead of the original scene pictures to eliminate recognition from idiosyncratic perceptual features of the photographs, instead testing more categorical memory. Next to the correct answer, two other scenes from the studied set were provided in the multiple choice question. To avoid the possibility of identifying the correct answer by solely remembering that the object had been shown with a congruent or incongruent scene, multiple choice options were manually arranged to have, additional to the correct answer, both one congruent and one incongruent incorrect option. All scenes were distributed to appear equally often as an option. Lastly, participants had to indicate how confident they were of their answer (3 choices: 'guess', 'not sure', and 'very sure'). Each response had to be given within 6 seconds.

2.5 Localizer experiment

A functional localizer experiment for objects and for scenes was conducted before the encoding task. Participants were shown colour photographs of objects, natural scenes, scrambled objects and scrambled scenes in a blocked design. The photographs used in the localizer experiment were different from the ones used in the associative memory experiment. Participants saw the same set of 24 pictures for each stimulus type five times, equalling 20 blocks with a total of 480 stimulus presentations. Blocks were presented in the same pseudo-random order for each participant. Each picture was presented for 0.7 seconds, followed by an intertrial interval of 0.4 seconds duration, during which a grey background was shown. During the localizer experiment, participants performed a 1-back task. They were instructed to press a button if the same picture appeared twice consecutively. Participants were told that they would not have to remember the pictures shown during the localizer task.

2.6 Behavioural analyses

According to the congruency ratings given on day 1, study phase trials were grouped into three congruency levels individually for each participant. The third of trials that was rated as worst fitting was labelled 'incongruent', while the third that was rated best fitting was labelled 'congruent'. All other trials were labelled 'intermediate'. Trials in which no congruency rating was given, i.e. the red bar was

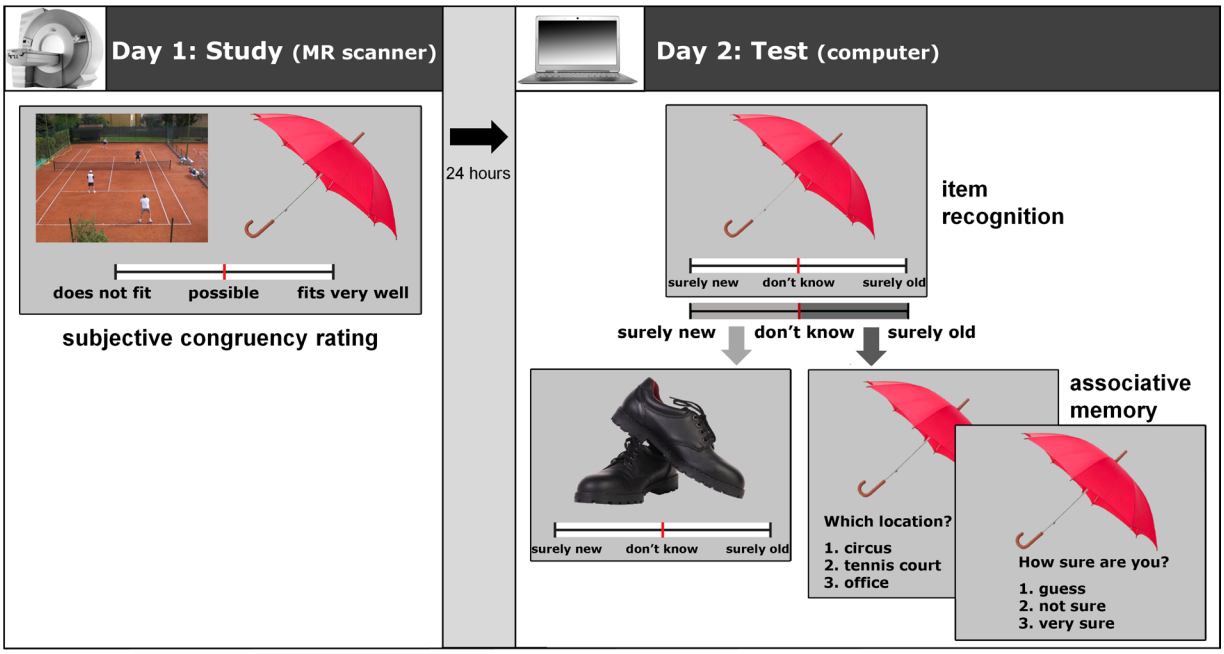

Fig. 1 Experimental design of associative memory experiment. The experiment was conducted on two consecutive days. On day 1, participants encoded a series of consecutively presented pairs of photographs in the MR scanner. They were shown pairs of one object and one scene each and asked to indicate how well they thought the two pictures matched by moving the red bar on a congruency scale. We used these ratings to assign trials to congruency levels according to the pairs' subjective congruency with the participants' prior knowledge. Each pair was shown for 3.5 seconds, followed by a jittered intertrial interval of 2-6 seconds duration. Participants returned 24 hours later to be tested on their memory for the pairs. This test was performed outside the MR scanner. Participants were shown old and new object pictures and had to indicate whether they had seen the object the previous day by moving the red bar on a confidence scale (item recognition). For objects judged as 'old', they had to choose one of three one-word descriptions of scenes (associative memory). Subsequently, they were asked to rate their confidence in the decision ('guess', 'not sure', 'very sure'). Participants had to answer each question within 6 seconds.

not moved, were excluded from the analysis. To capture timing differences between trials, we defined two measures of reaction time. First, we used the time it took participants to decide how congruent they thought a particular pair was (decision time), reflected in the time it took them to start giving a response, i.e. the duration from the onset of pair presentation until initiation of the answer. Second, we calculated the duration of the actual response (response time), i.e. the duration from the first until the last button press within a trial. The time it took to give the response was necessarily connected to a pair's congruency, as the red bar was positioned in the middle of the scale at the beginning of each trial and had to be moved further towards the ends of the scale the more incongruent or congruent a pair was judged.

In the memory test, all responses on the object recognition question from scale part 1 to 15 were classified as 'new' answers, while all responses from scale part 19 to 33 were classified as 'old' answers. All trials scoring answers in between, i.e. scale parts 16 to 18, were discarded from the analysis, to exclude trials in which no choice had been made, i.e. the red bar had not been moved, as well as the adjacent very unsure decisions. Item recognition performance (d-prime) for each congruency level was then calculated as the z-transformed proportion of objects in each congruency bin included in the analysis that were correctly identified minus the z-transformed proportion of false alarms. Subsequently, associative memory performance was calculated as the proportion of correctly recognized objects for which the scene was correctly identified.

To analyze the behavioural measures we used Predictive Analytics SoftWare (PASW) Statistics 18 (SPSS Inc., Chicago, IL, USA). We performed Student's t-tests to assess whether memory scores differed from chance level (one-sample t-tests against 50% for item recognition accuracy and 33% for associative memory performance) and whether memory scores, reaction times, beta weights (see below), and confidence of associative memory differed between congruency levels (paired-sample t-tests). For this purpose, the verbal confidence ratings of associative memory answers were transformed to an ordinal representation by scoring 'guess' as 1 point, 'not sure' as 2 points, and

'very sure' as 3 points. To assess whether scores changed consistently across congruency levels, we ran repeated-measures analyses of variance (textscANOVAs), testing for linear trends in the data. All measures were considered significant at a threshold of alpha=.05.

2.7 fMRI data acquisition

Participants were scanned using a 1.5 Tesla Siemens Magnetron Avanto system equipped with a 32 channel phased array head coil (Siemens AG, Erlangen, Germany). For blood-oxygen level dependent (BOLD) fMRI images, we used a T2* weighted gradient echo multi-echo Echo Planar Imaging (EPI) sequence (Poser, Versluis, Hoogduin, & Norris, 2006) with the following parameters: repetition time (TR)=2.64 sec, echo time (TE)1=6.9 ms, TE2=24.2 ms, TE3=33 ms, TE4=43 ms, TE5=52 ms, 34 slices, ascending slice order, 3mm slice thickness, 0.51 mm slice gap, matrix size=64*64, field of view (FOV)=224 x 224 x 199 mm, flip angle=80textdegree , voxel size=3.5 x 3.5 x 3.0 mm. Slices were angulated in an oblique axial manner to reach whole brain coverage. To allow T1 saturation to reach equilibrium, the first 3 volumes were discarded. Additionally, T1 weighted anatomical scans at 1 mm isotropic resolution were acquired using an Magnetization Preparaed Rapid Gradient Echo (MPRAGE) scan with TR=2250 ms, inversion time (TI)=850 ms, flip angle=15 textdegree and FOV=350 x 263 x 250 mm.

2.8 fMRI data preprocessing

For both encoding task and localizer experiment, raw multi-echo fMRI data were first processed using in-house software written in Matlab 7.5 (The Mathworks, Inc., Natick, MA, USA), which used 32 separately acquired weighting scans to calculate the optimal echo time for each voxel. Motion correction was performed on the first echo by using iterative rigid body realignment to minimize the residual sum of squares between the first and all further functional images. Then the calculations of optimal echo time for each voxel were used to combine multi-echo fMRI data into single-echo images. The combined images were further processed using Statistical Paramatric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm). Functional images were realigned to a mean functional image, and coregistered to the corresponding individual anatomical scan by using mutual information optimization. These images were subsequently spatially normalized and transformed to a common space, as defined by the SPM8 Montreal Neurological Institute (MNI) T1 template, as well as spatially smoothed by convolving them with an 8 mm Full-Width Half Maximum (FWHM) 3D kernel.

2.9 fMRI data analysis

2.9.1 Localizer experiment

The blocked design of the localizer experiment yielded four conditions (objects, scenes, scrambled objects, and scrambled scenes), which we modelled by convolving the block durations with a canonical haemodynamic response function. In a general linear model (GLM), we included these four conditions and six motion parameters. We entered these statistical maps into a full factorial second level analysis (type x scrambling), and then calculated object (objects - scrambled objects) and scene (scenes - scrambled scenes) contrasts. Statistical maps for the localizer experiment were analyzed at a threshold of family-wise error corrected p-value (p_{FWE})=.05.

2.9.2 Encoding task

According to the behavioural outcome from the retrieval test, we classified study phase trials as item misses (object not recognized), associative misses (object recognized but associated scene incorrect) and associative hits (item recognized and associated scene correct) for each of the three congruency levels. For each level, trial categories were modelled by convolving a boxcar function of pair presentation duration with a canonical haemodynamic response function. In a GLM, we included these nine trial types, the baseline period and six motion parameters. Additionally, we included decision time (the duration from the onset of the presentation of a new pair until the first button press within a trial) and response time (time from first button press until last button release within a trial) as parametric modulators for all trials, and confidence of associative hits (1, 2, or 3 points) as parametric modulators for associative hits only, yielding a design matrix with 38 regressors.

If not indicated otherwise, brain activation was analyzed at a threshold of p=.005 (uncorrected (unc.)) and considered significant at cluster-level corrected p_{FWE}=.05. For region of interest (ROI) analyses, we adopted a small volume corrected (SVC) threshold of p_{FWE}=.05 (at peak-level). All brain coordinates are given in MNI space.

2.9.2.1 Activity analysis

We calculated a subsequent memory contrast from the difference between associative hits and both item and associative misses (subsequent hits - subsequent misses), separately for each congruency level, on the single subject level. To test for a congruency-unspecific subsequent memory effect on the second level, we entered all resulting statistical maps into a 1-sample t-test and analyzed the results at a threshold of p=.001 (unc.). For ROI analyses we used an anatomical mask of the MTL, comprising bilateral hippocampi and parahippocampal gyri, constructed from the Anatomical Automatic Labeling (AAL) template as implemented in SPM8 (Tzourio-Mazoyer et al., 2002).

To investigate which brain regions showed an interaction between subsequent memory and congruency, we entered the statistical maps for the subsequent memory contrast, grouped by congruency level, into multiple regression analyses. We tested for a linear effect of congruency, either in the direction of congruency, i.e. linearly increasing from incongruent over intermediate to congruent, or in the direction of incongruency, i.e. linearly decreasing with increasing congruency level. To control for inter-individual associative memory performance differences, we entered individual performance as a covariate into all regression analyses. Associative memory performance was normalized to the mean performance within each congruency level for this purpose. Results of the multiple regression analysis in the direction of incongruency were analyzed at a threshold of p=.001 (unc.).

Beta weights of brain activation were extracted using in-house software written in Matlab 7.11 (The Mathworks, Inc., Natick, MA, USA) making use of the SPM toolbox MarsBaR (Brett, Anton, Valabregue, & Poline, 2002). It extracted a mean beta weight for each condition in the design matrix; these were then combined according to the contrasts of interest. Post-hoc tests on the beta weights for the subsequent memory contrast were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) (see above).

Small volume corrections in the activity analyses were performed using ROIs derived from van Kesteren and colleagues (2012b), who have shown that activity in mPFC and bilateral MTL correlates with successful encoding of schema-congruent and -incongruent information, respectively. Accordingly, we constructed an 8 mm sphere around their reported peak voxel in mPFC ([2,46,0]), as well as the overlap of 20 mm spheres around their reported peak voxels in bilateral MTL ([-28,-18,-28], [22,-16,-28]) with the anatomical mask of the MTL described above, and used these for ROI analyses.

2.9.2.2 Functional connectivity analysis

We furthermore computed psycho-physiological interactions (PPI), using the findings from the activity analyses as seed regions, testing for brain regions that showed an interaction between the factors of subsequent memory and congruency in their connectivity to the seed regions (mPFC or left MTL). We thus performed the PPI analyses on two seed regions: A sphere with a radius of 8 mm centred at the peak voxel in mPFC ([-4,40,2]) reported in the activity analyses (see below), and the cluster in left MTL, extracted at p=.001 (unc.) using MarsBaR, which comprised 51 voxels (see below). Separately for all congruency levels, we computed the connectivity of the seed region with all other voxels, testing for regions that showed an interaction between the time course of the seed region's BOLD response (physiological variable) and the subsequent memory contrast as used in the activity analyses (psychological variable). The resulting statistical maps, indexing regions whose connectivity with the seed was stronger for hits than for misses for one particular congruency level, were entered into further multiple regression analyses, again probing an increase with either congruency or incongruency as described above. Hence, we tested for regions whose connectivity with the seed region grew stronger with increasing or decreasing congruency more during hits than during misses.

To perform small volume corrections in the connectivity analyses, we computed task-relevant sensory representation areas within our population sample from the functional localizer experiment. Lateral occipital complex (LOC), which is sensitive to visual perception of objects (Malach et al., 1995), was defined from the contrast between objects and scrambled objects. For small volume corrections concerning object localizer regions, we used the overlap of each of two spheres with a radius of 8 mm centred at bilateral peaks of the object contrast ([-38,-46,-22], [38,-44,-20]) with the object contrast thresholded at p_{FWE}=.05 as ROIs. The parahippocampal place area (PPA), implicated in visual perception of scenes (Epstein, Harris, Stanley, & Kanwisher, 1999), was defined from the contrast between scenes and scrambled scenes. For small volume corrections concerning regions activated by the scene localizer, we used the overlap of each of two spheres with a radius of 20 mm centred at

bilateral peaks of the scene localizer ([-26,-48,-8], [24,-40,-12]) with the scene contrast thresholded at p_{FWE}=.05 as ROIs.

3. Results

To investigate whether the congruency of new information with prior knowledge affects the way this information is encoded, we conducted an experiment to measure brain activity during the encoding of differentially congruent object-scene pairs. On day 1, participants performed an associative long-term memory task in the MR scanner. Before the encoding task, they completed a localizer experiment that allowed us to locate sensory representation areas for objects and scenes in our sample of participants. In the encoding task, they were shown pairs of photographs - one object paired with one scene - and gave subjective congruency ratings for these pairs. Participants' ratings were used to individually assign each trial to one of three congruency levels (incongruent, intermediate, and congruent). Twenty-four hours later, participants returned and were tested on their memory for the pairs outside the MR scanner. For objects judged as old, they chose the associated scene from three options, and subsequently indicated their confidence of that choice.

3.1 Behavioural measures

3.1.1 Item recognition accuracy

Item recognition accuracy (d-prime, Fig. 2A) was above chance level (0) for all congruency levels (incongruent pairs: $t(25)=18.19$, $p<.001$; intermediate pairs: $t(25)=16.99$, $p<.001$; congruent pairs: $t(25)=20.32$, $p<.001$). Item recognition measures increased linearly with congruency (linear component: $F(1,25)=15.37$, $p=.001$; quadratic component: $F(1,25)=3.61$, p=n.s.). Items from congruent pairs were better remembered than items from incongruent pairs ($t(25)=3.92$, $p=.001$) and intermediate pairs ($t(25)=3.79$, $p=.001$). Item recognition accuracy of incongruent and intermediate pairs did not differ significantly from each other ($t(25)=0.35$, p= n.s.).

3.1.2 Associative memory performance

Associative memory performance (% correct, Fig. 2B; incongruent pairs: M=58%, SEM=3%; intermediate pairs: M=63%, SEM=2%; congruent pairs: M=78%, SEM=2%) was above chance level (33%) for all congruency levels (incongruent pairs: $t(25)=7.9$, $p<.001$; intermediate pairs: $t(25)=13.9$, $p<.001$; congruent pairs: $t(25)=21.8$, $p<.001$). Associative memory performance increased linearly with congruency ($F(1,25)=46.1$, $p<.001$) and differed significantly between all three levels (incongruent pairs - intermediate pairs: $t(25)=-2.5$, $p=.019$; intermediate pairs - congruent pairs: $t(25)=-7.1$, $p<.001$; incongruent pairs - intermediate pairs: $t(25)=-6.8$, $p<.001$).

3.1.3 Confidence of associative memory

Confidence of associative recognition (minimum 1 point, maximum 3 points) showed a significant linear increase with congruency, averaging across hits and misses (linear component $F(1,25)=37.80$, $p<.001$; quadratic component: $F(1,25)=1.11$, p=n.s.), but any interaction between memory (hits vs misses) and congruency failed to reach significance ($F(2,24)=2.47$, p=n.s.). To control for effects of confidence on brain activity, confidence of associative hits was included into the analysis of the fMRI data as a parametric modulator.

3.1.4 Reaction times

Decision times (duration from the beginning of a pair presentation until the first button press within a trial) differed significantly between congruency levels (incongruent pairs: M=1.66 seconds, SD=0.22 seconds; intermediate pairs: M=1.99 seconds, SD=0.29 seconds; congruent pairs: M=1.57 seconds, SD=0.23 seconds; $F(2,50)=142.4$, $p<.001$), as did response times (duration from the first to the last button press within a trial) (incongruent pairs: M=0.78 seconds, SD=0.18 seconds; intermediate pairs: M=0.41 seconds, SD=0.20 seconds; congruent pairs: M=0.71 seconds, SD=0.14 seconds; $F(2,50)=9.8$, $p<.001$). To control for any effects due to these differences in reaction times, both measures were included in the analysis of the fMRI data as parametric modulators.

3.2 Brain activity

3.2.1 Subsequent memory

Our analysis revealed a congruency-unspecific subsequent memory effect in left hippocampus (peak [-30,-16,-12], SVC pFWE<.05), left inferior orbitofrontal cortex (peak [-38,38,-4], $p_{FWE}<.05$),

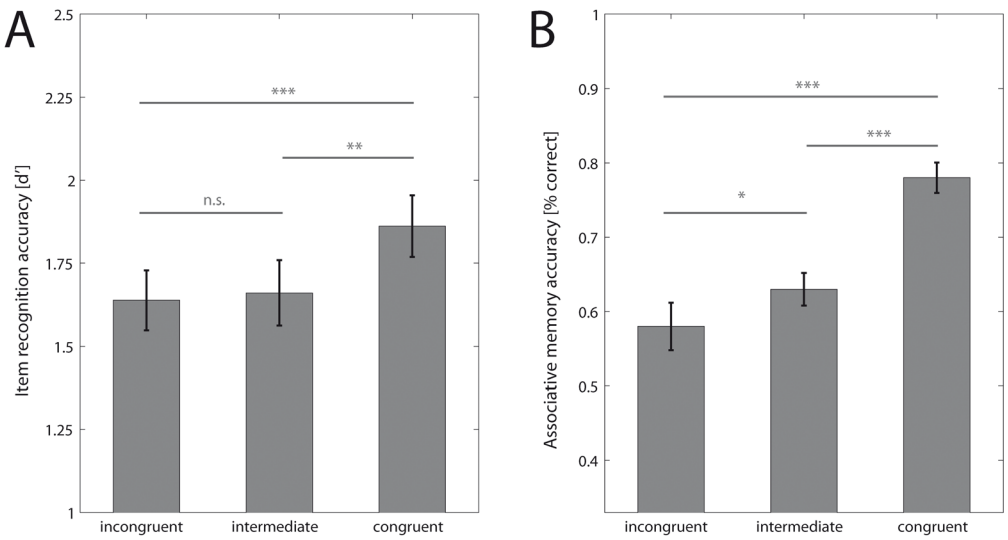

Fig. 2 Memory performance. **A.** Mean (and SEM) of item recognition accuracy (d-prime) for each congruency level. Performance was different from chance level (0) for all congruency levels (see main text). **B.** Mean (and SEM) of associative memory performance for each congruency level. Performance was different from chance level (33%) for all congruency levels and increased linearly with congruency (see main text). A + B Horizontal bars indicate paired-samples t-tests between two congruency levels; * $p < .05$; ** $p < .01$; *** $p < .001$; n.s. $p > .05$. SEM: standard error of the mean.

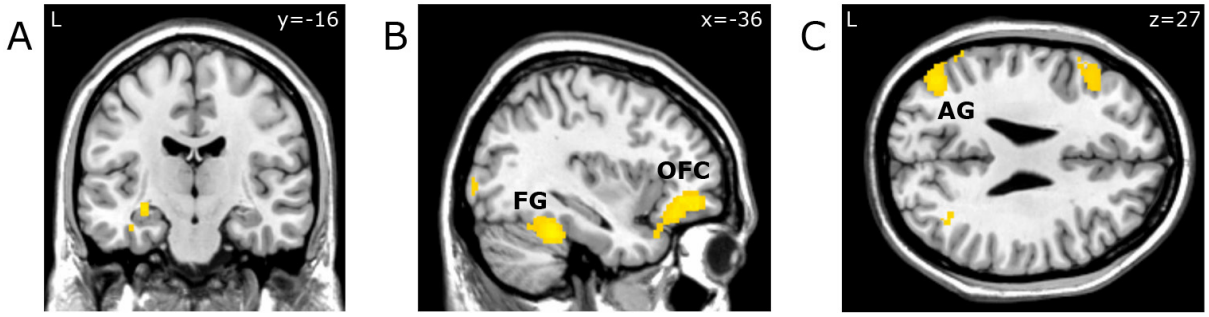

Fig. 3 Main effect of subsequent memory for object-scene associations. The congruency-unspecific subsequent memory contrast revealed enhanced activation in left hippocampus (peak [-30,-16,-12], SVC $p_{FWE} < .05$) (A), left inferior orbitofrontal cortex (peak [-38,38,-4], $p_{FWE} < .05$) (B, OFC), left fusiform gyrus (peak [-38,-44,-24], $p_{FWE} < .05$) (B, FG) and left angular gyrus (peak [-44,-70,28], $p_{FWE} < .05$) (C, AG). All maps are shown at $p = .001$ (unc.).

left fusiform gyrus (peak [-38,-44,-24], $p_{FWE}<.05$) and left angular gyrus (peak [-44,-70,28], $p_{FWE}<.05$) (Fig. 3). Masking with the object localizer contrast thresholded at $p_{FWE}=.05$ revealed that the cluster in left fusiform gyrus overlapped with regions found to respond preferentially to visual perception of objects in our participants (overlap not shown).

3.2.2 Interaction between subsequent memory and congruency

We first computed subsequent memory contrasts independently for each congruency level on the single subject level. To reveal brain regions that exhibited an interaction between congruency and subsequent memory, we then performed second level linear regression analyses on the subsequent memory contrasts for the three congruency levels.

Testing for a subsequent memory x congruency interaction yielded enhanced activity in the mPFC (peak [-4,40,2], SVC pFWE<.05) (Fig. 4A, C). Another region that we observed with this interaction contrast was the mid cingulate cortex (peak [4,-8,44], $p_{FWE}<.05$) (not shown).

In contrast, testing for a subsequent memory x incongruency interaction revealed stronger activation specifically in left MTL (peak [-14,-18,-20], $p_{FWE}<.05$) (Fig. 4B, D). This cluster was located in the left parahippocampal gyrus, and was distinct from the hippocampal activation reported for the congruency-unspecific subsequent memory effect (Fig. 3).

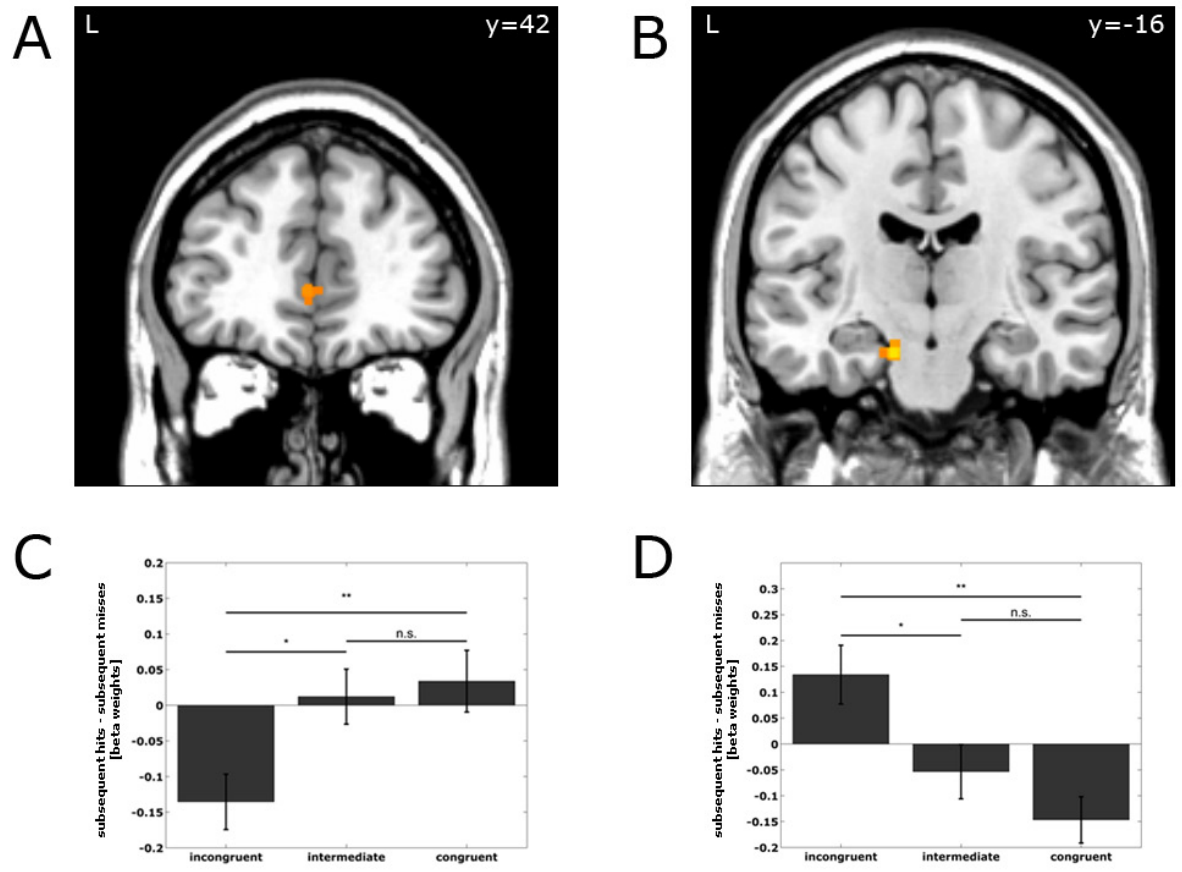

Fig. 4 Effect of congruency on subsequent memory. Statistical maps show the results of testing for brain regions which show an interaction between subsequent memory and congruency in their activation patterns. Linear regression analyses were performed on subsequent memory contrast for each congruency level, either testing for an increase with congruency (A) or incongruency (B). **A**. Activity in the mPFC during successful encoding increased with congruency (peak [-4, 40, -2], SVC $p_{FWE} < .05$; displayed at $p = .005$ (unc.)). **B**. Activity in the left MTL during successful encoding increased with incongruency (peak [-14, -18, -20], SVC $p_{FWE} < .05$; displayed at minimum cluster size 10 voxels, $p = .001$ (unc.)).**C**. Beta weights for the subsequent memory contrast extracted from the cluster in mPFC (A). D. Beta weights for the subsequent memory contrast extracted from the cluster in left MTL (B). **C & D** Horizontal bars indicate paired-samples t-tests between two congruency levels; * $p < .05$; ** $p < .01$; n.s. $p > .05$.

These results show that mPFC was more strongly activated during the successful encoding of congruent than incongruent pairs, while left MTL contributed more to the successful encoding of incongruent than congruent pairs.

3.2.3 Localizer experiment

The localizer experiment revealed distinct, bilateral brain regions that were more strongly activated during processing of objects and scenes, respectively, in a 1-back working memory task (Fig. 5). Activity in LOC was enhanced bilaterally in response to objects (peaks [-38,-46,-22], [38,-44,-20], p_{FWE}<.05) (Fig. 5A, B). Activity in PPA was enhanced bilaterally in response to scenes (peaks [-26,-48,-8], [24,-40,-12], p_{FWE}<.05) (Fig. 5A, C).We used these results to perform small volume corrections in the connectivity analyses (see methods section).

3.2.4 Functional connectivity

To investigate how connectivity of the areas shown to correlate more strongly with the encoding of congruent - mPFC - and incongruent - left MTL - pairs differed across congruency levels, we ran PPI analyses. These analyses tested for regions which showed an interaction between subsequent memory and congruency in their connectivity to mPFC or left MTL.

These tests revealed bilateral regions within the PPA whose connectivity with the mPFC during successful encoding increased with congruency of the encoded pairs (peak [14, -38, -8], SVC p_{FWE}<.05; peak [-16,-34,-14], SVC p_{FWE}<.05) (Fig.6). Furthermore, we found a region within LOC

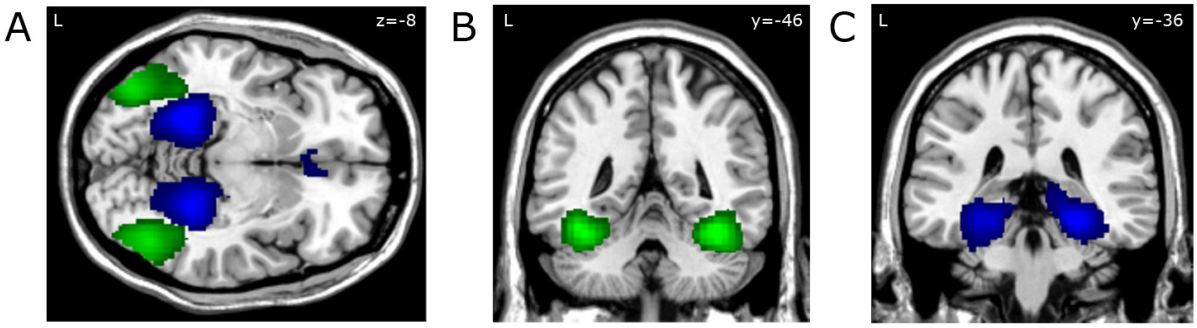

Fig. 5 Results localizer experiment. Activation during a 1-back working memory task was used to localize brain areas selectively responsive to objects and scenes. Activity in lateral occipital complex (LOC) was enhanced bilaterally in response to objects (objects - scrambled objects) (shown in green, peaks [-38,-46,-22], [38,-44,-20], $p_{FWE} < .05$). Activity in parahippocampal place area (PPA) was enhanced bilaterally in response to scenes (scenes - scrambled scenes) (shown in blue, peaks [-26,-48,-8], [24,-40,-12], $p_{FWE} < .05$). **A.** objects - scrambled objects (LOC) and scenes - scrambled scenes (PPA), displayed at pFWE = .05. **B.** objects - scrambled objects (LOC), displayed at $p_{FWE} = .05$. C. scenes - scrambled scenes (PPA), displayed at $p_{FWE} = .05$.

whose connectivity with left MTL during successful encoding increased with incongruency of the encoded pairs (peak [-42,-36,-24], SVC $p_{FWE} < .05$) (Fig. 6). These changes in functional connectivity were specific to task-relevant sensory representation areas, which was reflected in the absence of whole brain significant peaks or clusters in both analyses. Moreover, increased functional connectivity with sensory representation areas was also specific to the directions tested in mPFC and left MTL. This was shown by multiple regression analyses testing changes in connectivity of mPFC and left MTL with incongruency and congruency, respectively, which did not yield significant activation in LOC or PPA at a lenient threshold of $p = .05$ (unc.) (not shown).

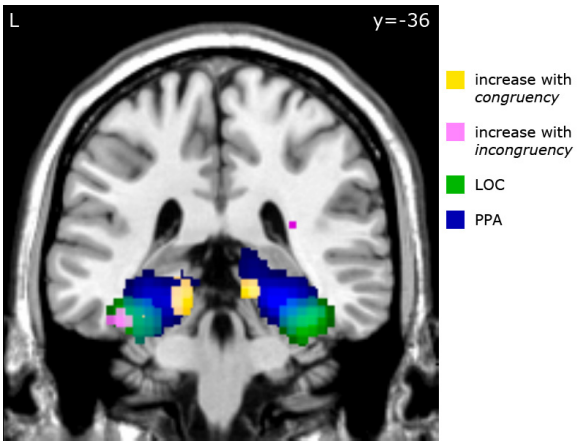

Fig. 6 Effect of congruency on functional connectivity during memory encoding. Statistical maps show the results of a linear regression on the interaction between connectivity of a seed region and subsequent memory for each congruency level, imposed on localizer experiment results (Fig. 5; green: lateral occipital complex (LOC), blue: parahippocampal place area (PPA)). Connectivity of the mPFC with bilateral PPA during successful encoding increased with congruency (shown in yellow, peaks [14, -38, -8], SVC $p_{FWE} < .05$; [-16,-34,-14], SVC $p_{FWE} < .05$). Connectivity of the left MTL with LOC during successful encoding increased with incongruency (shown in purple, peak [-42,-36,-24], SVC $p_{FWE} < .05$). Displayed at $p = .005$ (unc.).

4. Discussion

Recent studies in animals and humans have given insight into how activated schemata affect the neural correlates of memory processing (Tse et al., 2007, 2011; van Kesteren et al., 2010a, 2010b, 2012b). The SLIMM framework (van Kesteren et al., 2012a) proposes that two neural mechanisms for memory encoding exist, which encode new information depending on its congruency with prior knowledge. Accordingly, SLIMM predicts differential brain activity and connectivity for information on different extremes of the schema-congruency spectrum. In this experiment, we investigated how brain activity during encoding of new associations is altered as a function of the subjective congruency of those associations with prior knowledge.

We found a retention benefit for schema-congruent information in both item recognition accuracy and associative memory performance (Fig. 2), which is consistent with earlier results (van Kesteren et al., 2010a, 2010b, 2012b).

In line with previous studies (Sperling et al., 2003; Jackson & Schacter, 2004; Tendolkar et al., 2007), the fMRI-data analyses revealed congruency-unspecific subsequent memory effects (Fig. 3) in left hippocampus and left inferior orbitofrontal cortex. Moreover, we found activation in left fusiform gyrus, overlapping with a region that was preferentially

activated during visual perception of objects in our functional localizer experiment, to be predictive of congruency-unspecific subsequent memory. Thus, better subsequent memory was reflected in stronger involvement of task-relevant sensory representation or processing areas during encoding, regardless of the learned information's subjective congruency with prior knowledge. Furthermore, the left angular gyrus exhibited a subsequent memory effect. This region has been shown to be activated by semantic processing and suggested to promote verbal associations (Binder & Desai, 2011), processes which conceivably could have led to better associative memory.

Conversely, different brain regions were implicated in successful encoding when subjective schema-congruency was taken into account. The fMRI data showed, that activity in the mPFC during encoding of subsequently remembered associations increased linearly with subjective congruency of the information (Fig. 4A). Moreover, a subsequent memory x congruency interaction was also observed in mid cingulate cortex, a brain region that has been implicated in memory retrieval (Huijbers, Pennartz, Cabeza, & Daselaar, 2009; Sajonz et al., 2010; Huijbers, Pennartz, Rubin, & Daselaar, 2011). We relate this to the fact that congruent pairs (necessarily) depicted more common associations than incongruent pairs, potentially causing more retrieval of related information during encoding, which simultaneously could have been beneficial for memory encoding.

With subjective incongruency of new information, brain activity during successful encoding increased linearly in the left MTL (Fig. 4B). Unlike the MTL activation in left hippocampus reported for congruency-unspecific subsequent memory, this subsequent memory x incongruency interaction was found in left parahippocampal gyrus. This is consistent with previous studies that report subsequent associative memory effects there (Law et al., 2005; Tendolkar et al., 2007; Staresina, Duncan, & Davis, 2011), using tasks that required participants to learn arbitrary associations, which fall rather onto the incongruent extreme of the schema-congruency spectrum as conceptualized here.

Furthermore, we found that functional connectivity of mPFC and left MTL interacted with subjective schema-congruency of encoded information (Fig. 6): During successful encoding of increasingly congruent information, functional connectivity of the mPFC with task-relevant sensory representation areas was enhanced, while during successful encoding of increasingly incongruent information, stronger functional connectivity of the left MTL with a task-relevant sensory representation area was found. This implies that when mPFC and left MTL contributed to successful encoding, i.e. for more congruent and more incongruent information, respectively, their ability to integrate task-relevant information was enhanced, further supporting the notion that they contributed to successful encoding.

These results show that both memory performance and its neural correlates are affected by a parametric change in subjective schema-congruency of new information in a linear fashion. As subjective congruency with prior knowledge increased across multiple congruency levels, so did memory performance. Activation of mPFC and MTL during successful encoding exhibited oppositional developments, with mPFC contributing more strongly to encoding of increasingly schema-congruent information, and MTL correspondingly contributing more strongly to the encoding of increasingly schema-incongruent information. Obtaining these results using subjective congruency judgements indicates that we were able to exploit genuine trial-by-trial variability in brain activity.

Our results are mostly consistent with the SLIMM framework proposed by van Kesteren and colleagues (2012a), which suggests existence of two distinct neural mechanisms for the encoding of new information, whose contribution to memory formation is governed by the extent to which this information is congruent with activated schemata. Specifically, encoding of schema-congruent information is hypothesized to be mediated by the mPFC, while encoding of schema-incongruent information is proposed to depend on the MTL. Here we show that with a manipulation of subjective schema-congruency over three levels, both activity and functional connectivity in brain regions that were expected to mediate encoding of new information changed linearly. This extends earlier findings showing that mPFC and MTL are more strongly activated during the encoding of congruent relative to incongruent material and vice versa (van Kesteren et al., 2012b). Moreover, while it has previously been reported that during retrieval of congruent information functional connectivity of the mPFC to task-relevant sensory representation areas is enhanced (van Kesteren et al., 2010b), our results show that functional connectivity in mPFC and left MTL is affected already during encoding in a fashion that supports their suggested role in successful memory formation.

SLIMM predicts that with encoding of differentially congruent information by distinct

neural mechanisms, the nature of the created memories will differ. Congruent information is hypothesized to be incorporated directly into cortical networks via the mPFC without the formation of a strong episodic memory for this event, as it would be expected for MTL-dependent encoding of incongruent information, and it is proposed that therefore memory performance across the congruency scale will depend on the way that memory retrieval is cued. Different susceptibility of memories across the congruency spectrum to retrieval cues could potentially make memory performance a non-monotonic function of schema-congruency, either within one test or if the results of multiple ways of testing retention were integrated. Retention benefits for irregular information seem to arise especially under incidental encoding conditions (Kormi-Nouri, Nilsson, & Ohta, 2005) and particularly affect details, as e.g. demonstrated by Davis, Love, & Preston (2011), who reported better recognition memory for exceptions than for rule-following items in a two-alternative forced-choice task using foils greatly resembling the target stimuli. In contrast, we used intentional encoding and tested memory on a superficial level, using no highly similar lure objects or scene depictions. This may account for why we observed a retention benefit for congruent material only.

One potential caveat of this study is the way associative memory was assessed. To reduce effects of visual recognition, we used verbal descriptions as options in a multiple choice test, providing one incongruent and one congruent incorrect option additional to the correct scene. For some objects, which were very congruent with the associated scene, it was hardly possible to find a congruent incorrect option that was as plausible as the correct option. Although we were most cautious to eliminate guessing as a successful strategy for identification of congruent associated scenes, it is possible that we did not completely succeed. However, given the restrictions placed on the incorrect options, we strove to reach the optimal distribution of multiple choice options across trials. Additionally, item recognition assessment was not affected by any confounding factors, and yet item recognition accuracy showed a retention benefit for congruent information. Altogether, we are therefore confident that the congruency benefit we reported for both memory retention measures is genuine, as are the effects we showed on brain activation and functional connectivity.

It remains an open question to which extent memories for congruent information, formed with contribution of the mPFC, and memories for incongruent information, dependent on the MTL, differ in quality. SLIMM predicts that fast assimilation of schema-congruent information into existing neocortical networks leads to less contextual memory than MTL-dependent encoding. While mPFC is hypothesized to mediate selective encoding of schema-congruent aspects of an experience (van Kesteren et al., 2012a), resulting in a coarse memory trace for this event, which comprises little contextual information, MTL is assumed to non-selectively bind information present at the time of encoding into a detailed episodic memory trace (Eichenbaum, 2000). A qualitative difference in memory depending on the mediating encoding mechanism imposes the possibility, that memory performance may be a non-monotonous function of congruency with activated schemata. Memories of disparate nature may yield better performance in different measures of retention (e.g. accuracy, confidence) and be differentially susceptible to the way retention is assessed (e.g. free recall, cued recall). This question deserves further investigation using different memory tests to reveal the boundary conditions of mPFC- and MTL-dependent encoding.

Another prediction of SLIMM that requires further testing is whether the mPFC indeed inhibits the MTL during the encoding of congruent information. This warrants investigation using an experimental paradigm that allows for the analysis of causal connectivity between brain regions.

5. Conclusion

In conclusion, this study revealed that modulation of subjective congruency of to-be-learned information with prior knowledge over three levels affected memory performance, brain activity and functional connectivity in a linear manner. We provide support for the SLIMM framework, which proposes that two distinct neural encoding mechanisms, instantiated in mPFC and MTL, interact in memory encoding, where the extent to which each contributes to encoding is governed by the congruency of the learned information with prior knowledge. Corroborating this hypothesis, our results from analyses testing for interactions between congruency and subsequent memory in both brain activity and functional connectivity indicate that schema-congruent information is encoded predominantly via the mPFC, while encoding of schema-incongruent information is predominantly dependent on the MTL, with a linear change in

contribution of these two brain regions across the congruency spectrum.

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Motor System Involvement During Error Observation in 8-month-old Infants

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Although observing and evaluating behavior is especially important during the first years of life, when many novel skills need to be developed, little is known about the early neurocognitive mechanisms underlying error monitoring in infancy. Research has shown that similar regions in the adult brain are involved in evaluating one's own and observed behavior. Moreover, a recent study suggests that the adult motor system is activated more strongly by the observation of an erroneous compared to a correct action. In our current electroencephalography (EEG) study, we investigated to what extent the infant motor system is recruited during error observation and whether the infants' own action capabilities influence motor system involvement. We presented 8-month-old infants, who were at the verge of acquiring the pincer grasp, with videos of an actor who either failed or succeeded to pick up a small object using the pincer grasp. Enhanced attenuation in the mu frequency band of the EEG, which is associated with increased activation of the motor system, was found during the observation of correct compared to erroneous actions. In addition, the size of the differential activation was negatively correlated to the infants' pincer grasp proficiency. The results indicate an involvement of the motor system during error observation in 8-month-old infants, who still need to acquire proficiency in the observed action. Integrating the present findings with previous research on error observation, we propose a U-shaped relationship between differential motor system activation and action proficiency.

Keywords: motor system, development, error observation, electroencephalography

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1. Introduction

The detection of errors is a capacity that is essential for behavioral adaptation and the acquisition of new skills. Crucially, we are not only able to learn from our own mistakes but we also gain valuable information from observing others. In the first years of life infants need to acquire a range of new gross and fine motor skills. In order to become proficient in these skills, infants constantly need to adapt their own behavior and this process in guided by the ability to monitor themselves and others. Infants learn to sit independently, gradually become able to crawl, and some children might even be walking when they celebrate their first birthday (WHO Multicentre Growth Reference Study Group, 2006). In addition to the gradual improvements of the infants' gross motor capacities, their fine motor skills also develop rapidly during their first year of life (Von Hofsten, 1989). From about 4 months of age, infants start reaching for objects using their whole hand and their grasps become more proficient throughout the second half of their first year. Eventually, around 9 months, infants acquire the pincer grasp, enabling them to pick up and manipulate small object using their thumb and index finger (Butterworth, Verweij, & Hopkins, 1997). Although acquiring these motor skills requires the infants to continuously monitor themselves and others in order to adapt their behavior, action monitoring and its neurocognitive underpinnings have rarely been investigated in infancy.

In adults, however, the neurocognition of error monitoring and action observation has been studied extensively (Holroyd & Coles, 2002; Van Schie, Mars, Coles, & Bekkering, 2004; Calvo-Merino, Glaser, Grèzes, Passingham, & Haggard, 2005; Yu & Zhou, 2006; Cross, Kraemer, Hamilton, Kelley, & Grafton, 2009). Whereas earlier research mainly focused on how adults monitor their own behavior and adapt their behavior after committing an error (e.g., Gehring, Goss, Coles, Meyer, & Donchin, 1993), more recent studies have also looked into the processing of others' actions. These studies suggest that the observation of own and others' behavior recruits similar brain regions, including the medial prefrontal cortex (Van Schie et al., 2004), premotor and parietal regions (Cross et al., 2009) as well as the superior temporal sulcus (STS) (Calvo-Merino et al., 2005). In addition, the human motor system has recently been shown to be sensitive to the correctness of an observed action as well (Koelewijn, van Schie, Bekkering, Oostenveld, & Jensen, 2008). Using magnetoencephalography (MEG), the researchers showed that the motor system was more strongly activated by the execution and observation of an erroneous compared to a correct action. A study by Aglioti, Cesari, Romani and Urgesi (2008) indicates that this monitoring function of the motor system is modulated by the amount of experience the observer has with the given action. The researchers used transcranial magnetic stimulation (TMS) to compare motor system excitability in basketball expert players, expert watchers and novices who observed players throwing a basketball that either hit or missed the goal. Interestingly, the expert players were not only better in predicting the outcome of the observed action, they also showed enhanced motor system excitability during the observation of the erroneous compared to the correct shots.

Although adult findings thus indicate the involvement of prefrontal, parietal and premotor areas in monitoring and evaluating own as well as observed behavior, it is only recently that scientists have started to translate these findings to developmental populations. A number of researchers made use of adapted speeded response tasks from adult paradigms to assess the development of error monitoring in children (Jones, Rothbart, & Posner, 2003; Rueda, Posner, Rothbart, & Davis-Stober, 2004; Santesso, Segalowitz, & Schmidt, 2006). To date, however, most studies have focused on the investigation of own error processing in preschoolers and older children, whereas research on monitoring others' errors is limited. In addition, only few studies have investigated the neural correlates of error monitoring during early infancy. One of those few studies was conducted by Berger, Tzur and Posner (2006) who investigated arithmetic error processing in 6- to 9-month-olds using electroencephalography (EEG). Interestingly, infants showed a greater negativity in the event-related potential (ERP) and a power increase in the theta and alpha band of the time frequency spectrogram, which was similar to what was previously found in adults.

Although this study suggests that even in young infants, neurocognitive mechanisms involved in error processing can be detected, the results are limited to the processing of arithmetic errors and it remains unknown how this translates to the processing of other types of errors such as action slips. This study extends the research on error monitoring in young infants by investigating the role of the motor system during the processing of erroneous actions. In adults the motor system was shown to be modulated differentially by the observation of erroneous compared to correct actions (van Schie et al., 2004;

Aglioti et al., 2008; Koelewijn et al., 2008) and the present study aims to investigate this relationship in young infants.

The infant motor system can be investigated by analyzing the mu rhythm of the EEG (see Marshall & Meltzoff, 2011 for a review). The mu rhythm was first identified in adults as an oscillation in the frequency range of 8-13 Hz, similar to the classical occipital alpha (for a review on the adult mu rhythm and its interpretation see Pineda, 2005). In contrast to the occipital alpha, which is localized at occipital sites and associated with visual processing, the mu rhythm is localized above sensorimotor areas and is associated with activation of the motor system (McFarland, Milner, Vaughan, & Wolpaw, 2000; Pineda, Allison, & Vankov 2000; Muthukumaraswamy & Johnson 2004). More specifically, several studies have shown that the power in the mu rhythm is attenuated during action execution as well as during the observation of actions and imagination of movements (e.g., Cochin, Barthelemy, Rouw, & Martineau, 1999; Pineda et al., 2000; Oberman, Pineda, Ramachandran, 2007). An infant mu rhythm within a somewhat lower frequency range (6-9Hz) has been detected in infants as young as 5-6 months (Orekhova, Stroganova, Posikera, & Elam, 2006; Nyström, 2008; Marshall & Meltzoff, 2011). Several studies have shown that the infant mu rhythm – similar to adults – is attenuated when the infant is moving, reflecting activation of the motor system (Southgate, Johnson, Osborne, & Csibra, 2009; Berchicci et al., 2010).

Interestingly, the infant mu rhythm has shown to be attenuated by the observation of another person's actions (Van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008; Southgate, et al., 2009; Stapel, Hunnius, van Elk, & Bekkering, 2010; Nyström, Ljunghammar, Rosander, & Hofsten, 2011) which indicates that also in infancy, the motor system is involved in processing observed behavior. Furthermore, two recent studies have found additional similarities between the infant and adult motor system. Firstly, a study by van Elk, van Schie, Hunnius, Vesper and Bekkering (2008) showed that motor system activation in 14- to 16-month-old infants is influenced by the amount of experience the infants have with a given action. This is consistent with findings in adults (Calvo-Merino et al., 2005; Aglioti et al., 2008). The infants were presented with videos of other infants that were either walking or crawling. The researchers found that observing videos of other infants crawling elicited a stronger attenuation of the power in the mu and beta frequency band compared to the walking videos. Interestingly, the infants' own crawling experience was positively correlated to the size of this differential activation. This study was the first to show that the amount of motor system activation during observation is experience-dependent in young infants. Secondly, a study by Stapel, Hunnius, van Elk and Bekkering (2010) suggests that the infant motor system might also play a role in predicting actions and their outcomes. In this study, EEG data of 12-month-old infants were recorded while they were presented with videos of usual and unusual actions (for example a women bringing a cup to her mouth (usual) or to her ear (unusual)). It was found that the infants' motor system was differentially activated for the two action types, showing enhanced activation for the unusual actions. The adult motor system has been suggested to be involved in predicting action outcomes (Kilner, Friston & Frith 2007) and the authors argue that their results provide evidence for such a predictive role of the motor system already at 12 months of age.

The present study aims to extend the current research by investigating the involvement of the infant motor system during error observation. During their first years of life, infants acquire a range of new motor skills (Von Hofsten, 1989; Butterworth et al., 1997; WHO Multicentre Growth Reference Study Group, 2006) and in order to do so they need to constantly monitor and adapt their behavior to reach proficiency. Thus, it is especially interesting to investigate in how far the infants' motor system is already involved in error monitoring as it appears crucial for skill acquisition. In addition examining infants who are developing a novel motor skill provides the unique opportunity to directly study the influence of action experience and action proficiency on the neurocognitive processes involved in action monitoring.

Our main research question was whether the motor system is involved in error observation in infants. We expected that the infants would show enhanced motor system activation (reflected in a decrease of mu frequency power) when they observed an erroneous compared to a correct action. Especially since the study by Aglioti and colleagues (2008) suggested that in adults, action experience alters the ability to predict the correctness of an observed action, we expected action experience to influence motor system activation in infants acquiring a novel motor skill. More specifically, we expected that infants with greater proficiency would show enhanced differential activation when observing erroneous compared to correct actions.

We focused on investigating 8-month-old infants since they were in the phase of acquiring the pincer

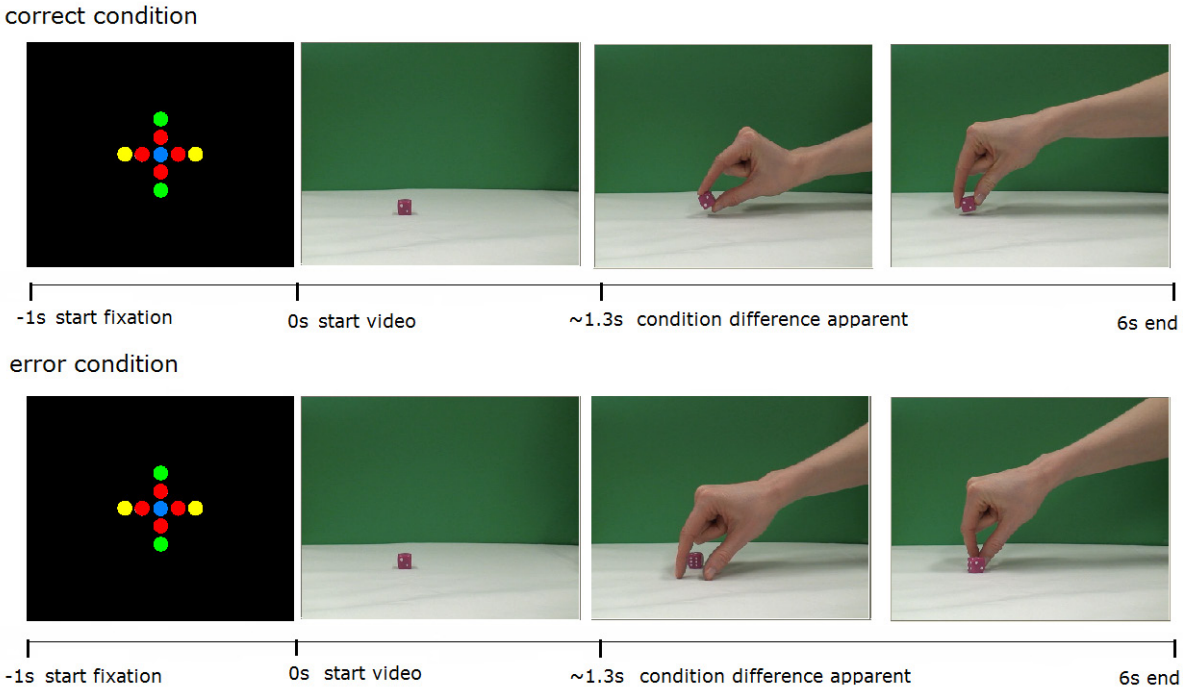

Fig. 1 Stimulus videos shown during the action observation phase of the experiment. After the presentation of a colored fixation cross the infants were presented with a video showing either a correct (upper illustration) or an erroneous pincer grasp (lower illustration).

grasp, a motor milestone that allows them to pick up and manipulate small objects using their thumb and index finger. As not all infants achieve this milestone at exactly the same age, there is a naturally occurring variability in proficiency, which allows studying the influence of action experience on motor system activation within one age group. Based on earlier research and a behavioral pilot study, we decided to test 8-month-old infants as we expected that at this age some infants would already able to grasp small objects using the pincer grasp whereas others would not (yet) be able to do so (see also Touwen, 1971; Von Hofsten, 1989; Butterworth et al.,1997). We recorded EEG data while the infants were presented with videos of either correct or erroneous pincer grasps and we consecutively measured the infants' own pincer grasp proficiency. As mentioned above, we expected motor system activation to be influenced by the correctness of the observed action and we anticipated the effect to be modulated by the infants' action experience.

2. Material and methods

2.1 Participants

In total 26 infants (13 female, mean age 8.2 months; SD= 0.29) participated in the study. All infants were first presented with the video stimuli and consecutively participated in a pincer grasp proficiency test. Finally, all parents completed a pincer grasp proficiency questionnaire. Data analysis was based on a final sample of seven infants (3 female, mean age 8.2 months; SD=0.22). The remaining infants had to be excluded from analyses, either because of technical problems (3), fussiness (2) or because the amount of valid EEG trials was not sufficient (14) (see Analysis of the EEG data section for more details).

The infants were recruited from a data base of the Baby Research Center Nijmegen containing contact information of families living in the surrounding area who had shown interest to participate in scientific experiments with their children. All participants received a small present or a monetary compensation for their participation. Before testing, all parents were informed about the nature and goal of the study and gave written informed consent. The research was approved by the local ethical committee.

2.2 Task and material

2.2.1 Video stimuli

The infants were presented with videos showing an adult female hand grasping a small object using a pincer grasp. Each video started with the hand approaching the object and the different conditions became distinct after approximately 1.3 seconds

(see Figure 1). In the correct condition the hand approached the object and grasped it successfully. In the error condition the actor repeatedly tried but failed to grasp the object.

We used four different objects which were grasped from the right side by two female actors. For each actor-object combination both the error and correct condition were recorded, leading to 16 different videos (actor (2) x object (4) x condition (2)). In addition, each video was also used in a horizontally flipped version, leading to a final set of 32 videos. Using the visual processing utility Virtual Dub 1.9.11 (http://virtualdub.org/) each video was edited and cut to a length of approximately 6 seconds.

As we wanted to make the error look as realistic as possible, we instructed the actors to close their eyes while grasping for the object during the recordings of the error condition videos. The actor consecutively tried to pick up the object but often failed because they were not able to see the objects' location. The displayed failure in our error condition therefore reflected an actual failure to pick up the object.

To keep the amount of motion in the videos equal for both conditions, the trajectory of the hand in space was matched. In addition, we compared the average amount of motion for the videos of the two conditions using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). The amount of motion per video was determined using Matlab (version 7.14.0.739 (R2012a), Matworks, Inc., Natick, MA; http://mathworks.com) by calculating motion changes frame by frame for each video (see Schippers, Roebroeck, Renken, Nanetti, & Keysers, 2010 for a similar analysis). A paired sample t-test revealed no significant differences in motion between the videos of the two conditions ($t(7)= -1.67$ $p=0.14$). A similar procedure has been used in previous research to ensure that observed differences in brain activation were not due to differences in low-level action features between the stimulus videos (Cross et al., 2011).

2.2.2 Pincer grasp proficiency assessment

A behavioral test and a parent questionnaire were used to assess the infants' pincer grasp proficiency. Figure 2 shows the device that was used for the behavioral assessment. In the middle of the device a little colored bead was attached with a string that the infant could pull out using the pincer grasp. Importantly, the bead was too small to be grasped and pulled out successfully with a full hand grasp. Two sizes of beads were presented to the infants to manipulate the difficulty of the task. In addition to the behavioral test, a questionnaire for the parents was constructed which contained five questions (see Appendix A). First the parents were asked to indicate whether the infant was able to move their index finger independently from the other fingers, which is thought to be a precursor of the pincer grasp (Butterworth et al., 1997). In addition, it was assessed whether and how frequently the parents observed their infant picking up small objects using the pincer grasp. If the parents reported that their infants were using the pincer grasp at home, they were asked to indicate at which age they had first observed this behavior. Furthermore, parents were asked to indicate how their children were usually picking up small objects.

Fig. 2 Proficiency assessment during the action execution phase of the experiment. Left: Pincer grasp proficiency device. Right: An infant is presented with the device to assess his ability to grasp small objects using the pincer grasp.

2.3 Procedure

Parents and infants were invited to the lab for a testing session of about one hour. After the infant had adjusted to the lab environment, the infant was fitted with an EEG cap and the experimenter controlled the impedances. Then the infant was brought to a shielded room and was seated in a child seat on the lap of the parent. This specific set-up was used to minimize parental influence and to ensure that the infant was sitting stable. Once the infant was feeling comfortable the testing started. The experiment consisted of two phases: an action observation phase and an action execution phase.

During the action observation phase the infant was presented with the stimulus videos on a computer screen which was placed at a distance of approximately 50 cm from the infant (see Figure 3 for the experimental set-up). As illustrated in Figure 1 every trial started with the presentation of a colored fixation cross before one of the stimulus videos was presented. All stimuli were presented in a pseudorandom order using Presentation Software (Neurobehavioral Systems; http://www.neuro-bs.com). The randomization was created per participant using the Windows randomization programme MIX (http://www.mrc-cbu.cam.ac.uk/ personal/ maarten.van-casteren/Mix.htm) which ensured that there were maximally four consecutive videos of the same condition. Additionally, at least two different videos were presented in between the repetition of the same actor-object combination. If the infant seemed to lose interest in the videos, the experimenter could trigger the appearance of a colored animation which was designed to regain the attention of the infant to the screen. The action observation phase of the experiment ended after each video had been shown twice (64 videos, approximately 10 minutes) or when the infant continued to show disinterest, started to cry or showed other signs of discomfort. Throughout the stimulus presentation soft music was played in order to keep the infant calm and interested, which has been done in previous studies (e.g., Llyod-Fox, Blasi, Volein, Everdell, Elwell, & Johnson, 2009).

After the infant finished watching, the experimenter entered the room and conducted the behavioral proficiency test (action execution phase). Two versions of the proficiency device were presented to the infant, first one with a big bead and then one with a small bead. The infant was seated on the parent's lap and the device was placed in front of the infant (see Figure 2). Once the infant was paying attention to the device the experimenter pulled out the bead, demonstrating a successful pincer grasp. Consecutively, the infant was allowed to freely play with the device for one minute, after which the second version of the device was presented in the same way. Whenever the infant lost interest in the task, the experimenter would point at the bead and verbally encourage the exploration of the device. Throughout the entire experiment EEG was recorded and the infant was videotaped using three video cameras for later coding and analysis. After the EEG session the parents were asked to fill in a proficiency questionnaire (see Appendix A).

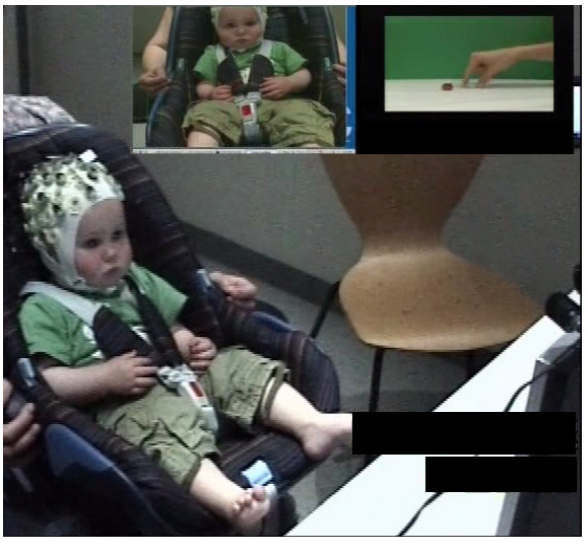

Fig. 3 Experimental set-up. An infant is presented with the video stimuli on a computer screen while seated in an infant seat on their parent's lap.

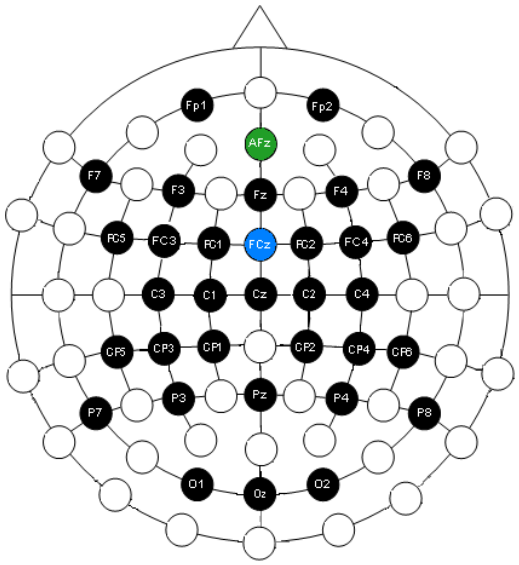

Fig. 4 Overview EEG configuration. Electrode FCz was used as a reference (blue) and Electrode Afz was used as ground (green).

2.4 Data acquisition

EEG was recorded from 32 active Ag/AgCL electrodes using infant-sized caps (ActiCAP), Brain Amp DC and Brain Vision Recorder software (Brain Products GmbH, Germany). The electrodes were arranged according to an adapted configuration based on the 10-20 system (see Figure 4) to increase measurement density above sensorimotor areas. The data was sampled at 500 Hz and online filtered using a 0.016 Hz high-pass and a 125 Hz low-pass filter. Electrode FCz was used as an online reference and the impedance was kept below 20 kΩ. For analysis, the EEG data was re-referenced to the average of all channels.

2.5 Data analysis

The EEG data was analyzed using FieldTrip, an open source Matlab toolbox, which has been developed at the Donders Institute for Brain, Cognition and Behaviour (http://www.ru.nl/neuroimaging/fieldtrip). Statistical analyses were performed using IBM SPSS (statistical package of the social sciences), version 19.0.0. Prior to any parametric statistical analysis, we validated that the data was normally distributed.

2.5.1 Analysis of pincer grasp proficiency

Three different measures of pincer grasp proficiency were used in this study. First the video recordings from the behavioral proficiency test in the action execution phase of the experiment were evaluated (behavioral proficiency). As the behavior of the infants did not differ between the small and the big bead, the data was collapsed for further analysis. None of the seven infants in our final sample managed to pull out the bead using the pincer grasp and we therefore decided to use a qualitative measure to evaluate the infants' behavior. Earlier studies have shown that infants' grasping behavior improves gradually from power grip to precision grip (Butterworth et al., 1997). More specifically, when grasping objects infants first use their whole hand before they start to use individual fingers to manipulate and pick up small objects. Therefore it was assessed how often the infants were touching the bead with their thumb and/or index finger (pincer grasp touch) and how often they touched the bead using other fingers or their whole hand (hand touch). Then the duration of both touch types was calculated and a ratio of pincer grasp touches per child was computed. More precisely, we divided the duration of pincer grasp touches by the sum of the duration of both touch types (duration pincer grasp touch / [duration pincer grasp touch + duration hand touch]). This ratio was used as first measure of pincer grasp proficiency and will be referred to as behavioral proficiency.

The second measure of proficiency was a score derived from the parental questionnaire which can be found in Appendix A (proficiency questionnaire). Points were assigned for question 2, 4 and 5. For question 2 the parents had to indicate whether they had ever observed the infant performing a pincer grasp. Answering this question with yes resulted in one point, answering it with no resulted in zero points. In question 4 parents were asked to indicate whether the infant was often, sometimes or only rarely grasping small objects using the pincer grasp. For this question two, one or zero points were assigned respectively. Finally, in question 5 parents had to indicate whether the infant was rather picking up small objects using his thumb opposed to the other fingers or using the tips of his thumb and index finger. Zero points were received for the former answer, whereas one point was received for the latter. Every infant therefore received a total score between zero and four on this proficiency measure which will be referred to as proficiency questionnaire.

In addition, parents were asked to indicate at which age they had first observed their child picking up small objects using the pincer grasp (acquisition age). As we were especially interested in the effect of action experience on motor system activation, we used the difference between the infants' age at the date of testing and the indicated acquisition age as third measure of proficiency which will be referred to as proficiency span.

2.5.2 Analysis of the EEG data

2.5.2.1 Segment selection

For the analysis of the action observation phase, windows of interest for the correct and error condition were selected from each stimulus video. More specifically, for each video four to five 1-second-windows, starting from the point where the condition difference became apparent, were evaluated. Only valid EEG segments of these time windows of interest were further chosen for the data analysis. Infants were included in the final analysis if they had at least 9 valid segments for the correct and error condition each. Segments were defined as

valid if all of the following three criteria were met: 1) The infant was attending the screen 2) The infant showed no overt body movements 3) The segment did not contain any EEG artifacts (like eye blinks, eye movements, noise or electrode drifts). Artifact rejection was done manually on every individual EEG segment. The criterion of 9 valid segments per condition was met by seven infants and only those data will be reported in the following part. On average, the infants completed 20 valid segments for the correct condition (SD=20.83) and 24 valid segments for the error condition (SD=20.17). In general, there was a large variability in the number of valid segments between subjects, ranging from a minimum of 9 to a maximum of 69 segments.

For the fixation period, segments were selected in a similar way, resulting in 13 segments on average (SD= 7.37). The lower amount of segments in the fixation period compared to the other two conditions was expected as the experiment contained longer periods of action video presentation than fixation cross presentation. In line with the processing of the experimental conditions from the action observation phase, we selected EEG segments of 1 second from the action execution phase during which the behavioral proficiency test was conducted. Based on the video recordings, segments were selected in which the infant was grasping or reaching for the bead attached to our device. All but one infant provided valid segments during the action execution phase and on average, 16 segments were selected per infant (SD=11.64). These segments were later used to compare motor system activation during action execution with activation during action observation (i.e., video presentation) and determine the sample specific mu frequency range.

For each segment fast Fourier transforms (FFTs) were conducted and averaged across segments per condition. Consecutively the resulting power spectrum was averaged across 14 predetermined centroparietal electrodes (Fz, FC1, FC2, FC3, FC4, Cz, C1, C2, C3, C4, CP1, CP2, CP3, CP4; see Figure 4 for location of these electrodes). The electrode selection was based on previous research showing that in infants the mu-frequency peaks at the frontocentral to centroparietal electrode cites (Marshall & Meltzoff, 2011: Stapel et al., 2010; Van Elk et al., 2008; Southgate et al., 2009).

2.5.2.2 Determining the sample specific mu frequency range

Earlier research suggests that the power in the mu frequency range is more strongly attenuated during the execution of an action than during action observation (Southgate et al., 2009; Marshall & Meltzoff, 2011). This attenuation of power is thought to reflect activation of the motor system (Pineda, 2005; Marshall & Meltzoff, 2011). We therefore compared the EEG power during action

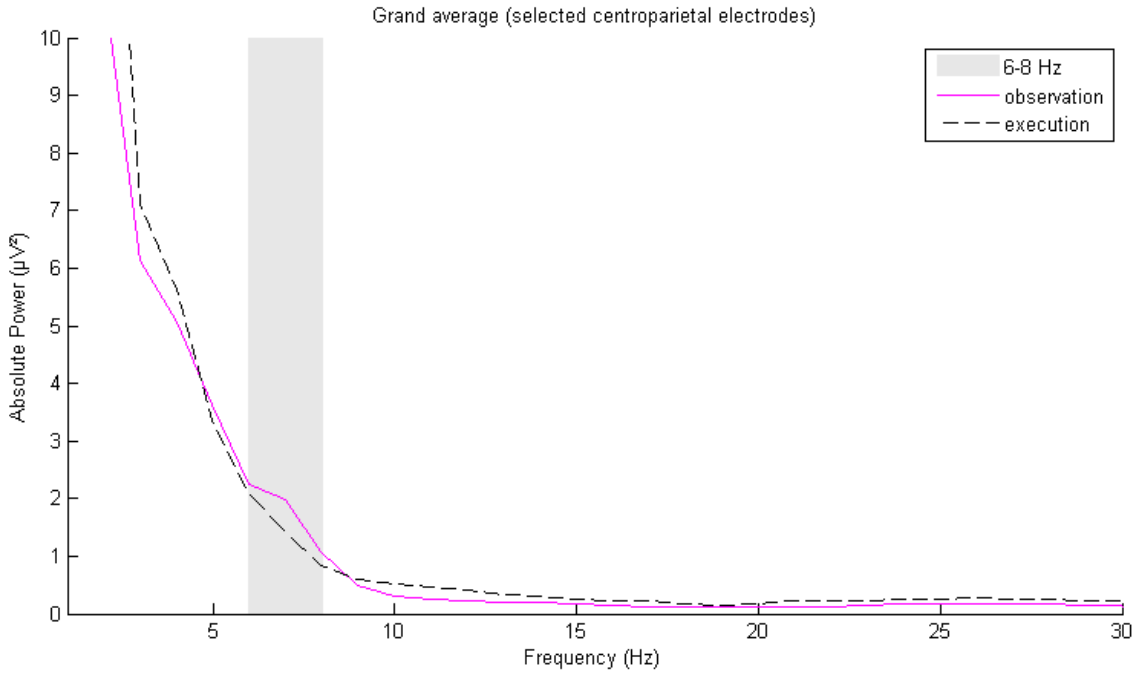

Fig. 5 Grand average of the powerspectrum of the observation and execution segments averaged across the 14 selected electrodes. A clear attenuation of the power during the action execution can be observed around 6-8 Hz.

Table 1.

Participants	Proficiency measures		
	Behavioral proficiency [duration pincer grasp touch/ (duration pincer grasp touch + duration hand touch)]	Proficiency questionnaire [parental questionnaire (score between 0 and 4)]	Proficiency span [age of testing-age of pincer grasp acquisition (in months)]
1	0.84	2	1.5
2	0.49	3	0.8
3	0.29	0	0.0
4	0.69	3	2.8
5	0.32	0	0.0
6	0.74	2	0.2
7	0.40	0	0.0
Mean	0.53	1.43	0.76

execution and observation in order to determine the specific mu frequency range of our sample. More specifically, we averaged the power spectrum of the fixation period, the correct and error condition and compared the grand average of these 'observation' segments to the grand average of the 'execution' segments acquired during the behavioral proficiency test. The grand average of the execution segments was based on six out of the seven infants because one infant did not provide usable EEG segments during this phase of the experiment. The resulting power was plotted against the frequency axis (Figure 5) and revealed a clear attenuation during action execution compared to observation between 6-8 Hz. This finding is in line with previous research which suggests that the mu frequency peaks around 6-9 Hz in infants of this age (Marshall, Bar-Haim, & Fox, 2002; Southgate et al., 2009; Berchicci, et al., 2010; Marshall & Meltzoff, 2011; Nyström et al., 2011).

2.5.2.4 Comparing motor system activation in the correct and error condition

To investigate motor system involvement during error observation, we used the determined frequency range (6-8 Hz) to compare mu frequency power during the observation of correct and erroneous pincer grasps. As mentioned above the FFTs of the segments were first averaged across the 14 electrodes of interest. Next, we averaged across the determined frequency range and calculated a normalized power difference of the two conditions per participant. A normalized power difference was used in order to control for baseline differences in mu frequency power between participants (see Meyer, Hunnius, van Elk, van Ede, & Bekkering, 2011 for a similar approach). The normalized power difference was computed by subtracting the power of the error condition from the power of the correct condition and dividing the result by the sum of both ([power correct - power error] / [power correct + power error]). The resulting normalized difference was compared using a one-sample t-test.

2.5.2.5 Correlation pincer grasp proficiency and motor system activation

One of the aims of this study was to assess in what way action proficiency influences the differential motor system activation. We therefore investigated the relationship between the three proficiency measures described above and the absolute normalized power difference of the two conditions. As we wanted to control for effects driven by maturation rather than experience, we evaluated the partial correlation of these measures controlling for the age of the infants.

3. Results

3.1 Pincer grasp proficiency

Table 1 gives an overview of the received proficiency scores per participant. None of the infants in the final sample received the maximum score for the parental questionnaire and neither did any infant manage to pull out the bead using the pincer grasp during the behavioral test. Although this indicates that in general our samples' proficiency was at the lower end of the proficiency spectrum, the infants nevertheless seemed to differ in their scores (see Table 1). This supports our assumption that variability in proficiency occurs around 8 months within the same age group.

Table 2.

		Behavioral p.	P. questionnaire	P. span
Behavioral proficiency	r	1	0.67	0.52
	sig (1-tailed)	-	*0.05*	*0.11*
Proficiency questionnaire	r		1	0.75
	sig (1-tailed)		-	*0.03**
Proficiency span	r			1
	sig (1-tailed)			-

In order to validate that our three proficiency measures were indeed assessing a similar construct (i.e. the level of proficiency in the pincer grasp), we calculated bivariate correlations of the three measures (Table 2). As we expected the three measures to correlate positively, we used one-tailed p-values to evaluate statistical significance. The score of the proficiency questionnaire correlated significantly with the proficiency span score (r=0.75, p=0.03) and the correlation with the behavioral proficiency score was marginally significant (r=0.67, p=0.05). The scores of the behavioral proficiency and the proficiency span however did not correlate significantly (r=0.52, p=0.11).

3.2 EEG analysis

3.2.1 Motor system activation in the correct and error condition

The main interest of this study was to investigate whether the motor system would be differentially activated during the observation of an erroneous compared to a correct action. Figure 6 shows the grand average of the powerspectrum (averaged across the 14 selected electrodes of interest) for the error and correct condition plotted against the frequency axis. To investigate differences in motor system activation, we inspected the difference in normalized power for the correct and error condition within the determined mu frequency range (6-8 Hz, see above). A one sample t-test revealed that the difference in normalized power between the two conditions was significantly different from zero (t(6)=3.09, p=0.02). More specifically, there was a significant decrease in mu frequency power for the correct compared to the error condition (see Figure 6). With an effect size of 0.78 this effect turned out to be large (r=0.78). As the analysis was based on a small sample of seven infants, we inspected the absolute mu frequency power of both conditions per participant (see Figure 7). Inspection of the individual data revealed that five

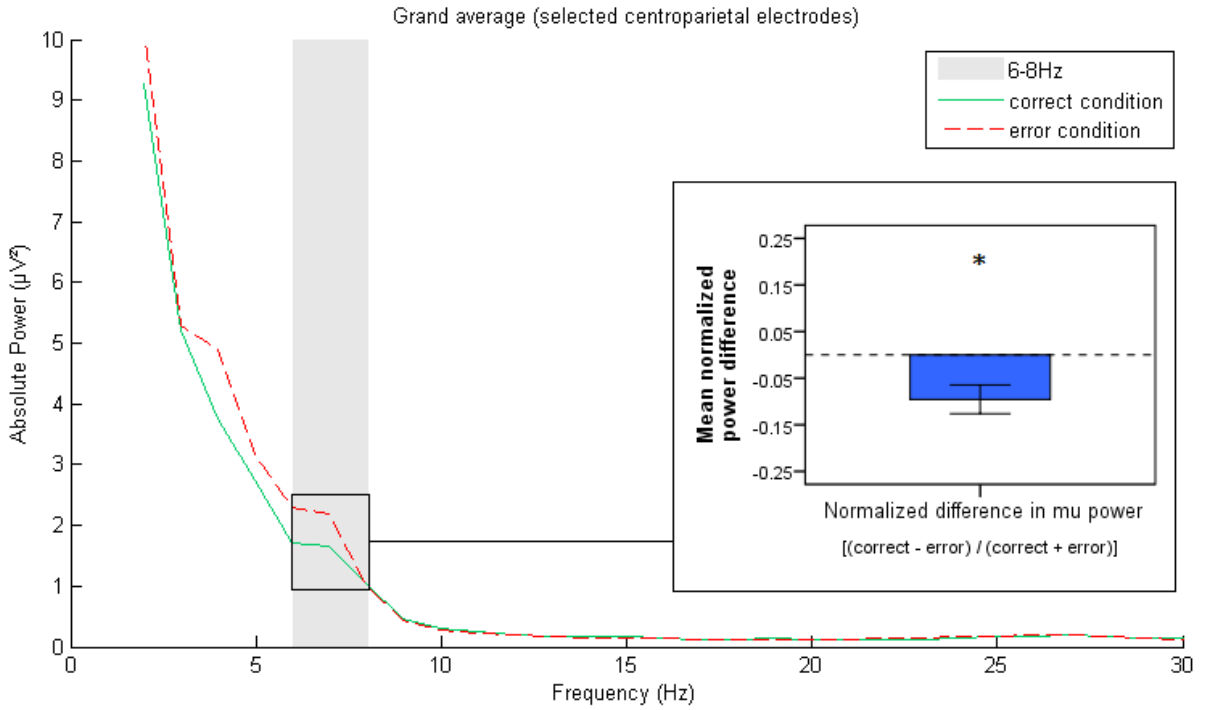

Fig. 6 Grand average of the powerspectrum of the error and correct condition averaged across the 14 selected. A closer inspection of the normalized power in the mu frequency range (6-8Hz) revealed a significant difference between the conditions.

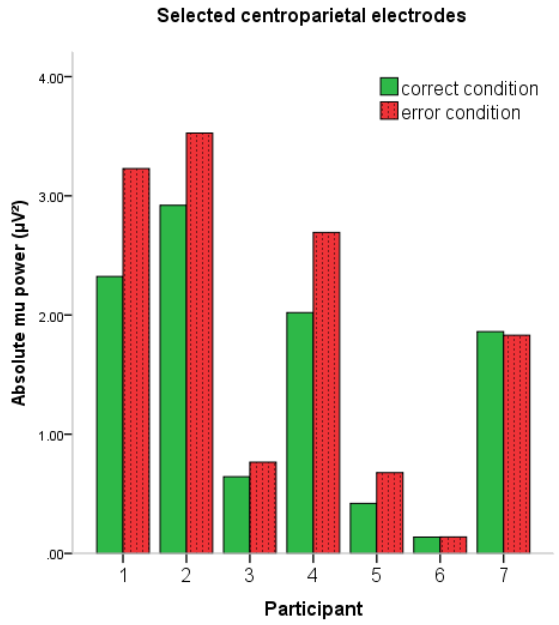

Fig. 7 Absolute mu frequency power in the correct and error condition per participant.

out of seven infants showed attenuated mu power in the correct compared to the error condition and none showed the opposite effect.

Visual inspection of the power spectrum (Figure 6) suggested that the difference between the conditions might extend into lower frequencies ranges. As Berger and colleagues (2006) also reported that the power in the theta frequency range was modulated by the correctness of an observed action, we decided to test for differences between the conditions in the theta range as well. In line with our main analysis we calculated the normalized power difference between the conditions for the theta frequency range (2-6Hz). Two one-tailed t-tests revealed no significant differences, neither above centroparietal areas (including the 14 a priori selected electrodes) ($t(6)=0.39$, $p=0.71$), nor above more frontal areas (including electrodes F3, Fz, F4, FC1, FC2, FC6, C1, C3, Cz, C4, C2, FC3, FC4, see Figure 4 for the location of these electrodes) ($t(6)=0.42$, $p=0.69$). These results suggest that the condition difference in our experiment was restricted to the mu frequency.

Topographic maps of the correct and error condition and the differential activation can be found in Figures 8 and 9, respectively. Visual inspection of theses maps suggested that condition differences in the mu frequency range were mainly restricted to centroparietal sites, associated with motor system activation.

In order to further validate that the effect in the mu frequency range was indeed distinguishable from possible differences in occipital alpha, we examined condition differences in the alpha frequency range (6-8Hz) at occipital sites. Similar to our main analysis, we computed the normalized power difference ([power correct - power error] / [power correct + power error]) for the alpha frequency range at three occipital electrodes (O1, Oz, O2). These electrodes were selected based on earlier research suggesting that occipital alpha is most pronounced in posterior electrodes (see Pineda, 2005). A one sample t-test revealed no significant difference between the conditions ($t(6)=1.56$, $p=0.17$), validating that the condition difference in the mu frequency was indeed restricted to the centroparietal electrodes.

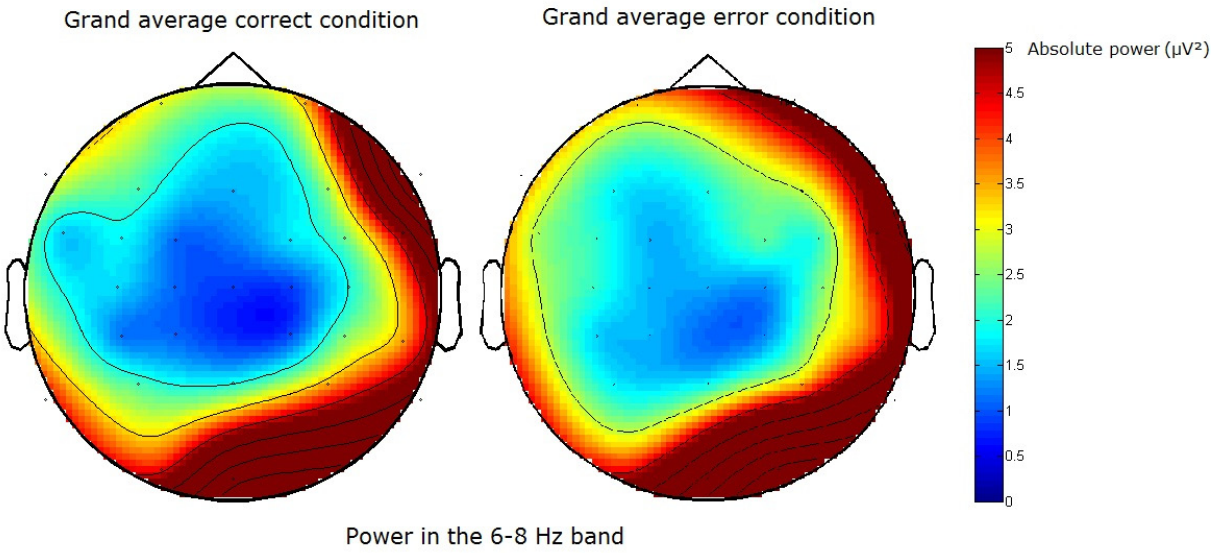

Fig. 8 Topographic map displaying the power in the mu frequency range for the correct condition (left) and the error condition (right). Dark blue colors represent a relative low power whereas green and red colors represent a relative high power.

3.2.2 Correlation pincer grasp proficiency and motor system activation

An additional aim of this study was to investigate in what way the motor system involvement was modulated by the amount of action experience. In order to investigate this relationship, partial correlations between the proficiency measures and the absolute normalized mu power difference were calculated, controlling for age. As can be seen in Table 3, the partial correlation between the behavioral proficiency score and the absolute normalized mu power difference reached significance (r=-0.83, p=0.04), while the other correlations were non significant. Interestingly, the behavioral proficiency correlated negatively with the absolute normalized mu power difference (see Figure 10), indicating that with increasing proficiency the difference in power between the correct and error condition decreases.

4. Discussion

This study investigated the role of the motor system during error observation in 8-month-olds. Previous research in adults suggests that the motor system is differentially activated when a person is observing an erroneous compared to a correct action (Koelewijn et al., 2008). In addition, a study by Aglioti and colleagues (2008) suggests that experience with the observed action also influences the motor system activation. The aim of the current study was to investigate how action experience in young infants influences their motor system response to observed actions.

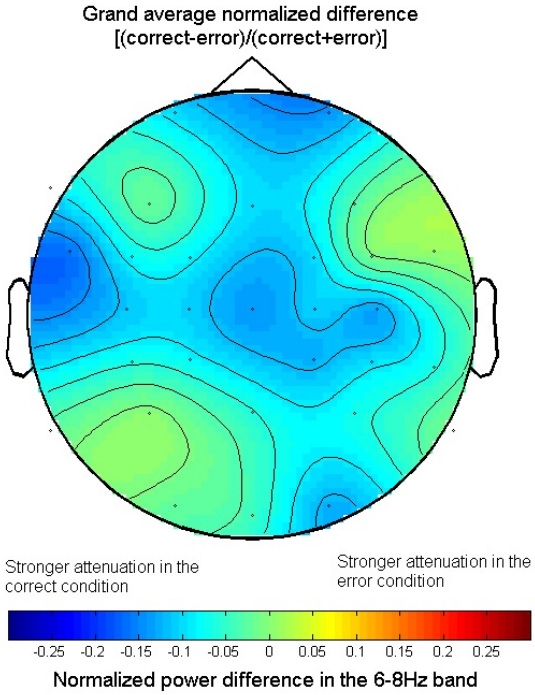

Fig. 9 Topographic map displaying the normalized power difference in the mu frequency range.

Table 3.

		Absolute normalized power difference
Behavioral proficiency	r	-0.83
	sig (2-tailed)	*0.04**
Proficiency questionnaire	r	-0.40
	sig (2-tailed)	*0.44*
Proficiency span	r	0.05
	sig (2-tailed)	*0.93*

In the present study, the infants were presented with video clips of correct and erroneous pincer grasps while their EEG was recorded. In addition, the infants' pincer grasp proficiency was measured in order to assess the influence of action experience on motor system activation in this context. Statistical analysis revealed that the observation of a correct action elicited stronger attenuation of the power in the mu frequency band compared to the erroneous action. This effect appeared consistent as it was apparent in 5 out of the 7 infants included in the final analysis. We conclude that the reported attenuation of mu power reflects activation of the motor system for several reasons. Firstly, we used a sample specific frequency range for the comparison of the two conditions which was based on recordings during action execution. Importantly, the selected frequency range (6-8 HZ) matched earlier findings investigating the mu rhythm in infants (Berchicci et al., 2010; Stapel et al., 2010; Marshall & Meltzoff, 2011). Secondly, visual inspection of the topographic maps showed that the distribution of the effect was restricted to centroparietal sites, associated with motor system activation (Pineda, 2005; Marshall & Meltzoff, 2011). Additional statistical analyses revealed no condition differences in lower frequency ranges, confirming that our effect was specific to the mu frequency range.

The reported difference between mu power in the correct and error condition thus suggests that in 8-month-old infants the motor system is involved during error observation. However, at first sight the finding that motor system activation was enhanced during the observation of a correct action seems to be contradictory to previous adult findings. Previous studies namely suggest that the observation of an erroneous action increases motor system activation (Aglioti et al., 2008; Koelewijn et al., 2008).

However, we propose that the present effect can

be explained in terms of a modulation of the motor system activation by the observer's stage of action proficiency (see Figure 11). The infants included in the current sample had a relatively low level of (pincer grasp) proficiency and none of the infants were yet able to perform the pincer grasp during the action execution phase of the experiment. The reviewed adult studies, however, have investigated the motor system in more proficient observers. Koelewijn and colleagues (2008) for instance, who found motor system enhancement during the observation of erroneous actions, presented adult volunteers with another person pressing a button. Although this study was not designed to investigate the influence of action proficiency, we can assume that all the adults were extremely proficient in this simple action of pressing a button. Aglioti and colleagues (2008) on the other hand compared basketball experts and novices who had to observe and judge basketball shots. Interestingly, in this study only the action experts showed an increase in motor system activation for the erroneous actions, whereas novices showed similar activation for both actions. Notably, although the novices had less experience with the presented actions, we can assume that they were generally able, but not specialized, to perform the observed action themselves and thus had a moderate level of proficiency. Yet, to our knowledge, it has not been investigated, how the motor system processes erroneous actions, which the observer is unable to perform. The present study provides neurocognitive data from infants who were at the verge of acquiring the observed action and were hence still low in

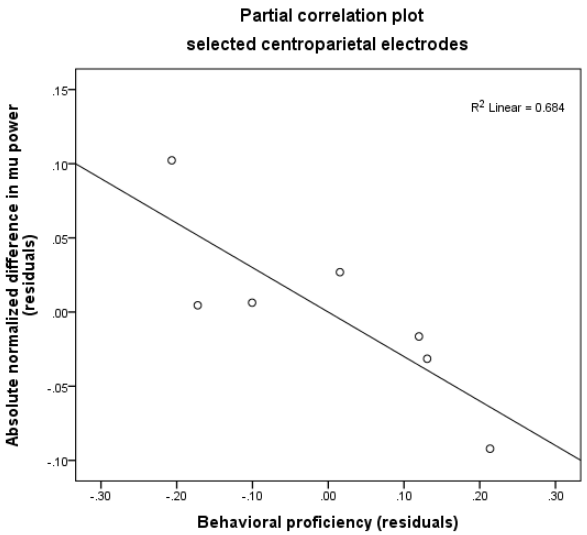

Fig. 10 Scatter plot of the partial correlation between the absolute normalized difference in mu power and the behavioral proficiency score.

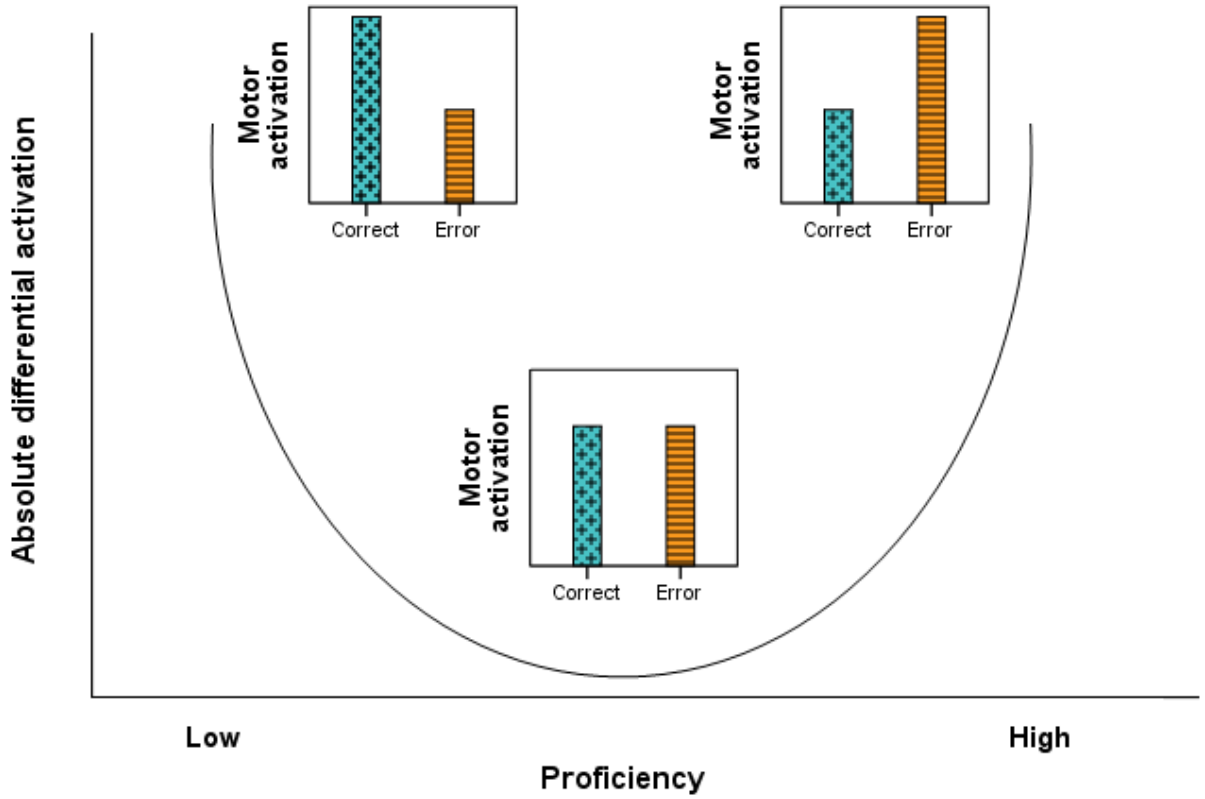

Fig. 11 Illustration of the proposed U-shaped relationship between motor system activation during error observation and action proficiency. We suggest that motor system activation might be modulated by the stage of the observers' action proficiency. This hypothetical model reconciliates the data from our current sample, representing lower stage of proficiency with earlier adult research, representing higher stages of proficiency (Aglioti, et al., 2008; Koelewijn et al., 2008).

proficiency. In fact, none of the infants in the present sample where able to pick up a small object using the pincer grasp. The present results therefore extend the previous findings on the relationship between action proficiency and motor system involvement during error observation (see Figure 11) by providing data from infants at the lower end of the proficiency spectrum. More specifically, in our low proficient sample an enhancement of motor system activation for the correct rather than the erroneous action was found. Together with previous work in adults these results suggest a U-shaped pattern, describing the relationship between proficiency and differential motor system activation. Figure 11 shows an illustration of this U-shaped relationship. At the beginning state of skill acquisition, the correct action activates the motor system more strongly, whereas with increasing proficiency the pattern flips and once expertise is acquired the erroneous action elicits stronger activation. Additional support for this proposed model comes from our partial correlation analysis. We found a significant negative partial correlation between the behavioral proficiency and the absolute normalized difference in mu power. In line with the hypothesized U-shaped relationship (see Figure 11), this suggests that within a sample of low proficient infants, differential motor system activation decreases with increasing proficiency.

Although, at this point, we can only speculate about the underlying mechanisms responsible for the shift in activation with increasing proficiency, we propose that it might be caused by a bias towards a certain action type depending on the stage of proficiency. As mentioned earlier, the infants from the current sample were relatively low in (pincer grasp) proficiency and at the beginning of acquiring this novel motor skill. It might therefore be the case that the infants were biased towards the correct action, displaying a motoric skill they were currently learning. Several studies with adults have shown that the intention during action observation can influence motor system activation (Grèzes, Costes, & Decety, 1999; Frey & Gerry, 2006; Muthukumaraswamy & Singh 2008; Suchan, Melde, Herzog, Hömberg, & Seitz, 2008). For example, a study by Frey and Gerry (2006) has shown that when the observer needs to learn from a presented action, activation in areas involved in motor representation is enhanced. Similarly, Muthukumaraswamy and Singh (2008) used MEG to investigate motor system activation during the observation of finger movements. The participants were either instructed to simply observe the movements or they were told that they had to reproduce the presented movements later. The researchers found an enhancement of beta band desynchronization, reflecting increased activation of the motor system, when participants were observing finger movements that they had to reproduce later, compared to passive observation condition. In line with these findings, the stronger motor activation for the correct condition in the present experiment might reflect that the infants were attempting to learn the pincer grasp from the presentation of the correct action. Notably, we observed several of the infants overtly imitating the observed pincer grasp during the presentation of our videos. Although we did not quantify this behavior it supports the idea that infants might have been trying to learn from the presented actions.

Furthermore, our model suggests that once proficiency with the observed action is acquired, infants would be biased towards the erroneous action. Based on previous research, we propose that this bias might be related to the involvement of the motor system in predicting action outcomes (Kilner et al., 2007; Stapel et al., 2010). Stapel and colleagues (2010) found that actions which developed differently than expected elicited enhanced motor system activation compared to usual actions. In line with these findings, once proficiency with an action is acquired, the observation of an erroneous action might violate the expectation of the observer and thus lead to an enhancement of motor system activation.

We are well aware that our current results are only tentative and that additional research will be needed to provide evidence for the proposed model. The model provides clearly defined and testable predications of the activation pattern at different stages of proficiency, which can be used to guide future research. More specifically, we suggest testing more proficient infants with the current set-up in order to provide additional support for the U-shaped relationship. In addition, we recommend testing adults with our stimuli in order to replicate earlier findings. Koelewijn and colleagues (2008) used visually identical stimuli, whereas our videos were visually distinct for the two conditions. We statistically validated beforehand that the amount of motion was similar for both conditions and this rules out that our results were only driven by low-level differences in the stimuli (Cross et al., 2011). Although we therefore expect our stimuli to evoke activation patterns comparable to earlier findings, collecting adult data would be valuable in order to test the predictions of the proposed model within one experimental design.

For future research, we also recommend to

include additional baseline periods. Due to the low number of fixation trials in this experiment, we directly compared the correct and error condition. In the future, however, it would be interesting to compare both conditions against a baseline period and to investigate whether effects are driven rather by suppression of activation in one condition or by enhancement of activation in the other condition.

Notably, the dropout rate of the current study was considerably high (73%). It was however comparable to previous studies implementing EEG in infants and young children (Striano, Reid, & Hoel, 2006; Stapel et al., 2010; Meyer et al., 2011). In the case of the present study, the small amount of included infants can be explained by the strict inclusion criteria. More specifically, as we were interested in motor system activation elicited by the observation of an action, it was necessary to exclude all trials containing overt movements of the infant.

Last, it is important to note that activation differences in our study were restricted to the mu frequency range. This is in line with a number of previous studies investigating action observation in infants (e.g., Southgate et al., 2009; Stapel et al., 2010). However, several studies with adults and infants have shown a reduction in oscillatory beta power during action observation as well (Koelewijn et al., 2008; van Elk et al., 2008). Similar to an attenuation in mu frequency power, a reduction of power in the beta frequency is also associated with activation of the motor system (Jurkiewicz, Gaetz, Bostan, & Cheyne, 2006; Parkes, Bastiaansen, & Norris, 2006). To date, it is unclear why some researchers investigating action observation in infants find modulations only in the mu frequency range, whereas other infant and adult studies report modulations in both mu and beta frequency ranges. One reason for these differences might be the distinct neural source and function associated with the two movement-related oscillations. Several studies suggest that the source of the mu rhythm is located near the somatosensory areas, whereas the origin of the beta rhythm is more anterior, close to motor areas (Pfurtscheller, Pregenzer, & Neuper 1994; Salmelin & Hari, 1994; Jurkiewicz et al., 2006; Parkes et al., 2006). Although the exact functional role of the two movement-related oscillations is still debated, the mu rhythm has been proposed to be involved in linking perception and action (Pineda, 2005). Beta band oscillations, on the other hand, are thought to be related to the maintenance of the current motor set (see Engel & Fries, 2010 for a review on the functional role of beta band oscillations). In addition, the absence of differences in the beta frequency range in the current study might be due to a general reduction of power in higher frequency bands in young infants (e.g., Marshall et al., 2002; Gaetz, MacDonald, Cheyne, & Snead, 2010). More specifically, Gaetz and colleagues (2010) reported that the attenuation of the movement-related beta frequency power was reduced in younger children and increased only at a later age. Hence, our measures might not be sensitive enough to detect beta frequency differences in 8-month-old infants. Notably, van Elk and colleagues (2008), who found differences in the beta frequency range in infants, tested 14- to 16-month-olds who were thus almost twice as old as the infants participating in the present study. However, further research will need to clarify the nature of the different findings and further establish the development and functional role of both movement-related oscillations.

5. Conclusion

The present study provides evidence for motor system involvement during error observation in young infants and suggests that activation is modulated by the observers' stage of proficiency. Combing the present findings with previous research, we propose a U-shaped relationship between differential motor system activation and action proficiency. Still, future research will need to extend our findings and validate the proposed model.

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Interaction of DNA Methyltransferase 3B Gene With Childhood Adversity on Hippocampal Volume

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The early environment plays an important role in the development of major psychiatric disorders. Emerging evidence suggests that DNA methylation is a key mechanism in the process by which the environment induces persistent change in the individual. Accordingly, variations in the epigenetic mechanism may modulate the impact of the environment across individuals. In mammals de novo DNA methylation is catalyzed by DNMT3A and DNMT3B.We investigated whether single nucleotide polymorphisms in DNMT3A and DNMT3B affect hippocampal volume depending on childhood adversity (CA) in two samples of healthy, young, adults. Genetic and magnetic resonance imaging data of 476 participants were used. A set-based analysis was used to test for effects of variations in DNMT3A and DNMT3B in the CA and no-CA groups separately. In both cohorts a trend ($p<0.10$) was observed for DNMT3B in the no-CA groups. Additional analyses combining the two cohorts revealed a significant association between DNMT3B and hippocampal volume in the no-CA group, while all other associations were non-significant. This potential interaction effect between DNMT3B and CA on hippocampal volume was further examined through analysis of the most significant single nucleotide polymorphisms (SNP) (rs2424932) within DNMT3B and a significant SNP-by-CA interaction was found for the combined cohort. This study shows that variations in DNMT3B may interact with CA on hippocampal volume in the healthy population and identifies a functional SNP of interest within this gene for further investigation.

Keywords: DNA methylation, childhood adversity, gene-environment interaction, DNMT3B, DNMT3A

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1. Introduction

It is widely accepted that complex diseases such as major depressive disorder are the product of interacting genetic and environmental factors. Though most disorders involve substantial genetic predisposition, it is clear that the environment also plays a crucial role in their development. For example, childhood adversity (CA) appears to increase the risk for developing psychiatric disorders (Hammen, 2005) and associated endophenotypes (Green et al., 2010; Rao et al., 2010). CA has also been shown to interact with disease relevant genes such as 5-HTT and BDNF (Aguilera et al., 2009; Gerritsen et al., 2012). How such environmental factors are translated to stable changes on a molecular level remains an open question however.

Epigenetics may shed light on the mechanisms through which the environment induces persistent change in organisms, and help us understand the etiology of complex disorders. Epigenetic mechanisms regulate gene expression by modifying the structure of DNA without changing the DNA sequence, and have been shown to react to environmental factors such as stress and neuronal activity (Ma et al., 2009; Martinowich et al., 2003; Weaver et al., 2004). Differences in gene expression can have major effects on the functioning of a cell and as a result lead to lasting alterations in brain function and behavior.

Historically, interest in epigenetic mechanisms has mostly come from the field of cancer research, where the heritability of gene expression patterns from parent to daughter cells is essential. In the brain such processes were considered only relevant during neural development and of little importance in post-mitotic neurons, since their epigenetic patterns were assumed to remain unchanged. More recent studies have highlighted the dynamic nature of epigenetic modifications throughout the lifespan, however (Siegmund et al., 2007), and the role of epigenetic processes in psychiatric disorders has received increasing attention (Schroeder, Hillemacher, Bleich, & Frieling, 2012; Toyokawa, Uddin, Koenen, & Galea, 2012). Evidence for involvement of epigenetic mechanisms in the pathogenesis of major depressive disorder and schizophrenia is accumulating, converging from both animal and human studies (Albert, 2010; Gavin & Sharma, 2010; Mill et al., 2008; Murgatroyd et al., 2009; Oberlander et al., 2008; Tremolizzo et al., 2002).

The two major mechanisms for epigenetic regulation are DNA methylation and histone modification (Schroeder et al., 2012). Histone modification refers to the addition of certain groups (like methyl or acetyl) to the tails of the histones around which DNA is wound. This process changes the chromatin to a denser or more open form, thus affecting the accessibility of genes to transcription factors. In DNA methylation, methyl-groups are attached to cytosine residues in the DNA. This process usually occurs at cytosine/guanine dinucelotide (CpG) regions and is catalyzed by DNA methyltransferases (DNMTs), which transfer and bind methyl groups to the residues. DNA methylation generally reduces expression of the affected gene; the methyl-groups block access of transcription factors by attaching closely to promoter regions, or make transcription more difficult by attracting other proteins that change the chromatin structure to a denser form. This is illustrated in Figure 1. DNA methylation is the most stable known form of epigenetic modification and can last a lifetime; an important characteristic given the long-lasting and recurrent nature of psychiatric disorders. Altered DNA-methylation patterns have been most notably associated with major depressive disorder, post-traumatic stress disorder and schizophrenia (see Toyokawa et al. (2012) for an overview).

DNA methylation in mammals occurs through the three active DNA methyltransferases (DNMTs) DNMT1, DNMT3A and DNMT3B (Bestor, 2000). They are crucial enzymes in establishing and maintaining the DNA methylation pattern. In general, DNMT1 is thought to be involved in the maintenance of the methylation pattern whereas DNMT3A and DNMT3B appear to be largely responsible for establishing new methylation patterns. Especially the latter two may play significant roles in pathogenesis of psychiatric disorders, since they are involved in (environmentally induced) changes in methylation. Therefore, the current study focuses on variations in DNMT3A and DNMT3B as potential modulators of environmental contribution to complex diseases. We use a gene-wide analysis to investigate interaction effects between common single nucleotide polymorphisms (SNPs) in DNMT3A/DNMT3B and CA on hippocampal volume in two cohorts of healthy participants. The hippocampus is a key intermediate phenotype for psychiatric disorders, and structural changes have often been reported in both psychiatric patients and high-risk individuals (Rao et al., 2010; Videbech & Ravnkilde, 2004; Wright et al., 2000). Moreover, DNA-methylation pattern changes in patients and animal models are also most commonly found in the hippocampus. Therefore, we considered

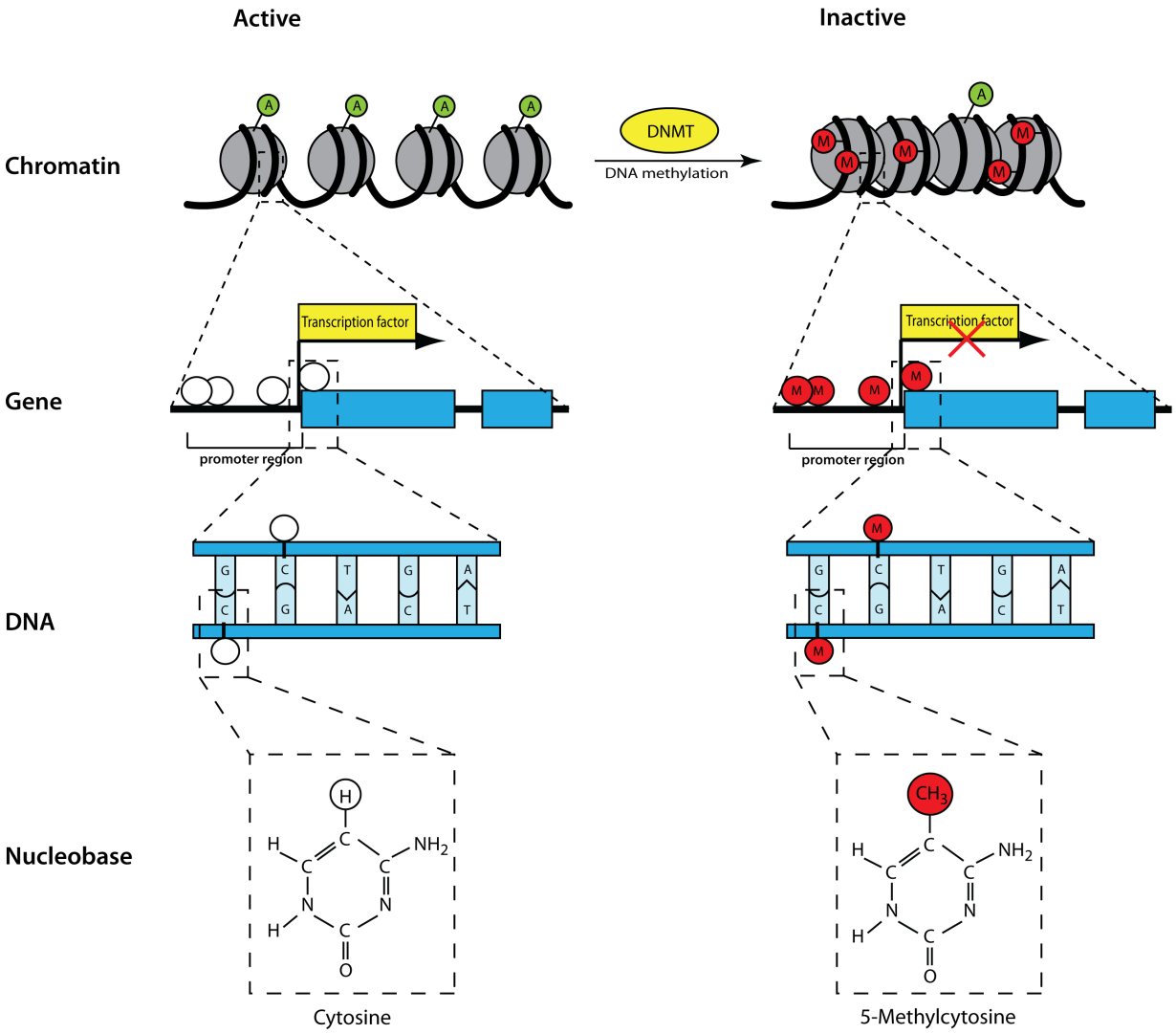

Fig. 1 A schematic overview of DNA methylation. **1A)** At the chromatin level DNA methylation represses gene transcription through interaction with histone modification proteins. DNA is wound around histone octamers, here visualized as spheres. Left shows a conceptualization of active chromatin; histones are acetylated (A) and a relatively large part of DNA is unmethylated. This is associated with an open structure allowing binding of transcription factors. Certain environmental
signals are associated with increased DNA methylation (M), catalyzed by DNA methyltransferase enzymes. Methylated DNA can recruit methyl binding domain proteins, which are associated with large complexes containing histone deacetylases and histone methylases. This process results in an inactive, condensed form of chromatin in which histones are deacetylated and DNA methylated. **1B)** At the gene level, DNA methylation may block transcription factors from accessing promoter regions. In the unmethylated state transcription factors can access the promoter region and start transcription of the gene. Methylation at the promoter prevents transcription factors from binding to the promoter region, reducing expression. **1C)** Effect of methylation on DNA level. Methylation typically occurs at CpG sites. **1D)** Methylation at the molecular level. A methyl-group is added to the 5-carbon position of Cytosine.

the hippocampus as the primary brain structure of interest to show an interaction of DNMT3A/DNMT3B genotype with CA.

2. Methods

2.1 Participants

The present study is part of the Brain Imaging Genetics project at the Donders Institute for Brain, Cognition and Behaviour at the Radboud University Medical Centre. Data were available for 481 healthy participants from Caucasian descent. The age in the sample ranged from 18 to 35 years (mean = 22.4 years, SD = 3.28) and 62.8 percent were female. All participants gave written informed consent and were screened for study inclusion using a self-report questionnaire. The exclusion criteria were current or past neurological or psychiatric disorder, medication, history of somatic disease with potential effects on the brain, substance abuse, illicit drug use during the past 6 months, current

or past alcohol dependence, pregnancy, lactation and menopause. The sample consisted of two independent cohorts, one scanned using 1.5 Tesla MRI scanners and one scanned using 3.0 Tesla MRI scanners. These cohorts were analyzed separately to provide an independent replication of results.

2.2 Neuroimaging Data

For all participants T1-weighted structural magnetic resonance imaging data were acquired at either 1.5 Tesla (n = 220) using Siemens Sonata and Avanto scanners or at 3.0 Tesla (n = 261) using Siemens Trio or TrioTim scanners. All T1-weighted structural MRI data covered the entire brain and had a voxel-size of 1 mm3. Neuroimaging procedures and sequences are as described by Franke et al. (2010) and Gerritsen et al. (2012).

Raw DICOM MRI data were converted to NIFTI format using the conversion as implemented in Statistical Parametric Mapping package 5 (SPM5) (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). Normalization, bias correction and segmentation into grey matter, white matter and CSF were performed using VBM5.1 Toolbox version 1.19 (http://dbm.neuro.uni-jena.de/vbm/) in SPM using default settings. The total volume of grey matter, white matter and CSF was determined by adding the tissue probabilities resulting from this analysis. Total brain volume was defined as the sum of white matter and grey matter volume.

Hippocampal volume was determined through automated segmentation using the FIRST module from FSL (FMRIB's Integrated Registration and Segmentation Tool version 4.1.5). The module uses a Bayesian model for shape and appearance of the hippocampus based on 336 manually-labeled T1-weighted MR images. Using the learned models, FIRST gives the most probable shape given the observed intensities in the T1 image (Patenaude, Smith, Kennedy, & Jenkinson, 2011). Subsequently, volumes were determined by multiplying the number of voxels with the voxel volume of 1 mm3. Volumes of bilateral hippocampus were summed for analysis.

2.3 Genetic Data

DNA was extracted from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to manufacturer protocol. Genotypes for DNMT3A and DNMT3B were available from genome-wide genotyping using Affymetrix GeneChip SNP 6.0 arrays and subsequent imputation using HapMap2 as a reference sample. The call rate threshold was set at 90% for the arrays. Sixty-six SNPs were detected within DNMT3A or 25kb up- and downstream of the gene (capturing regulatory sequences). For DNMT3B, 77 such SNPs were found. After filtering out SNPs with a minor allele frequency lower than 0.05 or Hardy-Weinberg equilibrium p-value lower than 1.0E-06, 64 and 67 SNPs were left in DNMT3A and DNMT3B respectively.

2.4 Childhood Adversity

Childhood adversity (CA) was assessed retrospectively using a short version of the List of Threatening Life Events, as developed and validated by Brugha, Bebbington, Tennant, and Hurry (1985). The adapted list by Brugha et al. (1985) was used because of its reduced length compared to the original, and was shown to account for the majority of reported life events with moderate to high long-term threat. Participants indicated whether they had experienced any events from the list of events before the age of 16, such as parental loss or abuse. This variable could not be normalized and therefore we dichotomized the scores such that presence of CA was defined as having experienced at least one such event. The questionnaire was part of a larger web-based test battery of the Brain Imaging Genetics project (http://www.cognomics.nl/big).

2.5 Analysis

Using Predictive Analytics Software version 18.0.0 (PASW 18) the hippocampus to total brain volume ratio was converted to standardized Z-scores for the two cohorts separately. Five participants, two from the 1.5T cohort and three from the 3.0T cohort, were excluded from analyses because their ratio scores deviated from the mean by 3 standard deviations or more.

Associations between DNMT3A and DNMT3B with hippocampal volume were assessed using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007) using set-based tests with DNMT3A and DNMT3B as separate sets (Neale & Sham, 2004; Soerensen et al., 2012). This gene-based framework aggregates single SNP statistics and corrects for multiple testing within the gene. Specifically, for each SNP a standard linear regression is performed, and the linkage disequilibrium (LD) r^2 with every other SNP is determined. For each gene up to 10 SNPs with p-values below 0.05 are selected, with the best SNPs

being selected first. If certain SNPs are in high LD (r^2>0.80), only the SNP with the lowest p-value in the LD-block is selected. The statistic for a gene is calculated as the mean of the selected SNPs. Significance of the statistic is estimated using a permutation test; the analysis described above was repeated with permuted phenotype labels 10000 times. The empirical p-value reflects the number of times a permuted gene-statistic exceeds the original one, giving an approximation of the chance that our current gene statistic would be found purely by chance and not due to a relationship between the gene and the phenotype. Using this method the p-value cannot be determined if none of the SNPs pass the initial threshold. The advantage of combining information from markers within a gene is that associations between gene and phenotype can be detected even if each SNP only carries a modest amount of information or in the case of allelic heterogeneity.

To test whether the effect of the DNMT3A or DNMT3B was contingent on CA, the analysis was done in the CA and no-CA groups separately. Due to limitations of the software package the interaction could not be tested straightforwardly while including the covariates. DNMT3A or DNMT3B was taken as the independent variable, hippocampal volume as the dependent variable and age, sex and total brain volume as covariates. The same analysis was done for the 1.5T and 3.0T cohorts combined to increase power. For this analysis Tesla was included as a covariate.

Following the gene-based approach, SNPs that may interact with CA on hippocampal volume were further examined using a general linear model using PASW 18. The model included CA and the relevant SNP as independent variables, hippocampal volume as the dependent variable, and age, gender and total brain volume as covariates. The analyses were repeated for the 1.5T and 3.0T groups combined with Tesla as an additional covariate. Significance level was set at p<0.05.

3. Results

Table 1 shows the demographic data and childhood adversity scores for our sample. We assessed whether there is an effect of DNMT3A or DNMT3B genotype on hippocampal volume contingent on CA presence by performing a setbased analysis separately for the CA and the no-CA group. The analyses showed a trend for DNMT3B on hippocampal volume in the no-CA group in the 1.5T cohort (p=0.10), but not in the CA group (p-value unavailable because no SNPs exceeded significance threshold). These findings were confirmed in the 3.0T cohort, with p=0.09 for no-CA group and no SNPs exceeding the threshold in the CA group. For DNMT3A no significant associations were found in either group in either cohort. Main effects of DNMT3A or DNMT3B on hippocampal volume were also tested using PLINK. As expected the gene-wide test revealed no evidence for a main effect in either cohort.

To explore the effects further, additional analyses were done with the two cohorts combined to increase statistical power. If the correlations between DNMT3B SNPs and hippocampal volume reflect random fluctuations, one would expect that such effects become smaller when the two groups are combined. However, the gene-wide test on combined cohorts revealed a significant effect of DNMT3B on hippocampal volume in the no-CA group (p=0.04), but not in the CA group (p-value unavailable because no SNP exceeded threshold).

Next, the SNPs that showed a significant association in our sample were investigated in more detail. Only two SNPs exceeded the significance threshold in the single-SNP statistics in the combined group analysis of the no-CA group: rs2424932 (p=0.005) and rs437302 (p=0.02). The location of the SNPs was identified using the Database of Single Nucleotide Polymorphisms (dbSNP, Build ID: 137 for human, Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine). Rs2424932 showed the strongest

Table 1. Characteristics of current study sample.

	1.5T cohort (N=218)	**3.0T cohort (N=258)**	**Combined (N = 476)**
Age (range 18 – 35 years)	22.57	22.33	22.44
	(3.428)	(3.149)	(3.278)
Female	62.40%	64.00%	63.20%
Childhood adversity (yes)	69.70%	71.30%	70.60%

association and is located on the 3' UTR region of DNMT3B, whereas rs437302 is in a noncoding region flanking the DNMT3B gene. Therefore, our main interest for further analysis was in rs2424932 as a potentially functional SNP. To assess whether this SNP has functional consequences and to better understand the differences between each group, the relationship between rs2424932 and hippocampal volume was further examined with a general linear model (GLM) using PASW18. Because of the small number of participants in the homozygous minor allele (A) group when taking into account presence of CA, the homozygous minor allele group was grouped with the heterozygous. Table 2 shows the distribution of participants according to CA and SNP. The interaction effects in the 1.5T and the 3.0T cohorts, perhaps due to limited statistical power, are not significant separately (Figure 2A, p=0.168 and p=0.058 respectively), but in the combined cohort a significant interaction (F=5.651, p=0.018) was found between CA and rs2424932. Post-hoc tests showed that participants who have experienced childhood adversity, have smaller hippocampal volume compared to those who have not, but only for the GG-genotype (homozygous for the major allele). The effect seems mainly due to larger hippocampi in GG individuals who have not experienced CA compared to the other groups (Figure 2B).

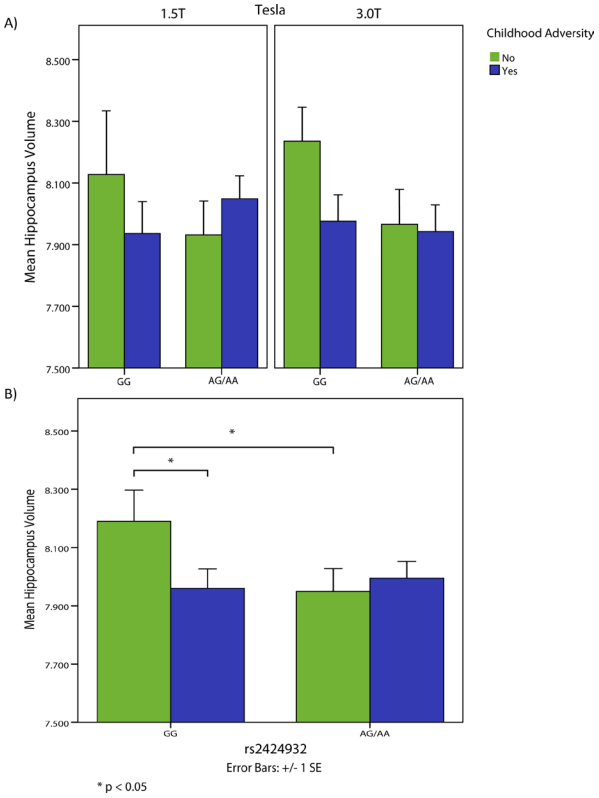

Fig. 2A. Interaction of rs2424932 with childhood adversity on hippocampal volume in the 1.5T (left) and 3.0T (right) cohorts. **2B.** Interaction of rs2424932 with childhood adversity on hippocampal volume in the combined cohort. Hippocampal volume decreases with presence of CA for the major allele homozygotes (G) only. The major allele(G) homozygotes who have not experienced CA have a significantly larger hippocampal volume than the heterozygotes and minor allele(A) homozygotes who have no CA experience.

4. Discussion

One mechanistic explanation of how environmental factors contribute to psychiatric disorder is that stressful events cause altered DNA methylation and subsequently altered gene expression in the brain. Some DNMT3B polymorphisms have been implicated in increased risk for early onset schizophrenia (Zhang et al., 2009) and suicide attempt (Murphy et al., 2013), but no functional polymorphisms in DNMT3A and DNMT3B have been firmly established. Given the importance of these enzymes in constructing the epigenetic pattern and the assumed role of epigenetics in complex diseases, genetic variance in these genes may modulate the impact of environmental factors

Table 2. Distribution of sample across rs2424932 genotype, childhood adversity and scanner field strength.

		rs2424932		Total
		GG	AA/AG	
1.5T	no CA	20	46 (10/36)	66
	CA	53	99 (15/84)	152
	Total	73	145 (25/120)	218
3.0T	no CA	27	47 (10/37)	74
	CA	82	102 (18/84)	184
	Total	109	149 (28/121)	258
Combined	no CA	47	93 (20/73)	140
	CA	135	201 (33/168)	336
	Total	182	294 (53/241)	476

on disease vulnerability substantially. Effects may mainly be asserted through interaction with other environmental or genetic factors however, leading to a failure to detect the gene through analyses that focus on main effects only, such as genome-wide association studies. Here we used a gene-wide approach to explore the hypothesis that common variants in DNMT3A or DNMT3B influence the effect of childhood adversity on hippocampal volume in healthy, young adults.

Childhood adversity was chosen as environmental factor because it has often been identified as a risk factor for psychiatric disease. This fits with the diathesis-stress model, which posits that a mix of genetic predisposition and life adversity may increase the vulnerability of the nervous system to subsequent insults and eventually lead to disease. Hippocampal volume was taken as an intermediate phenotype because of its firmly established link to psychiatric disorders. It has been hypothesized that the hippocampus (along with the prefrontal cortex) is especially sensitive to adversity because the region is one of the latest to develop fully (Lupien, McEwen, Gunnar, & Heim, 2009). Moreover, the mechanisms through which changes in hippocampal structure and function occur are suggested to be epigenetic (McClelland, Korosi, Cope, Ivy, & Baram, 2011) and stress-induced changes in DNA methylation have also mostly been found in the hippocampus, commonly in association with major depressive disorder or schizophrenia.

Reduced hippocampal volume has previously been related to an increased vulnerability to develop major depressive disorder. Rao et al. (2010) reported smaller hippocampal volume both in depressed and high-risk (parental depression) adolescent groups compared to control subjects. The subjects were subsequently followed for up to 5 years, and mediation analysis of the data showed a significant association between CA and hippocampus volume, as well as hippocampus volume and the development of a depressive episode during follow-up. Animal models suggest that reduced hippocampal volume due to chronic stress exposure is mainly caused by elevation of glucocorticoid levels, which suppresses neurogenesis and causes dendritic retraction and neuronal death (Czeh & Lucassen, 2007). DNA-methylation may play an essential role in this process. Weaver et al. (2004) showed that poor maternal care in rat offspring changes DNA methylation patterns in the glucocorticoid receptor (GR) gene promoter, reducing GR expression in the hippocampus. This was associated with a higher stress response persisting through adulthood. The decrease in GR gene expression is thought to impair glucocorticoid negative feedback sensitivity, elevating stress responses. The study shows that stress early in life can result in hypersensitivity to stress later in life through epigenetic mechanisms. Similarly, increased DNA methylation and decreased expression of the human GR gene was found in the hippocampi of suicide completers who had experienced child abuse compared to nonabused suicide victims and control subjects (McGowan et al., 2009).

A trend for an association between DNMT3B and hippocampal volume was found in both cohorts separately (both $p<0.10$) for the individuals who had not experienced CA, but not for those who had experienced CA. An analysis of the two cohorts combined revealed a significant effect for DNMT3B on hippocampal volume in the no-CA group ($p<0.05$). This indicates that the direction of the potential SNP effects was the same in the two cohorts and the failure to find a significant effect in the separate cohorts may be due to lack of statistical power. The results suggest that common variants in DNMT3B modulate the effect of CA on the individual. This may be due to differences in DNA-methylation activity in response to stressful events around, for example, the GR gene.

The gene-wide analysis did not show a main association between DNMT3A or DNMT3B and hippocampal volume. Given the role of DNMT in translation of environmental signals to cellular changes, this finding is to be expected. The combination of factors is critical in causing a measurable effect. It once more stresses the importance of understanding gene x environment (GxE) interactions in explaining endophenotypes and understanding the etiology of psychiatric disease. Such interactions may mask the effect of important factors and could help to explain why genetic studies can yield seemingly conflicting results.

Significantly associated SNPs in DNMT3B were further investigated and rs2424932 was identified as a potential SNP of interest. An interaction between rs2424932 and CA was found such that experience of CA was associated with a smaller hippocampal volume only in the major allele homozygotes (GG) of rs2424932. This interaction effect appears mainly due to a larger hippocampal volume in the GG individuals who have not experienced childhood adversity compared to the AA/AG individuals. If decreased hippocampal volume is a vulnerability marker for psychiatric disorder, it may indicate a lower risk for the major allele homozygotes to develop such disorders under 'normal' development circumstances compared to the other individuals.

Interestingly, Murphy et al. (2013) very recently investigated the association between DNMT3B and DNMT3L variations in psychiatric patients who had attempted suicide versus those who did not, and found only rs2424932 to be significantly related to suicide attempt. They showed that patients with the A/A genotype were almost twice as likely to have a history of attempted suicide. These and our findings may be complementary, reflecting different ends of the spectrum given that we only included healthy participants without history of psychiatric illness while Murphy et al. (2013) investigated psychiatric patients.

The observation that environmental factors such as stress change DNA methylation levels of disease-related genes, and that these changes are associated with altered gene expression and disease phenotype, provides compelling evidence that epigenetic mechanisms play a fundamental role in psychiatric disorders. Because epigenetic patterns are highly dynamic and different across cells, it is difficult to establish definite causal links in humans. Most studies to date rely on animal models or post-mortem examinations. Causal studies in animal models are made more difficult by the lack of specific DNA methylation inhibitors. Peripheral cells have also been used in linking methylation with psychiatric disease (Uddin et al., 2011), but the relationship between epigenetic patterns in peripheral cells and neurons is not well understood. Many studies are yet to be replicated, but many lines of evidence suggest a central role for epigenetic mechanisms in the pathogenesis of complex diseases. Adding to the current literature on the role of DNA methylation in psychiatric disorders, this study supports a role of DNMT3B in a disease-relevant endophenotype. The impact of DNMT3B in this specific interaction may be present but limited in healthy participants, given that the interaction effect between DNMT3B and CA was only significant in the combined cohort analysis.

In general GxE, gene x gene, or even gene x gene x environment studies will be faced with the problem that a much larger number of participants is needed than in conventional genetic studies if effects of similar sizes are to be detected. Cell sizes dramatically decrease with an increasing number of factors, such that even for moderate effects one needs a reasonably large number of participants. Large consortia already exist that may provide enough power for targeted interaction studies. However, given that failure of replication is a problem in many gene association studies, it is important that the functionality of a variation is assessed through different methods, from molecular to behavioral. Even then findings may be inconsistent. For example, the Met-allele for the well-researched BDNF Val66Met polymorphism is commonly associated with susceptibility to psychiatric disorders, but some studies have failed to replicate this finding while others even find the opposite effect (Groves, 2007).

We chose to use an intermediate phenotype approach in healthy, young adults instead of using patient/control groups. This avoids confounding factors such as treatment, chronic disease, and comorbidity; and the intermediate phenotype strategy can elucidate biological mechanisms underlying complex disorders that cannot be readily understood from behavioral data. Traits such as hippocampal volume are expected to be closer to the genetic substrate, enhancing our ability to understand the genetic pathways that lead to disease vulnerability and phenotype. Intermediate phenotypes are commonly assumed to be affected by fewer factors than the highly complex disorders we seek to understand, providing a method through which the problem may be parsed into smaller sub-processes.

Presence of CA was assessed retrospectively through a questionnaire. The current study therefore cannot exclude the possibility of inverse causality; instead of CA and DNMT3B causing a change in hippocampus volume, it is for example possible that the volume differences lead to over- or under-reporting of CA. However, animal studies at least suggest a causal effect of CA on hippocampal volume. For example, Andersen and Teicher (2004) reported that induced postnatal stress in rats leads to attenuated hippocampal development compared to controls. Whether this is translatable to human cases must be assessed using prospective studies, which are rare, unfortunately.

Much remains to be done in order to understand the functionality of DNMT3B in relation to psychiatric disorders. First of all, there are eight alternatively spliced transcript variants have been documented for DNMT3B (Gene information available at http://www.ncbi.nlm.nih.gov/gene, National Center for Biotechnology Information). Expression may differ across the body, allowing for complex SNP effects. One SNP may affect the function of one or multiple splice variants, or the balance between alternative splice variants. DNMT3B is important in neurodevelopment, so it is not surprising that mutations in the gene can cause serious disease phenotypes such as ICF syndrome. However, other variants in the gene may have much more subtle or specific effects that are not immediately apparent.

Secondly, there is a large cascade of interacting proteins underlying the epigenetic pattern. The interaction between these genes is also essential in understanding the translation of environmental factors to lasting changes in the organism and the brain. Moreover, DNMT genes may also interact with important disease-related genes such as BDNF and 5-HTT. For example, Roth, Zoladz, Sweatt, and Diamond (2011) demonstrated with a rat model of post-traumatic stress disorder that changes in BDNF gene expression in the hippocampus occur after stressful episodes. This finding is significant because a lower BDNF level in the hippocampus has often been associated with major depressive disorder in patients, and antidepressants appear to raise the BDNF level (Duman & Monteggia, 2006). The study by van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, and Philibert (2010) shows an association between increased risk of unresolved loss or trauma and high levels of methylation in homozygotes of the long allele variant of the 5-HTT serotonin transporter promoter, while lower levels of methylation are associated with more unresolved loss or trauma in the short allele homozygotes. Kinnally et al. (2010) showed that higher methylation of 5-HTT occurs with maternal separation in non-human primates, and Beach, Brody, Todorov, Gunter, and Philibert (2010) found that child abuse is associated with higher serotonin transporter gene methylation in humans. 5-HTT has been repeatedly implicated in higher susceptibility to anxiety and major depressive disorder. These findings highlight the fact that DNMT variations could have profound consequences on the overall regulation of many genes affecting many different processes. Thus it would be interesting to see whether an interaction between variations in DNMTs and such genes are present. Finally, other environmental factors may have different effect on DNA-methylation than childhood adversity does. Though childhood adversity has received much attention in gene-environment interaction research, other environmental factors such as urbanicity and migrant status (associated with for example schizophrenia; McGrath et al., 2004) have also been shown to affect risk of developing psychiatric disorder. Including these factors helps us understand the generality or specificity of certain gene-environment interactions.

In conclusion, the current study shows that common variation in DNMT3B affects the impact of CA on hippocampal volume, an endophenotype often associated with psychiatric disorders. It warrants further investigation into functional variations in DNMT3B and identifies the SNP rs2424932 as a candidate of interest regarding gene-environment interactions in psychiatric illness. Future studies may focus on interactions between DNMT3B and other measures of CA, other susceptibility genes such as 5-HTT and other environmental factors such as urbanicity.

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The Influence of Prior Information about Stimulus Distribution on Sound-Localization Performance

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Although the peripheral auditory system is a frequency analyzer, we succeed in localizing sounds highly accurately by making use of implicit spatial cues. However, this purely acoustic information can be unreliable due to noise in the environment and in the processing of those cues within the brain. Bayes' model of optimal integration suggests that both sensory information and prior knowledge can be integrated in a statistically optimal way that maximizes response precision and accuracy. In this study we investigated whether and how listeners adjust their sound localization behavior when the sound location distribution changes during the experiment, and whether this adaptation is done in a near-optimal way. We used three different paradigms in which the distribution of auditory stimuli (the experimental prior) i) varied across blocks, ii) changed abruptly within one block and iii) changed dynamically. Results indicate that listeners not only adjust their localization behavior by reducing their localization gain for narrow distributions, but also often tend to do so in a nearly optimal way.

Keywords: cue integration, Bayesian modeling, optimal, human, head movements

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1. Introduction

The peripheral auditory system is a frequency analyzer that provides no direct information about a sound's spatial origin. In order to estimate a sound's location the brain makes use of implicit acoustic cues: interaural time and level differences (ITDs and ILDs, respectively), which vary systematically in azimuth (Akeroyd, 2006; Blauert, 1997), and monaural spectral cues provided by the pinna, which diffracts sounds from different elevations differently (Bremen, Van Wanrooij, & Van Opstal, 2010; Hofman, Van Riswick, & Van Opstal 1998; Kulkarni & Colburn, 1998; Middlebrooks & Green, 1991). These cues enable humans to accurately and rapidly localize sounds (Frens & Van Opstal, 1995). Nevertheless, due to the fact that the brain receives various sources of information at the same time (for example different sounds), and due to internal noise that perturbs the sensory and motor systems, the auditory system is vulnerable to generate localization errors (Langendijk, Kistler, & Wightman, 2001). In order to minimize errors, the system not solely relies on sensory evidence, but also is thought to incorporate prior knowledge. For example, when playing tennis and your opponent hits the ball, sensory evidence (vision) tells you that your opponent seems to play the ball straight towards you (on average), but as you have to react in a split second there is some doubt remaining (variance). You also know from prior experience in tennis matches with this opponent, that the ball is most likely to end up between certain boundaries in your playfield (on average), but this also varies from game to game. It seems therefore reasonable to combine both sources of information.

Bayes' theorem states how to optimally do so, simply by multiplying the probability distributions of the prior information, P(x), and the sensory evidence, P(s|x), with s the sensory location, and x the estimated location:

$$P(x|s) = \frac{p(s|x) \cdot p(x)}{p(s)} \quad (1)$$

P(x|s) is the posterior distribution of estimated possible locations, given the sensory evidence.

If we assume that the prior and likelihood follow Gaussian distributions, given by N(μ,σ), the variance in the posterior estimate is determined by:

$$\frac{1}{\sigma^2_{posterior}} = \frac{1}{\sigma^2_{prior}} + \frac{1}{\sigma^2_{likelihood}} \quad (2a)$$

and the mean of the posterior by:

$$\mu_{posterior} = \left(\frac{\mu_{prior}}{\sigma^2_{prior}} + \frac{\mu_{likelihood}}{\sigma^2_{likelihood}}\right) \cdot \sigma^2_{posterior} \quad (2b)$$

We assume further that the prior assumption P(x) of the nervous system about x follows $N(0,\sigma_{prior})$, and that the likelihood function, P(s|x) follows $N(s, \sigma_{likelihood})$. In that case, the posterior belief, P(x|s), is also a Gaussian, with mean, μ, and variance, σ^2.

The above equations can be rewritten as:

$$\sigma^2 = \left(\frac{\sigma^2_{prior} \cdot \sigma^2_{likelihood}}{\sigma^2_{prior} + \sigma^2_{likelihood}}\right) \quad (2c)$$

and

$$\mu = \left(\frac{\sigma^2_{prior}}{\sigma^2_{prior} + \sigma^2_{likelihood}}\right) \cdot x \equiv G \cdot x \quad (2d)$$

where we defined G as the posterior gain. According to the Bayesian hypothesis, the optimal estimate ($\mu_{posterior}$) about a stimulus' location is a weighted product of both prior information (prior) and sensory evidence (likelihood), whereby the weighting depends on the sources' (estimated) reliabilities (σ^2) (eq. 2).

That humans efficiently use prior information has been shown for the perception of visual movement (Stocker & Simoncelli, 2006), visuomotor integration (Kording & Wolpert, 2004), movement planning (Hudson, Maloney, & Landy, 2007), audiovisual integration (Van Wanrooij, Bremen, & Van Opstal, 2010) and cue combination (Kording, Beierholm, Ma, Quartz, & Tenenbaum, 2007).

If we assume that humans match their response probability to the posterior belief probability from eq. 2 (termed probability matching; Burns & Demaree, 2009; Vulcan, 2000; Wozny, Bleierholm, & Shams, 2010), then we can derive a response gain, that depends on two variables, namely the variation of the stimulus distribution σ^2_{prior} and the variation of the responses $\sigma^2_{response}$:

$$G = 1 - \frac{\sigma^2_{response}}{\sigma^2_{prior}} \quad (3)$$

This model predicts a parabolic relationship for the response gain G and variance σ^2. The slope is determined by the inverse of the prior's variance.

Equation 3 gives the exact near-optimal relationship between the prior variance, response variance, and the response gain. According to equation 3 the near-optimal gain in a Gaussian distribution is close to 1, when the prior's variance is large compared to response variance. This intuitively

means that you should disregard your prior beliefs if you are uncertain about them, and rely on new sensory evidence. This also means that under varying external stimulus distributions, the response gain would have to be adjusted for the variance of that distribution in order to minimize errors.

The aim of our study was twofold: first, we investigated whether humans incorporate prior knowledge about stimulus distributions in sound localization; second, we investigated whether they adjust nearly optimal. We presumed that if prior information about the sound location distribution is incorporated, the response gain varies for distinct distribution ranges. We therefore designed and tested three paradigms. In the first paradigm we compared the responses towards the same auditory stimuli that were presented within three different stimulus distribution ranges across conditions. In the second paradigm we presented an abrupt switch from a narrow to a broad distribution (or vice versa) in the middle of the experiment. In the third paradigm we presented stimuli in a dynamically changing distribution range. For each paradigm we modeled the near-optimal gain, based on the measured response variance and given distribution variance. Here, the paradigm of equation 3 allowed us to quantify the prior's dynamics. Taken together, we investigated whether, how fast, and how listeners adapt to changes in sound localization distribution.

2. Method

2. 1 Participants

Data were collected for thirteen (eight male) listeners spread across three experimental designs (constant block distribution: five listeners; dynamic block distribution: 9 listeners; dynamic sine distribution: 10 listeners; see Paradigms section below). Subjects had normal or corrected-to-normal vision and no hearing dysfunctions, aged 20-35 (mean, 24.9 years). Two listeners are author of this paper; the remaining eleven listeners were naïve about the purpose of this study. Listeners did not receive any payment. Prior to their participation each participant was informed thoroughly about the procedure and their permission to stop the experiment at any time. The experimental procedures adhered to The Code of Ethics of the World Medical Journal of July 18, 1964.

2. 2 Set-up

2. 2. 1 Apparatus

The set-up has been described earlier (Bremen et al., 2010; Agterberg et al., 2011; Van Wanrooij, Bremen, & Van Opstal, 2010).

During the experiments, subjects sat comfortably in a chair in the centre of a completely dark, sound-attenuated room (3 x 3 x 3 m). The chair was positioned at the center of a vertically-oriented circular hoop (radius 1.2 m) on which an array of 58 small broadrange loudspeakers (SC5.9; Visaton GmbH, Haan, Germany) was mounted. A green light-emitting diode (LED) was mounted at the center of each speaker and could serve as an independent visual fixation stimulus. Head movements were recorded with the magnetic search-coil technique (Robinson, 1963). To this end, the listener wore a lightweight spectacle frame with a small coil attached to its nose bridge. Three orthogonal pairs of square coils (6 mm2 wires, 3 m x 3 m) were attached to the room's edges to generate the horizontal (80 kHz), vertical (60 kHz) and frontal (48 kHz) magnetic fields, respectively. The head-coil signal was amplified and demodulated, low-pass-filtered at 150 Hz, and digitized at 500 Hz/channel.

2. 2. 2 Calibration experiment

To establish the off-line mapping of the coil signals onto known target locations subjects accurately pointed a laser attached to the spectacle frame towards LED locations in the two-dimensional frontal hemifield that encompassed the stimulus range of the actual experiments.

2. 3 Paradigms

In all paradigms, listeners were instructed to first fixate a central light either by aligning the head-fixed laser pointer or by pointing with their nose. The fixation light was extinguished after a button press of the listener, and after a pseudorandom delay of 100 to 300 ms later a broadband noise (0.5-20 kHz, 150 ms) was presented. Listeners were instructed to "point the laser or their nose as fast and as accurately as possible towards the sound source" and they had to respond within 1500 ms, after which the trial ended, and a new trial was initiated. Each listener performed only one experimental session of 262 to 500 trials (~40 min each) per day.

Subjects participated in three different

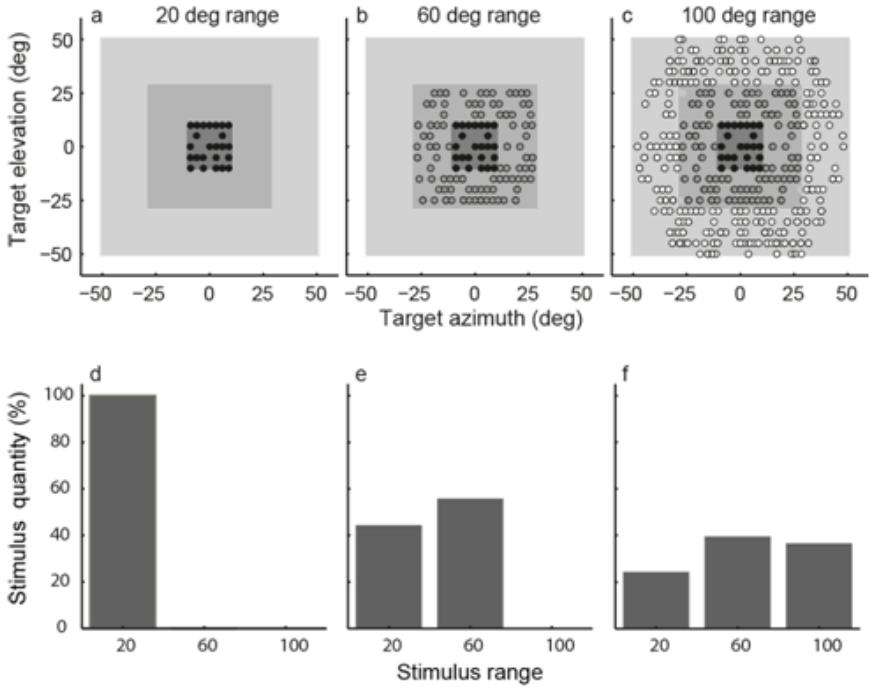

Fig. 1 Distributions of stimulus locations of the constant block paradigm a) for the 20 degree range, b) 60 degree range and c) 100 degree range. Note that the stimuli within the 20 deg range are exactly the same in all conditions. d-f) The relative amount of stimuli within a 20 deg range decreases for the 60 deg and 100 deg ranges.

experimental paradigms with varying distributions of target sound locations. The extent and the change of the distributions are detailed below. Listeners received no information about the stimulus distribution ranges nor were they told about possible changes.

2. 3. 1 Constant Block Distribution

In the first paradigm, sound locations were pseudo-randomly chosen from a boxcar distribution, in azimuth and elevation (Fig. 1 a-c). A boxcar distribution is a distribution in which the occurrence of a variable has the same probability at each possible location. The distribution of stimulus locations (the experimental prior) was kept constant within one block of trials, but the range of the distribution varied across blocks. Three ranges were presented to all five listeners (3 male) with normal or corrected-to-normal vision and no hearing dysfunctions, aged 22-34 (mean, 25.6 years):

i) 20 degrees (from -10 to +10 deg, 31 stimuli, repeated twice, 62 stimuli in total, Fig. 1a),
ii) 60 deg (-30 to +30 deg, 50 stimuli, Fig. 1b),
iii) 100 deg (-50 to +50 deg, 150 stimuli, Fig. 1c). The 100-deg range trials were divided in two equal-length blocks of 75 trials each.

The resulting four blocks were presented in one experimental session in pseudorandom order for every listener, with interleaved short breaks. Listeners were asked to point with the head-fixated laser. Completing one session took approximately 40 minutes. Importantly, the stimuli within 20 degree were the same in all conditions, but their relative occurrence decreased in the 60 deg and 100 deg conditions (Fig. 1 d-f).

With this paradigm we investigated whether listeners adapted to distinct sound location distributions. If the prior (knowledge about stimulus distribution range) is not taken into account, responses towards the same stimuli should not differ.

2. 3. 2 Dynamic Block Distribution

In the second paradigm, sound locations were pseudo-randomly chosen from a boxcar distribution, in elevation only and for each listener anew (Fig. 2). The distribution of stimulus locations switched after the first half of the experiment, either from small (-15 to +15 deg, 250 trials) to large (-55 to +55 deg, 250 trials) (Fig. 2 a) or from large to small (Fig. 2 b), excluding zero deg targets in order to elicit head movements on each trial. Each of the nine listeners (4 male) with normal or corrected-to-normal vision and no hearing dysfunctions, aged 22-26 (mean, 23 years) participated in one of the two

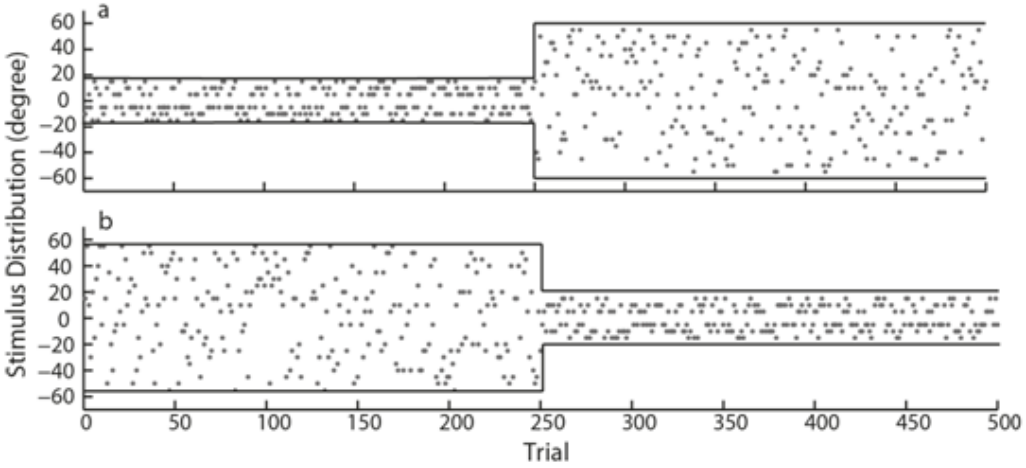

Fig. 2 Distribution of stimulus locations of the dynamic block paradigm. The stimulus distribution switched from a) narrow to broad or from b) broad to narrow after 250 stimuli (only elevation locations).

possible sessions per day, with no interleaved breaks. Listeners were asked to point with their nose and received no visual feedback. Completing one session took approximately 35 minutes.

With this paradigm we investigated whether and how listeners adapt to an abrupt change in stimulus distribution within the same run.

2. 3. 3 Dynamic Sine Distribution

In the third paradigm, sound locations were pseudo-randomly chosen from a Gaussian distribution, separately for azimuth and elevation (Fig. 3a-b), yielding a two-dimensional Gaussian target distribution centered at zero deg. In a Gaussian distribution the probability of occurrence is higher at a particular location and decreases for locations in the periphery. The distribution of stimulus locations varied dynamically following a squared sine wave at one of three frequencies (Fig. 3d-f). Each session contained 400 trials, resulting in 1.6 (Fig. 3 d, slow change), four (Fig. 3e, medium change) and eight cycles (Fig. 3f, fast change), swaying between a broad and a narrow distribution. Ten listeners (7 male) with normal or corrected-to-normal vision and no hearing dysfunctions, aged 22-35 (mean, 25.5 years) participated in this experiment and were asked to point with a head-fixed laser. There were no interleaved breaks and completing one session took approximately 40 minutes.

With this paradigm we investigated how fast listeners could adapt to dynamic changes in stimulus distribution.

2. 4 Analysis

The recorded voltage position signals were first low-pass filtered (cut-off frequency 75 Hz, filter order 50) in order to determine the gaze or head traces (note that these were identical because subjects were required to fix their gaze to the head-mounted laser pointer). A custom-written Matlab program detected head-movement onsets whenever their velocity surpassed 10 degrees/second and offset when they fell below 10 degrees/second. We took the end position of the first movement after stimulus presentation as a measure for localization performance, and excluded correction movements. Each movement-detection marking was checked by the experimenter and adjusted when necessary. Data analysis and visualization was performed in Matlab (The Mathworks Inc., Natick, MA, USA).

Target and response coordinates are expressed in azimuth and elevation coordinates of the double-pole coordinate system (Knudsen & Konishi, 1979).

2. 4. 1 Statistics

The optimal linear fit of the stimulus-response relation between saccade elevation and azimuth with target elevation and azimuth was found by minimizing the sum-squared deviation of

$$\epsilon_R = g \cdot \epsilon_r + b \tag{4a}$$

for elevation (ε) components and

$$\alpha_R = g \cdot \alpha_r + b \tag{4b}$$

for azimuth (α) components. g is response gain

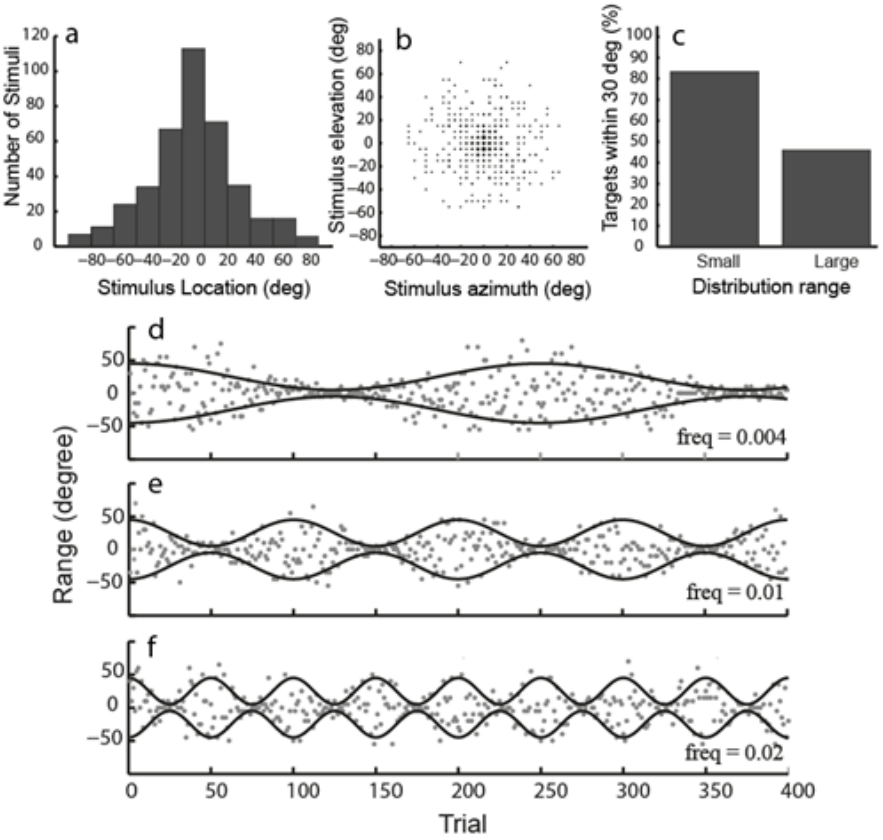

Fig. 3 Distribution of stimulus location of the dynamic sine paradigm a,b) Stimuli are selected from a Gaussian distribution with a mean of 0. The distribution varied dynamically following a squared sine wave of different frequencies, resulting in d) 1.6, e) 4, and f) 8 repetitions. c) The percentage of stimuli within 30 degree decreased for the large distribution ranges.

(slope), and b is response bias in degree (offset of the fitted line). Furthermore, we determined the standard deviation of the residuals, sigma. We also determined r^2 of the linear fits, with corresponding F- and p-values.

We conducted an ANOVA (paradigm 1) with factors experimental range and response gain and obtained F- and p- values to investigate whether the observed differences were significant.

2. 4. 2 Near-optimal gain

We calculated the predicted near-optimal gain which depends on response variance and experimental prior variance. The near-optimal gain was obtained from equation 3, assuming Gaussian distributions, while in two paradigms (constant block distribution, dynamic block distribution) we used boxcar distributions. We determined the standard deviations of simulated Gaussian and boxcar distributions and found that they differed by a factor of approximately 0.58. However, when we calculated the near-optimal gain by multiplying both, the standard deviation of the responses and the stimulus range with this factor (0.58), this difference in standard deviation was cancelled out. Thus, using a boxcar distribution did not change the predictions and correcting for it was not necessary.

2. 4. 3 Normalization

Because response gains can in principle be larger than 1 while the near-optimal gain based on Bayes' theorem can never exceed a value of 1 ($G_{pred} = 1 - \sigma^2_{response} / \sigma^2_{prior}$) we normalized the data by dividing the responses (ϵ_R) by the maximum gain (g) for every subject (eq. 5).

$$\hat{\epsilon}_R = \frac{\epsilon_R}{max(g)} \quad (5)$$

We assume that idiosyncratic factors other than minimizing the error variance influence the localization gain (e.g. leading to higher gains for small head movements). By this normalization step we try to remove these confounds, as well as the inter-subject differences. Hence, the presented gains are not the average gains, but their relative proportions.

3. Results

3. 1 Localization gain changes

In the first paradigm we presented three conditions with different stimulus distribution ranges, for which the stimuli within the 20 deg range were the same. Listeners participated in this paradigm between four and seven times. Thus, although we have data for five listeners, the results are based on 29 sessions in total. After pooling the data over all listeners and selecting the trials that contained stimuli presented within the 20 deg range in each condition, we performed an analysis of variance (ANOVA) with experimental range as independent variable and gain as dependent variable. The mean gains were different in azimuth ($F(2) = 4.47, p < .036$) and elevation ($F(2) = 4.29, p < .04$).

If listeners integrate prior information about the stimulus distribution range, the response gains towards the same stimuli should differ for narrow vs. broad target distributions. More precisely, the broader the distribution, the higher the gain should rise, and that is exactly what we find (Fig. 4, black circles). In elevation the effect is more pronounced, here, the gain increases from approximately 0.8 to 1 (Fig. 4b). In azimuth the gain increases from approximately 0.9 to 1 (Fig. 4a). The actual gains nicely follow the prediction of Eqn. 3 (Fig. 4, grey circles). Note also that the response variance is lower in azimuth than in elevation (Fig. 4c, d).

3. 2 Adaptation to abrupt changes in boxcar distribution width

In the second paradigm we presented an abrupt switch from a narrow (30 deg range) to broad (110 deg range) stimulus distribution (or vice versa) in the middle of the experimental run. Inspection of the stimulus-response plots for the entire data sets revealed that i) the gains for the broad vs. the narrow target-distribution epochs differed, and that ii) the overall pattern of gain changes appeared to differ across listeners (Fig. 5). For example, while listener S2 demonstrated a higher gain for the broad distribution range than for the narrow distribution

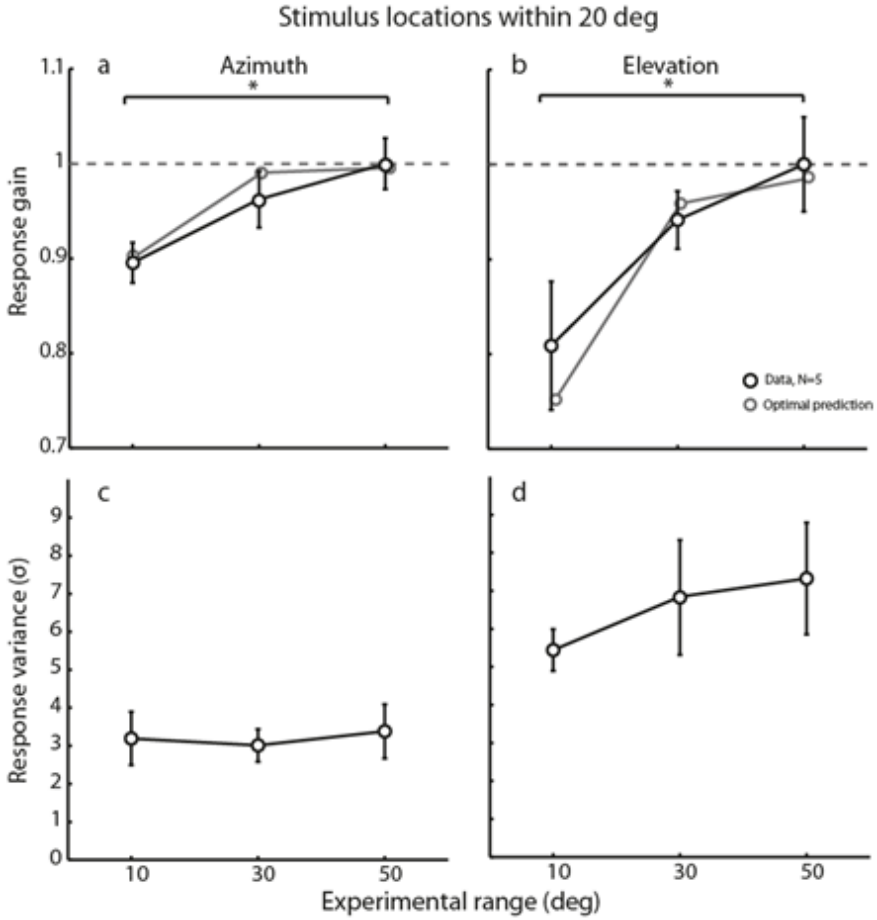

Fig. 4 Normalized response gains for targets within a 20 degree range for experimental ranges 20, 60 and 100. The gains based on the data (black) and the predicted near-optimal gains (grey) coincide both in a) azimuth and b) elevation, c,d) show the corresponding response variance. Error bars indicate standard error.

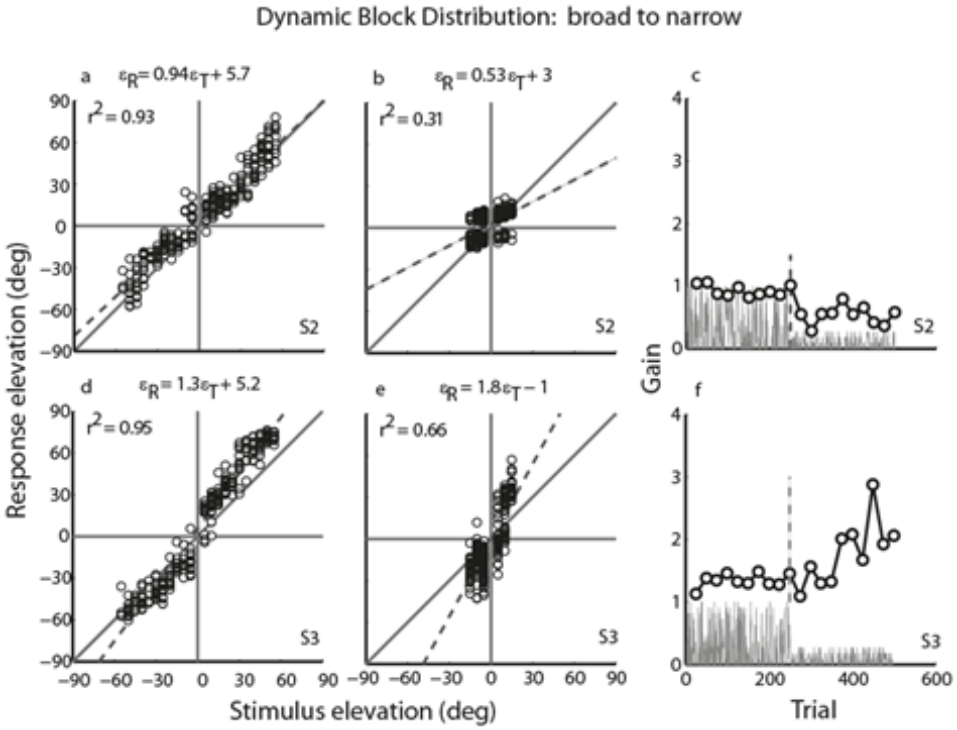

Fig. 5 Abrupt switch from a broad to narrow distribution after 250 stimuli. Stimulus response plots for two listeners for the a,d) broad and b,e) narrow distribution. c,f) Gain over the course of trials.

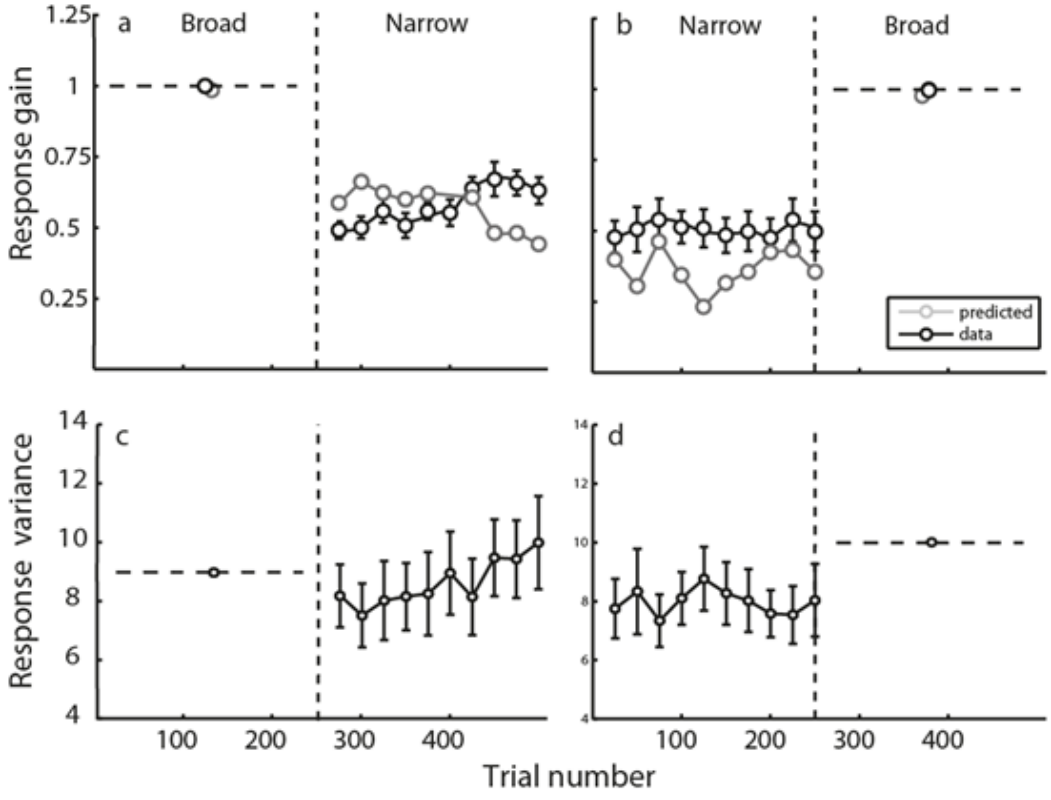

Fig. 6 Normalized response gain over the course of trials (black) and predicted near-optimal gain (grey) for the condition in which the distribution switched a) from broad to narrow and b) from narrow to broad. The results are based on selected data, in which stimuli where presented within a 30 deg range. c) The response variance increased over the course of trials, which explains why the predicted near-optimal gain decreased. d) The response variance and the predicted near-optimal gain demonstrate opposite patterns.

range (Fig. 5a,b; g = 0.94, g = 0.53, respectively), listener S3 demonstrated the opposite pattern (Fig. 5d,e; g = 1.3, g = 1.8, respectively. We calculated the gain over the course of trials (each gain is based on the previous 25 trials), whereby the difference becomes more apparent (Fig. 5c,f). We could not disentangle whether these differences in overall response gain occurred in response to differences in the stimulus distributions, or were simply due to differences in localization behavior with head movements for the narrow vs. the broad range. The finding that some listeners (~4) exhibited very large overshoots (gain > 1.5) for the narrow distribution epoch of this experiment suggested that the latter was more likely.

In order to directly compare the gains of the broad and narrow distributions, we selected those trials in which stimuli were presented within a 30 deg range in both epochs. We calculated one gain for the broad distribution part, because the selected trials were not evenly spread and relatively few in numbers (~76 trials per session). For the narrow distribution part we calculated the ongoing gains over the course of trials, whereby each gain was based on the previous 25 trials. We pooled the data of all listeners and averaged the results (Fig. 6a,b, black circles).

After the switch from a broad to a narrow distribution range, the gain decreased from 1 to approximately 0.5 within 25 trials. Over the course of the following 250 trials it remained flat (Fig. 6a). For the reverse condition in which the distribution switched from narrow to broad the response gain stayed relatively stable at 0.5 in the narrow distribution. After the switch it increased to 1, however, we cannot estimate how long this process took, because we only have one gain for the whole second part. Nonetheless, we find that listeners did adjust their gain in response to an abrupt switch (Fig. 6b). The patterns of the calculated near-optimal gain (Fig. 6a,b, grey circles) closely matched the results, as the measured gain was high for the broad distribution epoch and low for the narrow distribution epoch. Minor differences between the predicted near-optimal and the measured gain can be explained by changes in response variance over the course of trials (Fig. 6c, d): The higher the response variance, the lower the predicted optimal gain.

Taken together we find that i) listeners adjusted their response behavior after an abrupt switch of the stimulus distribution range, ii) this adjustment seems already to take place within the first 25 trials after the switch, and that iii) the actual gains closely follow the predicted near-optimal gains.

3.3 Adaptation to dynamic changes in Gaussian distribution width

In the third paradigm we presented stimuli in dynamically changing stimulus distributions that followed a squared sine wave form of one of three different frequencies (1.6, 4, and 8 repetitions). The stimulus-response plots for listener S3 demonstrate that responses were somewhat more accurate and precise in azimuth (Fig. 7a; g = 1, σ= 0.88 deg) than the responses in elevation (Fig. 7b; g = 1.1, SD (residuals) = 2.57 deg). This holds true for all listeners and seems to indicate (according to the Bayesian hypothesis) that listeners were more certain about stimulus locations in azimuth than in elevation. With this paradigm we investigated whether listeners adjusted their responses according to dynamic changes in the stimulus distribution. To that end, we calculated the gain over the course of trials (Fig. 7c, d; listener S3, each gain is based on the previous 5 trials). The gain was not stable across trials. Interestingly, the fluctuation generally followed the pattern of the stimulus distribution changes, particularly in elevation.

As in the previous paradigm some listeners demonstrated large overshoots for the narrow distribution ranges (Fig. 8, example for responses in elevation). For listener S2 the gain was higher in the broad distribution range than in the narrow distribution range (Fig. 8a, b; g = 0.99, g = 0.64, respectively). Listener S5, however, demonstrated an overshoot in the broad distribution range (g = 1.3) and an even larger overshoot for the narrow distribution range (g = 1.6) (Fig. 8, d,e, respectively). As a result, simply averaging across all listeners would not have yielded informative results. For illustration, we superimposed the data on one cycle and calculated the gain over the course of trials. Figure 8c, f shows the results for the condition with 1.6 sine repetitions with data superimposed on one cycle (250 trials) for listeners S2 and S5. After averaging the gains over the course of trials across all listeners it appeared that the gain stayed more or less stable during the experiment (Fig. 9a; N = 9). However, after dividing the listeners into two groups, namely those who demonstrated large overshoots (g > 1.5), and those, who did not (g < 1.5) revealed a very different pattern: the response gain increased for the high-gain listeners (Fig. 9b; N = 3), and decreased for the lower-gain listeners (Fig. 9c; N = 6) during the narrow target-distribution part.

Again, we cannot disentangle whether these differences reflected a response to changes in the

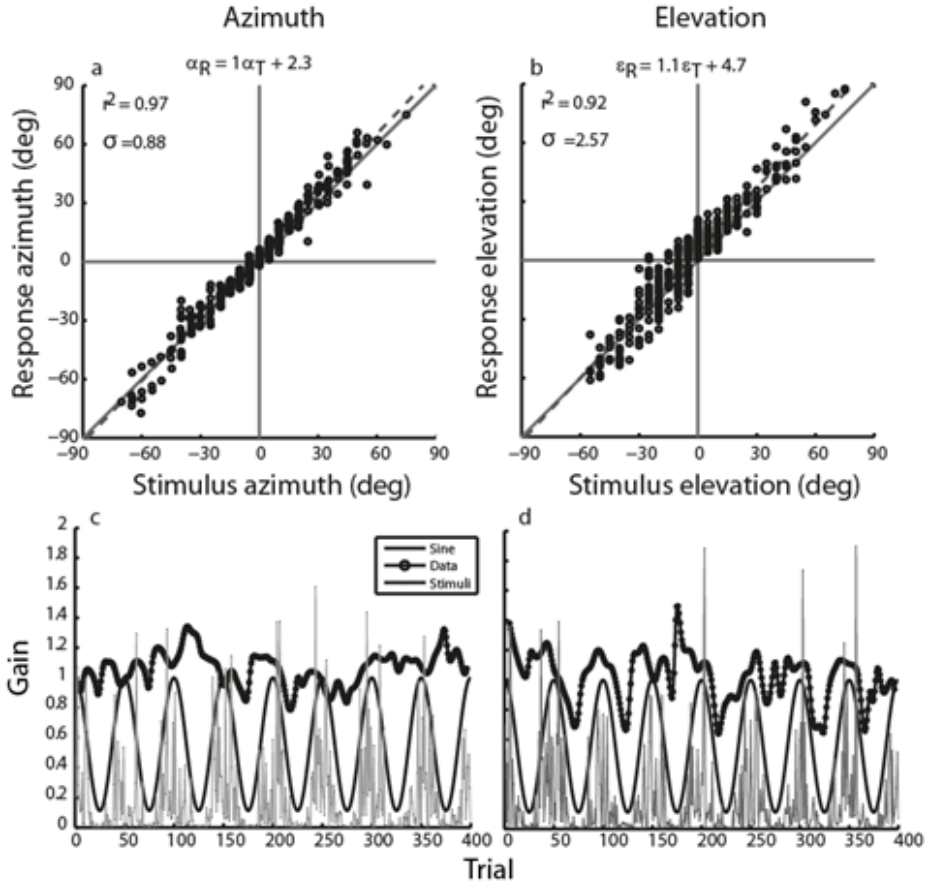

Fig. 7 Stimulus-response plot for listener S3 in a) azimuth and b) elevation, and the gain over the course of trials in c) azimuth and d) elevation. Indications of sine and stimuli are not true to scale, but illustrate how the distribution of stimuli dynamically changed during this experiment.

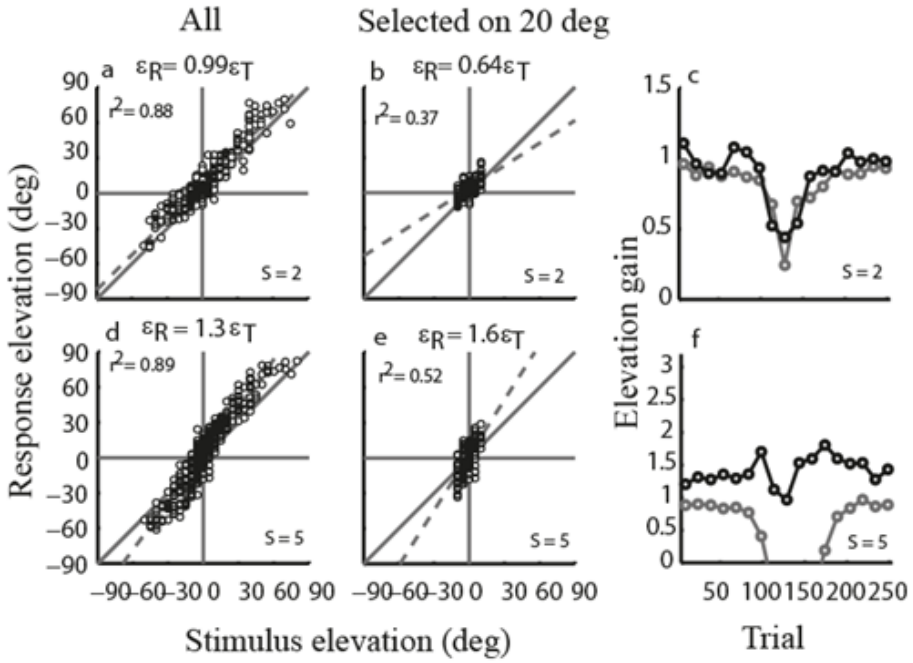

Fig. 8 Stimulus response plots for two listeners (S2,S5) in the a,d) broad and b,e) narrow ranges within the dynamic distribution in elevation. c,f) The actual gain (black) and predicted near-optimal gain (grey) over the course of trials. Results are shown for the condition with 1.6 sine repetitions with data superimposed on one cycle.

target distribution, or that listeners simply differ in their strategy to localize in broad and narrow distributions by head-movements.

To enable a better comparison, we therefore selected those trials in which the stimuli were presented within the same 20 degrees range. For the first and last quarter of each condition we calculated one gain, because we retained only a small number of trials that were not evenly distributed (Fig. 10; black circles on dashed line). The medium quarters mainly contained stimuli within 20 deg so that here we could calculate the gain over the course of trials. Figure 10 shows the normalized averaged results across all listeners. For the slow condition, the calculated gains were based on the previous 15 trials (Fig. 10a, d). For the medium condition the calculated gains were based on the previous 7 trials (Fig. 10b, e), and for the fast condition they were based on the previous 5 trials (Fig. 10c, f).

In elevation the response gain evidently changed over the course of trials for the slow, medium and fast distribution change conditions (Fig. 10a-c). When the stimulus distribution shrunk, listeners adjusted their response gain by lowering it accordingly. This effect was pronounced in the slow and medium change conditions, but could also be observed for the fast change condition. It seems likely that gain adjustments can take place within approximately 10 trials. In azimuth the gains were not stable during the experiment either, but the pattern differed across conditions. During the slow change condition, listeners slightly lowered their response gain when the distribution shrunk (Fig. 10d). For the medium and fast conditions the gain slightly increased when the distribution shrunk. (Fig. 10e, f).

The predicted near-optimal gains follow the same pattern as the actual gains in elevation. In azimuth the pattern of actual and predicted gains are relatively similar for the slow change condition, but seem to contradict each other for the medium and fast change conditions: the near-optimal gain is predicted to decrease for the narrow distribution range, while the actual gains stay high. Note that the response variance attains a minimum in the elevation responses when the gain is lowest, which is nicely in line with the Bayesian prediction. Interestingly, the response variance in azimuth is also minimal during the changed gains, although the Bayesian model would have predicted a lower gain to achieve this result. However, the response variances in azimuth are already quite low, when compared to elevation.

4. Discussion

The present study tested whether listeners adaptively account for changes in the stimulus distribution when programming a rapid head-orienting response. These changes could only be observed through ongoing feedback during the experiment, as subjects were not told or explicitly instructed (either verbally or visually) about the actual stimulus distributions in the experiment. We hypothesized that if the brain somehow keeps track of the stimulus distribution of prior trials to update its expectations of the current stimulus distribution, one should observe an effect of the imposed stimulus distribution on the orienting responses.

Indeed, our main finding was that head saccades were systematically altered by the stimulus distribution range. More precisely, listeners demonstrated lower response gains in narrow stimulus distribution ranges and higher response gains in broad stimulus distribution ranges. This was true i) for changes in

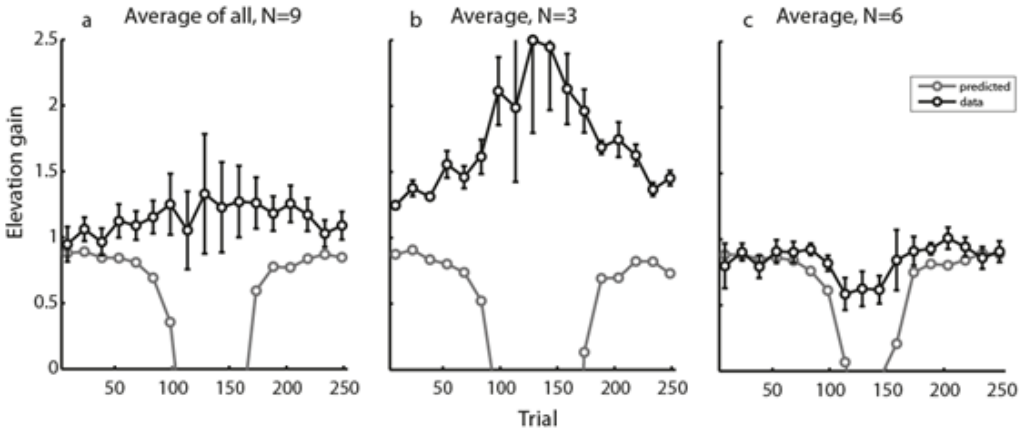

Fig. 9 Gain over the course of trials for one of the three conditions (slow change, one cycle = 250 stimuli) averaged over a) all listeners, b) listeners with a gain > 1.5 in the narrow range, and c) listeners with a gain < 1.5 in the narrow range. Results are shown for elevation only.

distribution range across blocks (paradigm 1), ii) for abrupt changes within one session (paradigm 2) and iii) for dynamic slow to fast sinusoidal changes. The response gain depended systematically on the particular stimulus distribution, which suggests that the brain indeed constructs a dynamic expectation regarding the stimulus distribution range of novel stimuli. Interestingly, the number of trials needed to update the estimated stimulus distribution is in the order of only a few (order five to ten) trials.

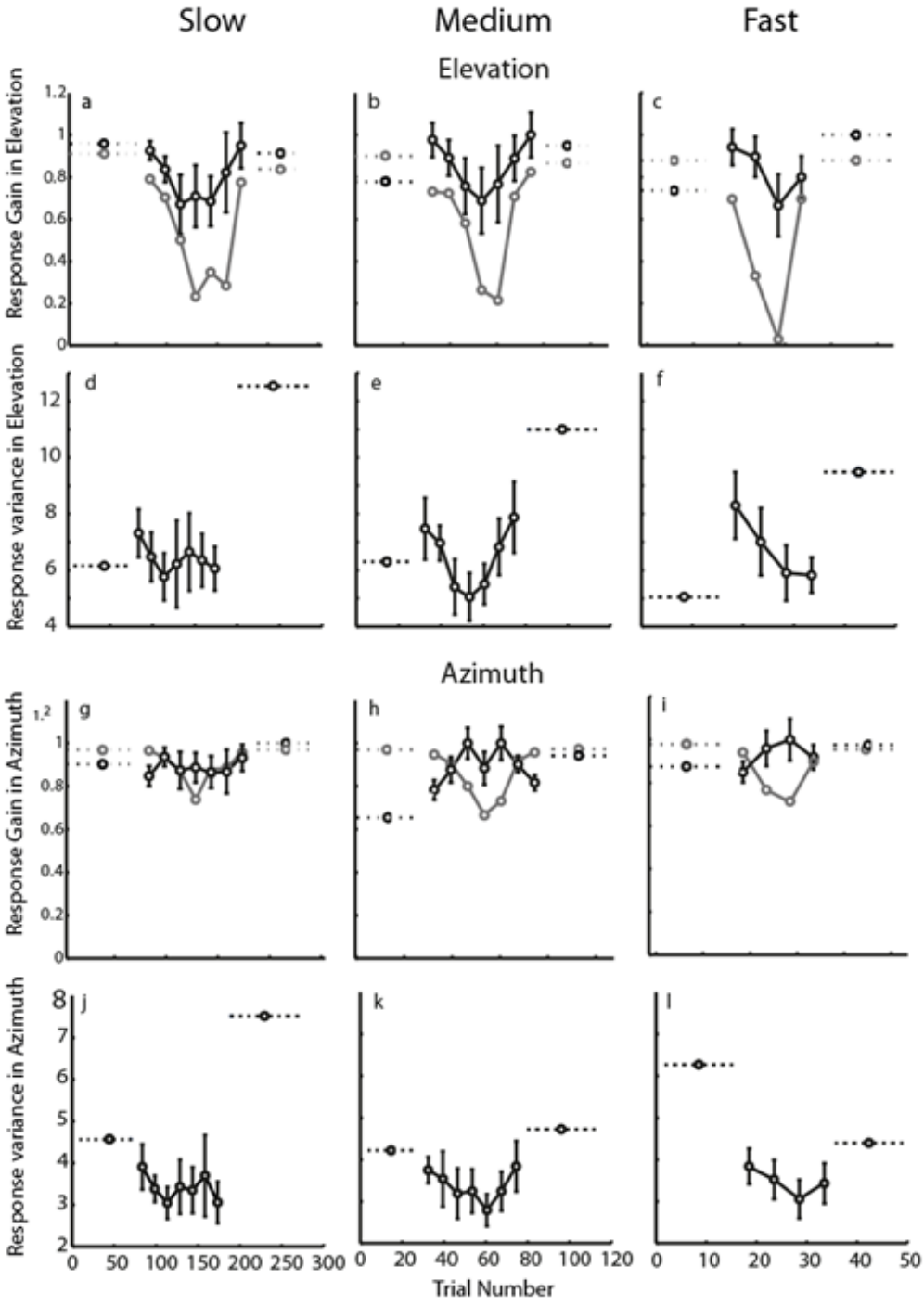

Fig. 10 Normalized average gain over the course of trials and corresponding response variance for the slow, medium and fast distribution change conditions in a-f) elevation and g-l) azimuth. In elevation the actual gain and the predicted near-optimal gain decrease when the distribution range decreases. This is more prominent in a) the slow change condition. In azimuth the actual and predicted gain also decrease when the distribution range decreases for g) the slow change condition. In the conditions in which the change was faster, the actual gain rather seems to increase. Response variances in azimuth are smaller than in elevation.

4. 1 Azimuth and elevation localization

In each of the paradigms in which stimuli were presented in both azimuth and elevation, the response gain varied stronger, and the response variance was higher, in elevation than in azimuth (Fig. 4). Because the spectral elevation cues are noisier than the robust multichannel binaural azimuth cues, the sensory uncertainty in elevation is expected to be higher, and more vulnerable to internal noise. Lower elevation gains in stimulus-response relations have been reported before (Hofman et al., 1998; Zwiers, Van Opstal, & Cruysberg, 2001), but have so far not been interpreted within the Bayesian framework. According to this theory, the larger endpoint variance is in line with a lower response gain in case the prior estimate is around straight-ahead (gain zero). The finding that elevation response gain varies more strongly with the different stimulus distribution is nicely in line with Bayes' theorem (Harris & Wolpert, 2006), and suggests that the degree of certainty about the stimulus location influences the degree of adjustment according to estimated, perceived sound-location distribution changes. This shows that the response gain indeed depends both, on distribution variance and response variance.

Note that the gain changes are made on the basis of noisy measurements by the auditory elevation system, which could be wrong. In fact, we would rather expect that the changes are not induced by a real physical change in the stimulus distribution (as in the current experiments), but could result purely on the basis of a perceptual change in the stimulus distribution. For example, if the auditory target distribution would be accompanied by (false) visual feedback signals, the weighting of the auditory and visual information sources ('ventriloquism') would determine the perceived sound distribution, which would be biased by the observed visual distribution. In other words, one could repeat the experiments by imposing a visual target change without an acoustic target change, and still observe gain changes in the auditory evoked responses (as well as in the visual evoked saccades). It would be interesting to study the interplay between the different sensory signals within the Bayesian theory by varying the expected alignment of auditory and visual stimuli in the experiment, and hence the reliability of the auditory and visual signals belonging to the same object in space-time.

4. 2 Feedback and Timing of Adaptation

In previous studies about Bayesian integration participants received feedback about their performance (Berniker, Voss, & Kording, 2010). Interestingly, although in our study listeners never received explicit feedback, their response gain nonetheless changed in the appropriate near-optimal way with changes in the stimulus distribution. This has never been demonstrated before. According to that, the brain estimates the errors and incorporates this knowledge in the planning of subsequent responses, independent from explicit feedback.

Further, Berniker et al. (2010) not only investigated whether subjects would integrate prior knowledge in an optimal way during a complex high-level task, but also the time course of learning a prior. They confirm that subjects adapted their prior, however, in their experimental paradigm subjects had as much time as needed to respond, whereas in our study listeners had to respond as fast and accurately as possible, leaving no time for cognitive deliberations. We therefore presume that the phenomenon is due to an automatic, bottom-up (exogenous) neural process rather than to cognitive, top-down (endogenous) factors, and we find that changes in response gain already occur within 10 to 20 trials (Fig. 6, Fig. 10). Van Wanrooij et al. (2010) studied the effect of expectation on the strength of multisensory integration. They modeled a leaky integrator with a memory of 15 previous trials and a decay time of 4 trials, which demonstrated good correspondence between estimated and actual data.

4.3 Distribution Type

We tested two types of target distribution, two boxcar distributions, and one Gaussian distribution. For the prediction of the near-optimal gain the differences in distribution variance did not matter because the difference factor was cancelled out in the calculation of the predicted near-optimal gain. Otherwise we could have been able to investigate whether listeners rather assume a boxcar or Gaussian distribution. We presented the auditory targets around the centre (mean = 0) and it would be interesting to test distributions for which the mean would differ from zero. Such a test might indicate whether listeners rather rely on central priors or not.

4. 4 Bayesian model

Our model suggests that prior-likelihood integration depends dynamically on the previously experienced stimulus distribution. Acquiring such prior evidence, however, takes time: here, listeners gradually adjust their gains within about 10-20 trials (Fig. 6, Fig. 10). Such a lag is not easily accounted for with an optimal Bayesian model. The Kalman filter, for example, assumes a static prior variance and becomes unstable if the underlying process is dynamic. A model with a leaky integrator, that favors the most recent trials, though not optimal, might predict the actual dynamics of the behavior even better.

4. 5 Decision strategies

Apart from the probability-matching strategy, which is often observed in human (Wozny et al., 2010) and animal (Sugrue, Corrado, & Newsome, 2004) behavior, we have also considered two other decision strategies. The maximum-likelihood strategy ignores any prior knowledge and falls short in this context. The maximum-a-posteriori strategy predicts different response gains, and even results in no exploratory behavior (both G and σ=0) when uncertainty is high. It seems very reasonable to adopt a less optimal strategy (e.g. with a probability-matching strategy), which allows exploring complex and dynamic environments, even if the variance in the errors increases to some extent.

4. 6 Suitability of the measurement

We have seen that some listeners demonstrated huge overshoots in responses towards stimuli in a narrow distribution range (Fig. 4, Fig. 5, Fig. 8). Listeners might have difficulties to point by head-movements. In the future it will therefore be important to refine the methods by measuring eye-movements. Eye-movements are faster and presumably more precise than head-movements. It will be interesting to see how the difference in response modality expresses in prior adaptation.

4. 7 Neural implementations

Brenner, Bialek and de Ruyter van Stevenick (2000) found that the input/output relation in dynamic environments changes with statistical properties to maximize information transmission when they investigated the coding of dynamic velocity signals in a motion sensitive neuron of the fly's visual system. Similarly, Maier and colleagues have shown that neurons in the auditory system also adjust their responses to the statistics of sound level and ITD distributions (Maier, McAlpine, Klump, & Pressnitzer, 2009; Maier et al., 2012). This is consistent with our behavioral data which shows that humans can localize sounds near-optimally in dynamically changing environments and we advocate that adaptive neural coding that incorporates prior knowledge about sound distribution brings about this efficient behavior. It would be interesting to verify whether and how the rules of prior-likelihood integration in dynamically changing stimulus distributions that emerge from our study would be reflected in sensory and/or preparatory activity epochs in neurons.

5. Conclusion

Our experiments support the idea that the brain makes a dynamic evaluation of the stimulus distribution to program rapid orienting responses in a near-optimal way that may be based on prior-likelihood integration. Such a strategy is particular useful in unpredictable, complex and dynamic environments.

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Slot Machines: Fun or Addictive? A Neurobiological Comparison Between Pathological Gamblers and Healthy Controls

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Known as a common recreational activity, slot machines are also associated with addictive effects as seen in "pathological gambling". The neurobiological mechanisms of pathological gambling are not clear yet. Certain properties of slot machines may contribute to disordered gambling, such as near-miss events in which a no-win is proximal to the jackpot. These near-misses are found to elicit brain responses similar to a reward and have been shown to invigorate gambling behavior. However, a previous study could not indicate functional activity differences in the brain to near-misses in gamblers compared to controls. Moreover, the idea that pathological gamblers have a distorted sensitivity to wins remains controversial, with some studies indicating a hypo-responsive reward system while others suggested a hyper-responsive system. Using a simplified slot machine task combined with functional Magnetic Resonance Imaging (fMRI), we sought to replicate previous results on near-misses (Chase & Clark, 2010), explore their effects in the nucleus accumbens and shed light on the neurobiological signature of pathological gambling on win outcomes. We replicated previous results showing that near-misses activate striatal and insula circuitry similarly as wins do and showed that gambling severity predicts near-miss-related activity in the midbrain and insula. The nucleus accumbens responded linearly with the distance to the jackpot. Compared to controls, gamblers displayed diminished blood-oxygen-level-dependent (BOLD) responses to wins compared to nonwins in the right anterior caudate nucleus. These findings provide neurobiological evidence for the invigorating role of near-misses on gambling in general and suggest that pathological gambling might be characterized by blunted striatal responses to wins.

Keywords: pathological gambling, near-misses, ventral striatum, fMRI, behavioral addiction

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1. Introduction

1.1 Pathological gambling

Pathological gambling (PG) is a psychiatric disorder described as "persistent and recurrent maladaptive gambling" leading to negative consequences on one's social and financial life. In the fourth edition of the Diagnostic and statistical Manual of Mental disorders (DSM), it is categorized as an Impulse Control Disorder which is expressed by the failure to inhibit impulsive actions and here specifically gambling (American Psychiatric Association, 2000). It shares many commonalities with substance dependence, which has stimulated a discussion whether the classification of disordered gambling should be changed to a nonchemical, behavioral addiction in the fifth edition of the DSM (Kessler et al., 2008; Petry, Stinson, & Grant, 2005). By classifying PG as a form of addiction, maladaptive gambling is described as less goal-directed and rather habitually-driven behavior. (Everitt & Robbins, 2005).

Many studies have pointed out that substance dependence and PG share common vulnerability factors such as genetic predispositions (Ibanez, Blanco, Perez de Castro, Fernandez-Piqueras & Sais-Ruiz, 2003) and clinical characteristics such as psychiatric comorbidities (Petry et al. 2005, Verdejo-Garcia, Lawrence, & Clark, 2008). Genetic studies, for example, suggest that carriers of the dopamine D2A1 allele receptor gene are at higher risks to develop PG and/or substance dependence (Comings & Blum, 2000). The reward deficiency hypothesis proposed that this genetic alteration is associated with changed dopamine D2 receptor signaling and is predicted to result in reward processing deficits in the mesocorticolimbic pathway that predispose individuals to more sensation-seeking behavior (Comings & Blum, 2000).

Neuroimaging studies have recently focused attention on PG and highlighted striking similarities with drug addiction. For example, both impairments are associated with the ventral frontostriatal circuit in the brain that plays a key role in reward processing (Reuter, Raedler, Rose, Hand, Glascher & Buchel, 2005; Potenza, 2008). Some functional neuroimaging evidences point to reward processing deficits in PGs that are consistent with the reward deficiency account (Reuter et al., 2005; de Ruiter , Veltman, Goudriaan, Oosterlaan, Sjoerds & van den Brink , 2009; Balodis, Kober, Worhunsky, Stevens, Pearlson, & Potenza , 2012). Reuter et al. (2005), for example, showed that pathological gamblers (PGs) exhibit decreased win-related responses in the ventral striatum and medial prefrontal cortex (PFC) in a monetary guessing task compared to healthy controls (HCs). However, the specific direction of BOLD responses to rewarding outcomes in PGs is far from being resolved as there are inconsistencies. In contrast to the findings of Reuter et al. (2005), others have shown increased responses to monetary outcomes in PGs relative to HCs (de Ruiter et al., 2009; Fehr, Meyer, & Herrmann, 2010; Holst, Veltman, Buechel, van den Brink, & Goudriaan, 2012). For example, using a quasi realistic blackjack, Miedl et al. (2010) demonstrated that PG subjects display enhanced activity in the reward pathway in gambling-naive and problem gamblers during winning events compared to loosing events. In order to quantitatively address these inconsistencies in the neuroimaging literature, we tried to perform a meta-analysis on reward outcome contrasts comparing HCs>PGs and PGs>HCs. Unfortunately, the number of published data and reported peak coordinates were insufficient to report any reliable conclusions.

Considering the contradicting findings on brain activity to reward outcomes in PGs, more studies on PG are needed in order to clarify the underlying neurobiological underpinnings of maladaptive gambling.

1.2 Effects of near-misses on gambling

Interestingly, there are also some external, task-specific properties that may contribute to PG as in the case of fruit or slot machines. Slot machines traditionally consist of three reels with a row of different symbols such as images of fruits, letters, etc. that lead to three outcomes: wins, near-misses and full-misses. In cases of wins, the symbols on the three reels are matched and the player receives money. Near-misses are no-win events that happen when the last reel stops one position away the other reels and are therefore proximal to the anticipated win. Full-misses include no-win outcomes where the last reel is far away from the other reels.

Despite their lack of monetary reward, near-misses have been shown to foster gambling persistence and motivation (Coˆte´, Caron, Aubert, Desrochers, & Ladouceur, 2003; Kassinove & Schare, 2001). In a computerized slot machine task, Clark, Lawrence, Astley-Jones & Gray (2009) reported that near-misses were experienced as less pleasant but more motivating compared to full-misses in healthy participants. These behavioral

effects were accompanied by enhanced reward-related activity in the ventral striatum, anterior insula and anterior cingulate cortex (ACC). Besides their rewarding effects, near-misses were also linked to cognitive components such as the agency of gambling actions (Wagenaar, 1988; Walker, 1992). For example, Clark et al. (2009) used a two reel slot machine where participants had to select a play icon on the first reel before the second reel started to spin. They investigated the effect of personal control by manipulating the involvement of the participant's choices comparing trials where the play icon was either chosen by the participants or the computer. Interestingly, the invigorating effects of near-misses on gambling motivation were restricted to trials where participants had personal control over the game compared to computer-chose trials. Many gambling studies observed similar effects and therefore emphasized the role of certain characteristics of chance games that foster the confidence to win (Davis, Sundahl, & Lesbo, 2000; Ladouceur & Mayrand, 1987). Clark et al. (2009) similarly interpreted their results, proposing that personal control over the game in combination with near-miss outcomes elicit a false belief of skill learning although the outcomes of the slot machine are solely determined by chance. The authors therefore described near-miss outcomes as promoting an "illusion of control". This assumption was strengthened by the results indicating that gambling-related cognitive distortions as the 'belief in luck' or 'illusions of control' were correlated with near-miss related activity in the insula (Clark et al., 2009). Within this framework, reinforcement learning mechanisms have been hypothesized to contribute to the belief of an acquired skill because near-misses may elicit brain responses which are in between an omission and receipt of a reward (D'Ardenne, McClure, Nystrom, & Cohen, 2008; McClure, Berns, & Montague, 2003). Such learning mechanisms have been often associated with reward prediction errors that signal the difference between the obtained reward and the predicted reward (Bayer & Glimcher, 2005). As a consequence, whereas a positive prediction error is beneficial in natural environments, slot machine near-misses may elicit maladaptive cognitions in circumstances where adjustments of (instrumental) behavior are not predictive for reward. This effect may be even stronger in individuals vulnerable to addictions. This hypothesis is partially strengthened showing that gambling-related cognitive distortions are more prevalent in PGs. (Toneatto, Blitz-Miller, Calderwood, Dragonetti, & Tsanos, 1997; Ladouceur & Walker, 1996). Additionally, Chase and Clark (2010) reported in their study that gambling severity was positively correlated with brain responses to near-miss outcomes in the dopaminergic synthesis region of the midbrain (i.e. the ventral tegmental area and the substantia nigra). The authors concluded that near-misses therefore may enhance dopamine transmission in disordered gambling.

Although all these evidences argue for a differential effect of near-miss outcomes in PGs compared to HCs, the comparison of the two groups did not reveal any significant functional changes in the brain. Contrasting wins against non-wins (including near-misses and full-misses) indicated diminished activity in PGs compared to HCs only. However, they were two potential problems with this study. First, gamblers differed significantly on gambling severity, ranging from minimal to modest gambling involvement. It is possible that this heterogeneity limited the authors to detect neurobiological differences relative to HCs. Therefore, it may be more beneficial to include a group of pathological gamblers who lie at the end of the continuum of disordered gambling, probably showing the most exacerbated reward processing alterations. The second problem with this study is that both groups, the gamblers and HCs, were not properly matched.

The current study first aims to replicate the main results of Clark et al. (2009) showing that near-misses elicit higher reward-related responses compared to full-misses in the ventral striatum and further strengthen the correlation of gambling severity with the near-miss response in the midbrain in PGs (Chase & Clark, 2010) as well as the correlation of gambling-related cognitive distortions with insula activity. Second, we will compare the brain responses between PGs and HCs. Concerning wins, the specific direction of neurophysiological functions within the reward circuitry is not clear reflected by many inconsistencies showing enhanced reward brain responses while others reported decreased responses in PGs. As a result, more research is needed to be done to clarify this relationship. Concerning near-misses, Chase and Clark (2010) were not able to demonstrate differential brain responses of gamblers relative to HCs probably due to their selection of gamblers and the not properly matched groups.

We will test this in an independent sample of a homogenous group of slot machine PGs and HCs with blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI). We reprogrammed a 3D version of the slot machine task

in order to elicit more realistic gambling experiences. The slot machine consisted of two reels, each displaying six icons and a payline. Participants could either win 5 euro or not win by selecting the play icon on the left reel and await the outcome.

1.3 Hypotheses

Based on previous results, near-misses are predicted to elicit higher reward-related responses compared to full-misses in the ventral striatum and the insula. Further, we expect to replicate the correlation of gambling severity with the near-miss response in the midbrain in PGs and further strengthen the correlation of cognitive distortions and near-miss related activity in the insula.

Win outcomes are hypothesized to elicit differential activity in the reward pathway, particularly in the ventral striatum and medial prefrontal areas, in PGs compared to HCs. According to the reward deficiency account, we predict hypo-responsive reward activity in PGs compared to HCs.

Near-misses are expected to stimulate stronger brain reward-responses in the ventral striatum and the insula in PGs relative to HCs.

2. Materials and Methods

2.1 General procedure

The entire experiment was conducted at the Donders Institute for Neuroimaging in Nijmegen, the Netherlands. Before scanning, each participant went through a screening session including a psychiatric interview. Because this study was part of an ongoing study focusing also on the role of dopamine on the same processes, it consisted of a pharmacological design, a double-blind, placebo-controlled, cross-over design. This necessitated that each subject was scanned twice on separated days. Test sessions started in the morning and the fMRI data acquisition was preceded by a training phase outside and inside the scanner in order to gain familiarity with the task requirements and the appropriate response buttons. The entire experimental day endured 5 hours including the slot machine tasks and other tasks which will be not addressed here.

2.2 Participants

We studied two groups, 13 PGs and 16 HCs, hence a total of 29 participants with normal or corrected to normal vision. This is not the definitive sample size as we did not finish the data acquisition yet. All participants were males because of the higher prevalence of PG compared to females. All subjects were right-handed, except for one PG.

All PG subjects met the DSM-IV-TR criteria for PG and had at least a score of 5 on the lifetime South Oaks Gambling Screen questionnaire measuring gambling severity (SOGS; Lesieur & Blume, 1987) (see Table 1). All HCs had a SOGS score of 0, except for 3 control subjects who scored 1, 1 and 2, respectively. The first question of the SOGS asking for the frequency and preference of gambling activities and the psychiatric interview enabled us to further assess whether PGs were still actively involved in gambling activities at the time of the experiment. More than half of the gamblers were still active indicated by scores of at least 5. All other PGs were either less active or non-active relative to the past.

We further asked for demographic variables as education, age, income, and reading ability which was used as a proxy of intelligence (NLV, Nederlandse leesvaardigheids test, Dutch Reading test for Adults; Schmand, Bakker, Saan & Louman, 1991). Additionally, we used the following questionnaires to assess related factors, the Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) which is an indicator of nicotine dependence severity, the Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, De la Fuente & Grant, , 1993) used to estimate alcohol consumption, the Hospital Anxiety and Depression scale (HAD; Zigmond & Snaith 1983) to assess current depressive and anxiety symptoms, the Gamblers Belief Questionnaire (GBQ), to examine gambling-related cognitive distortions (Steenbergh, Meyers, May, & Whelan, 2002), the Barratt Impulsiveness Scale (BIS-11) to evaluate impulsivity (Patton,Stanford & Barratt, 1995), the Kirby questionnaire which assesses delay discounting (Kirby & Maraković, 1996), and the listening span that measures working memory capacity (Daneman & Carpenter, 1980) and has been shown to be a proxy measure of dopamine synthesis (Cools, Gibbs, Miyakawa, Jagust, & D'Esposito, 2008). Lastly, we used the BIS BAS questionnaire which measures the sensitivity to reward and punishment (Carver & White, 1994).

Both groups did not differ on education, age, alcohol usage, anxiety and depression, income, and reading ability. Smoking behavior was not well matched between the groups, which is reflected in higher average scores in PGs as in HCs on the FTND. This is not really surprising since it has

been already indicated that nicotine dependence is one of the most prevalent comorbidities in PGs (Lorains, Cowlishaw, & Thomas, 2010). Exclusion criteria comprised substance abuse/dependence (except for nicotine dependence) and importantly all other DSM-IV-TR axis I disorders based on lifetime diagnosis. To ensure this, a structured psychiatric interview was conducted by a psychiatrist (MINI, Sheehan et al., 1998).

All participants were recruited via advertisement (flyers, newspaper, internet) and gave written informed consent, which was approved by the local ethics committee. Participants received a fixed amount of 50€ as a financial compensation. Additionally, they were told that they would receive the average amount of wins across all three runs of the slot machine task. Due to ethical considerations, this amount was a priori determined so that all participants received 25€ in cash after every scanning session. Finally, all participants were medication and alcohol free on both scanning days.

2.3 Task

We used a modified version of the slot machine task used in Clark et al. (2009). In order to manipulate outcome trials more easily, it resembled a two-reel slot machine with six symbols ordered in similar fashion on each reel and a horizontal 'pay line' across the center of the screen (see Figure 1). Each trial consisted of a selection phase, an anticipation phase, an outcome phase and a rating phase. In the selection phase, one of the six symbols on the left reel had to be selected by the participant using two buttons to scroll through the shapes and a third button to confirm the choice within a time window of 5s. Responses that were not completed within this time were followed by a "Too Late!" message. The selection phase was followed by an anticipation phase during which the reel was spun for a variable duration between 3.30 and 6.95s. When the reel stopped, the outcome phase, of duration of 3s, started. There were three different outcomes. Wins comprised trials where the symbols of both reels were matched. All other events were no-wins separated into two different categories. Near-misses constituted outcomes where the reel stopped one position above or below the designated win, whereas full-misses where composed of trials where the reel stopped more than one position away from the pay line. Wins were associated with a monetary gain of 5€ and cash-register sound. Near-misses and full-misses were associated with a 0€ outcome and a buzzer sound. In order to analyze these different outcomes and ensure a fair number of wins, near-miss and full-miss trials were pseudo-randomized with 1/6 wins, 2/6 near-misses and 3/6 full-misses. At the end of each trial a visual analogue scale appeared for participants to rate their motivation to continue gambling. A maximal time of 8 seconds were given to report their motivation on a 1 to 10 continuous scale (1=very little motivated; 10 =very highly motivated), otherwise a "Too late" message appeared. The task lasted for approximately 30 min and was separated into three runs of 30 trials. Each trial followed with an inter-trial interval between 2 to 5 seconds.

2.4 Behavioral analysis

The behavioral ratings comprising the participant's motivation to gamble were analyzed with SPSS using repeated-measures ANOVA. We included outcome type (win vs. near-misses vs. full-misses), session (testday1, testday2) and run (run 1, run2, run3) as within-subject factors, and group

Table 1. Demographic variables and clinical scales of the healthy controls and pathological gamblers.

Measures	HC (n=16)	Gamblers (n=13)	p-value (α=0.05)
Age	28.4(±8.95)	33.5(±8.75)	0.14
Education (number of years)	13.6(±2.4)	12.3(±3.2)	0.24
National adult reading test	88.068.08	82.92(10.88)	0.16
Net income (€)	1609.3(±1224.6)	1596.2(±826.25)	0.97
Fagerström Test for Nicotine Dependence	0.6(±1.45)	2.3(±2.75)	*0.04*
Alcohol Use Disorders Identification Test	6.1(±3.9)	6.85(±4.5)	0.62
Hospital Anxiety scale	3.2(±3.0)	3.7(±2.8)	0.65
Hospital Depression scale	2.0(±2.5)	2.8(±3.3)	0.48
South Oaks Gambling Screen questionnaire (lifetime)	0.25(±0.6)	10.6(±3.9)	*0.00*
South Oaks Gambling Screen questionnaire (last 3 months)	0.0(±0.00)	3.7(±2.3)	*0.00*

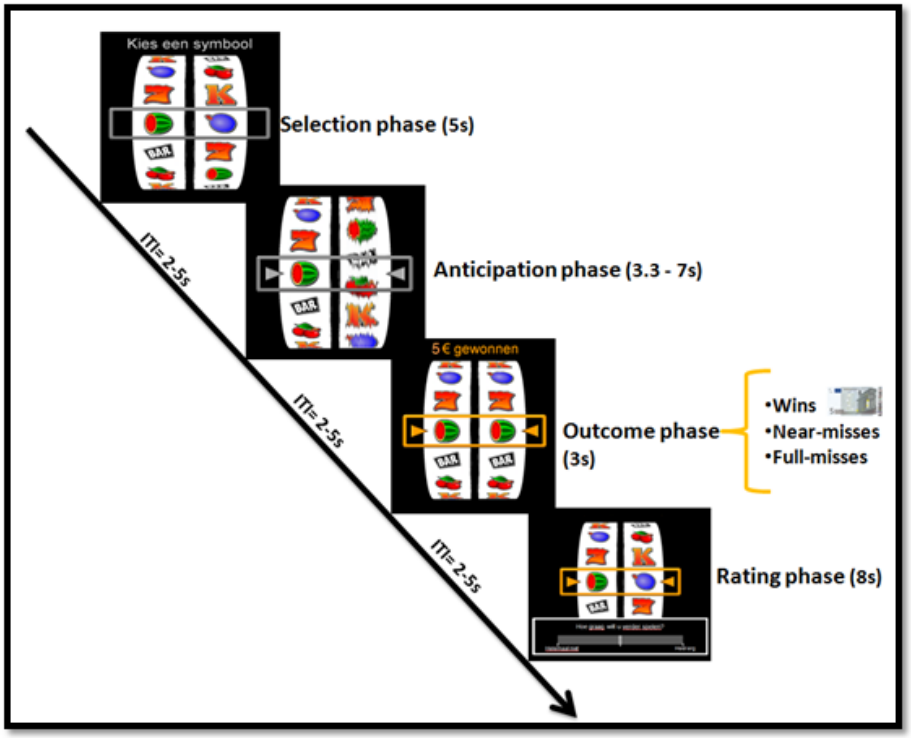

Fig. 1 The slot machine task: Each trial consisted of a selection phase, an anticipation phase, an outcome phase and a rating phase. In the selection phase, one of the six symbols on the left reel had to be selected, then the reel started to spin for a variable time after which the outcome phase followed consisting of wins, near-misses, and full-misses. After participants observed the outcome, they had to rate their motivation to continue to gamble.

(PGs vs. HCs) as a between-subject factor. Clark et al. (2009) used z-scores to account for the variability in anchoring across subjects which is useful when studying one group. Because this study is mainly aimed to focus on the differences between HCs and PGs we diverted from this approach and therefore focused on group means and standard deviations only.

2.5 FMRI data acquisition

Whole brain T2*-weighted BOLD fMRI data was acquired using echoplanar imaging (EPI) on a Siemens TIM Trio 3T scanner using an ascending slice acquisition sequence (38 axial slices, voxel size, 3.3x3.3x2.5mm, matrix, 64x64 repetition time (TR), 2.32 s flip angle, 90°,) . In order to benefit from reduced susceptibility artifacts at low echo times (TE), we used a multi-echo EPI sequence, with TE values of 9.0, 19.3, 30.0 and 40.0ms (Poser, Versluis, Hoogduin, & Norris, 2006). High-resolution structural images (1.0×1.0×1.0 mm) were acquired using a T1-weighted 3D gradient echo sequence (192 slices, TR, 2.3s, TE, 3.03s, flip-angle, 8°, matrix, 256x25).

2.6 fMRI data analysis

2.6.1 Preprocessing

Image preprocessing and statistical analysis of the fMRI data were performed using SPM8 (Welcome Department of Imaging Neuroscience, London). Volume-to-volume realignment parameters were estimated from the shortest TE-images and applied to all other TEs (Poser et al., 2006) using a least squares approach and a 6 parameter (rigid body) spatial transformation (Friston, Penny, & Glaser, 2005). Thirty volumes acquired before the start of the actual experiment were used to estimate weighting images representing BOLD contrast-to-noise ratio maps for each TE. Based on those images, a weighted summation was then performed to combine all four TEs into a single dataset (Poser et al., 2006). The resulting functional images were then coregistered to the bias-corrected anatomical image and slice-time corrected using the temporal middle slice as a reference. The anatomical image was segmented and normalized to the Montreal Neurological Institute space (MNI) using the grey matter for calculation of the nonlinear transformation matrix. This matrix was further used to normalize all functional images, which ultimately resampled into 2.5x2.5x2.5-mm isotropic voxels. Spatial smoothing was finally

performed with an isotropic Gaussian kernel of 8 mm full-width at half maximum.

2.6.2 Statistical analyses

First-level event-related analyses were run on individual datasets in the context of the general linear model (GLM). In a first GLM, the different event-related trial events were modeled and convolved with the canonical hemodynamic response function (HRF) of SPM8. We used independent regressors modeling the different trial phases with variable durations, the selection phase, based on the choice reaction time of the participant, the anticipation phase, determined by the variable duration the reel spun, the outcome phase (win, near-miss, and full-miss) modeled as a stick function and lastly the rating phase which was again determined by the participants reaction time choice (for detailed information on the durations, see Figure 1). Further, each run was modeled separately in order to account for different baseline effects. Realignment parameters were included to account for movement-related artifacts and a high-pass filter with a cut-off of 128sec was applied to the time series. Contrast images were generated at the single subject-level, including the orthogonal regressors wins vs. no-wins (i.e. near-misses and full-misses) in the first contrast, and near-misses vs. full-misses in the second contrast.

A second-level, random-effect analysis was then performed to assess consistent effects across participants. We used a flexible factorial design, modeling participants as a random factor and the two test days as a within-subject factor (of no-interest here, since we are still blind to the pharmacological manipulation). This was done within each group separately, and also across groups in order to directly compare HCs vs. PGs. Within-group results are reported at a peak-level threshold of $p<0.05$ corrected for multiple comparisons across the whole-brain using the family-wise error (FWE) method. Because of the high amount of data acquired and the resulting statistical power we decided to use a more explorative approach in form of a whole brain analysis. This diverted from Clark et al. (2009) methods where they used a win-related mask. Based on our a priori hypotheses concerning the role of the ventral striatum in PG, between-group results were thresholded using a FWE correction within small volumes of interest corresponding to this region. These volumes were defined as 12-mm spheres centered around peak voxels derived from an independent meta-analysis on reward processing (Liu, Hairston, Schrier, & Fan 2011; left: x,y,z=-10, 8, -4, right: x,y,z= 12,10, -6) .

We performed two additional analyses. First, in order to investigate whether the near-miss-related activity was linearly associated with gambling severity, we conducted a voxelwise univariate regression analysis using the SOGS scores of the PGs as independent variable. The results are reported at a threshold of $p<0.001$ uncorrected for multiple comparisons. Based on the results of Chase and Clark (2010), a small volume correction (SVC) was further applied in the midbrain, using 12-mm centered around the peak voxels reported by these authors (left: x,y,z=-6, -18, -16: right: x,y,z=10,-18,-12). Second, we also examined whether the near-miss-related activity correlated with gambling-related cognitive distortions. We therefore conducted a second voxelwise univariate regression analysis including the GBQ scores of the PGs as an independent variable. The results are reported at a threshold of $p<0.001$ uncorrected for multiple comparisons.

Lastly, a second GLM was used in order to explore the near-miss effect in further detail. Previous literature suggested that near-misses elicit (ventral) striatal activity. Based on this, we reasoned that the functional response of ventral striatum may be more generally and linearly locked to the distance between the two reels. We therefore decomposed the variable outcome as function of pay line distance with wins indexing 0-position distance, near-misses 1-position distance, full-misses with 2-position and 3-position distances from the pay line. By expecting linear effects in the ventral striatum to these reward-gradient, the variable outcome was parametrically modulated at the first level. Based on its key role in reward processing, we selected an anatomical region of interest (ROI) in the nucleus accumbens (Nacc) by the hammers probabilistic structural atlas (Hammers et al., 2003) and extracted the fMRI signal from this ROI by using rfxplot (Glaescher, 2009). Particularly, we extracted the time-course of the BOLD response using a finite impulse response (FIR) modeling approach. We tested for consistent effects within subjects and across groups in a second random effect analysis.

We aimed to determine whether the ratings on the different slot machine outcomes were correlated with the brain activity. We therefore calculated the mean ratings following wins minus no-wins and near-misses minus full-misses for each subject and then performed a multiple regression across all participants using the contrasts win>no-wins and near-misses>full-misses, respectively.

Table 2. Questionnaires- Personality for both healthy controls and pathological gamblers. Mean values in the cells, standard deviations indicated in brackets. WM = working memory.

Measure	HC (n=16)	Gamblers (n=13)	p-value (α=0.05)
Gamblers Belief Questionnaire			
-Overall	121.3(±18.1)	79.5(±19.7)	*0.000*
- Belief in luck/ perseverance	75.7(±9.85)	50.7(±12.3)	*0.000*
-Illusion of control	45.6(±9.65)	28.85(±9.0)	*0.000*
Barratt Impulsiveness Scale			
-Overall	57.5(±8.3)	69.3(±13.6)	*0.008*
-Attentional impulsiveness	13.4(±3.2)	17.3(±4.3)	*0.009*
-Motor impulsiveness	21.6(±4.7)	24.8(±4.95)	0.092
-Nonplanning impulsiveness	22.4(±3.7)	27.2(±6.2)	*0.015*
Behavioral inhibition system Scale	17.6(±3.3)	16.4(±2.9)	0.300
Behavioral activation system Scale	39.1(±4.3)	42.3(±3.7)	*0.045*
-Reward	14.6(±1.9)	16.3(±1.4)	*0.010*
-Drive	12.4(±2.4)	13.2(±1.7)	0.319
-Fun	12.1(±1.5)	12.8(±1.6)	0.280
Kirby (k-value)	0.0109(±0.0112)	0.0642(±0.0711)	*0.006*
Listening span (WM capacity)	4.4(±1.5)	3.15(±1.3)	*0.031*

3. Results

3.1 Psychometric measures

To test for variability of our groups on different psychological measures, we tested all participants on gambling-related cognitive distortions (GBQ), impulsivity (BIS11), sensitivity to reward and punishment (BIS-BAS), delay discounting (Kirby) and listening span (working memory measure) (Table 2). PGs and HCs significantly differed on most of these psychological questionnaires. Compared to HCs, PGs were found to have more gambling-related cognitive distortions. They showed significant higher scores on overall impulsiveness and on the subscales of attention and non-planning and a trend towards higher motor impulsiveness. In line with this, they additionally reflected significantly higher delay discounting. Furthermore, PGs scored higher on the reward-related and the general behavioral approach scores as assessed with the BAS scale. In general, this means that PGs have an enhanced sensitivity to reward compared to HCs (Gray, 1991). Interestingly, PGs scored lower on the listening span, a working memory test.

3.2 Behavioral results

3.2.1 Ratings on wins, near-misses and full-misses

After the outcome phase, participants were asked how much they wanted to continue to play as a measure of gambling persistence and motivation (Figure 2). The Repeated Measures ANOVA indicated a main effect of outcome on ratings ($F(2,28)=16.69$, $p<.001$) which was driven by higher ratings following wins compared to near-misses and full-misses. We also find a differential effect of near-misses and full-misses. An overall LSD post-hoc t-test indicated higher ratings for wins compared to near-misses ($p<.001$) and full-misses ($p<.001$). The ratings for near-misses were also higher than on full-misses ($p<.028$). Interestingly, we further detected a group difference driven by higher ratings in PGs compared to HCs ($F(1,28)=6.09$, $p=.02$). This effect was independent of outcome revealed by a non-significant interaction of group and outcome ($F(2,28)=0.36$, $p=0.7$) and reflects the intrinsic higher motivation in PGs. The analysis also revealed a significant main effect of run showing that increasing runs generally decreased the behavioral ratings ($F(2,28)=4.3$, $p=.025$). This may indicate that the motivation of the participants decreased over time. Session had no influence on the ratings ($F(1,28)= .863$, $p= .36$).

3.2.2 fMRI results on wins, near-misses and full-misses

Brain responses to wins, near-misses and full-misses are illustrated in Figure 3. Contrasting

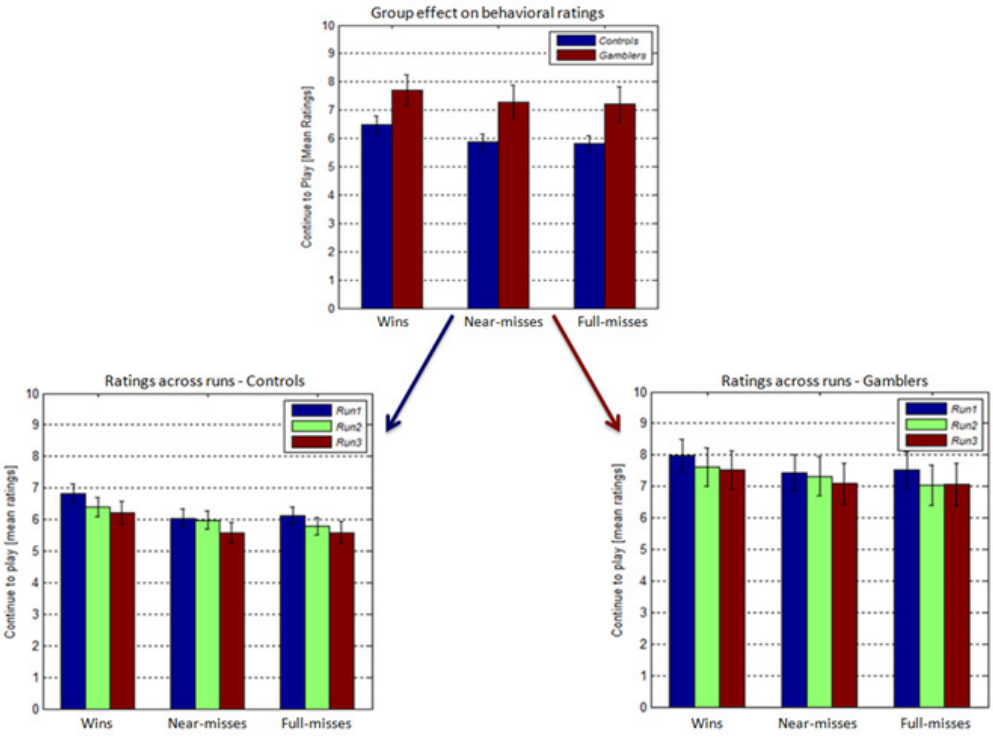

Fig. 2 Subjective ratings on the slot machine task. The ratings were intended to measure the motivation to continue gambling. The top graph shows the mean ratings across the three outcomes, wins, near-misses and full-misses, for each group (HCs and gamblers). The left and right graphs below illustrate the ratings across runs for each group separately (on the left for controls, on the right for gamblers). There was a significant effect of group, outcome and run. Error bars represent SEM.

monetary wins against all no-wins (i.e. near-misses and full-misses) indicated that PGs and HCs recruited similar brain regions (see Table 3 in the supplementary material for a detailed summary). Unpredictable monetary wins elicited significantly higher activity in structures frequently associated with reward and reinforcement learning, such as the ventral striatum, the anterior insula, the ventro-medial prefrontal cortex, and a midbrain region proximal to VTA. These results were FWE corrected for multiple comparisons ($p \leq .05$). Similarly consistent are the BOLD activities recruited with near-misses versus full-misses, showing again reward processing areas such as the ventral striatum, ACC, insula, medial and superior frontal regions, inferior frontal cortex, thalamus, and parietal regions but no midbrain activity when correcting for multiple comparisons across the whole brain. The neurobiological correlates for near-misses were similar to win events. These results replicate previous results with even higher statistical robustness (Clark et al., 2009; Chase & Clark, 2010).

Clark et al. (2009) investigated whether near-misses have distinct effects on reward processing when the play icon stopped one position before or after the pay line. He called those events pre- and post-near-misses, respectively. He reported greater recruitment of reward-related processing areas with pre-near-misses. We tested whether we could replicate these findings but our analysis revealed no significant differences between the two near-miss events, neither for behavioral or BOLD measures.

3.2.3 Parametric modulation of outcome: Reward sensitivity of the nucleus accumbens

In order to better characterize the BOLD response of the Nacc to the different reward outcomes, we built a second GLM in which all outcomes were grouped into one single regressor and parametrically modeled the distance between the two reels leading to the jackpot. Thus, wins were assigned a value of zero, near-misses were assigned a value of one, while full-misses were assigned a value of two and three positions away from the pay line. Based on its key role in reward processing, we extracted the fMRI signal from an anatomical ROI in the nucleus accumbens (Nacc) for each of these four event types (Figure 4). Interestingly, in both HCs and PGs, the amplitude and the peak latency of the BOLD response in the NAcc are proportional to the pay line distance and therefore to the proximity to the jackpot. The latency of the BOLD peaked earliest for full-misses three positions from the pay

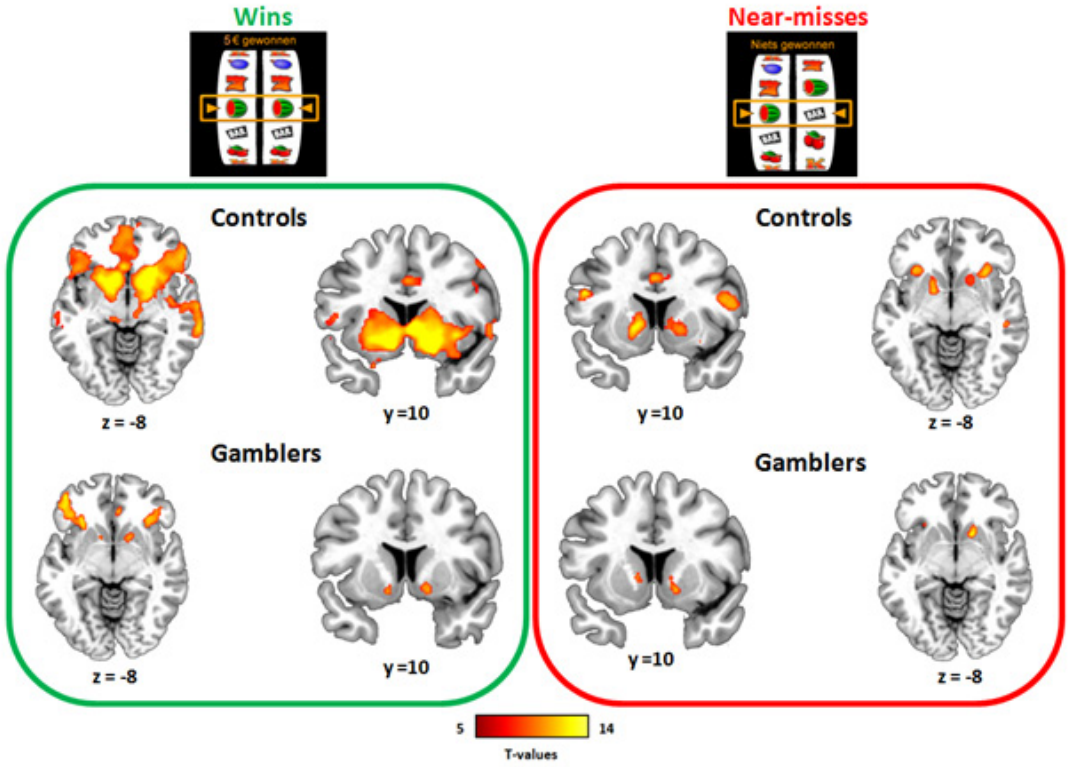

Fig. 3 Reward-related activity to wins and near-misses in HCs and PGs. Brain regions responding to monetary wins > nonwins are illustrated in the left green box whereas brain activities elicited to near-misses > full-misses are shown in the right red box. Results are displayed at $p < 0.05$ FWE whole-brain corrected. The T-map indicates the voxels with peaks of significance in the bilateral ventral striatum, ventromedial prefrontal regions and the bilateral insula. Activations are overlaid on an average T1 scan.

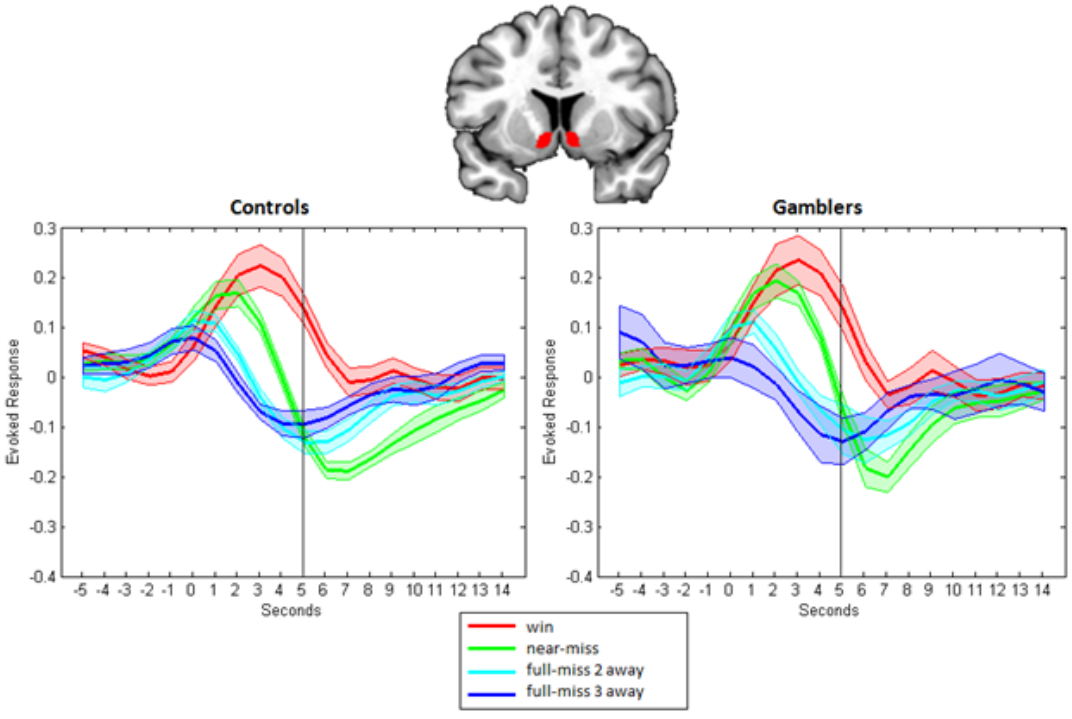

Fig. 4 Extraction of BOLD response using a FIR model in an anatomical ROI corresponding to the NAcc. The parametrically modeled factor reward outcome is displayed involving four levels of different pay line distances: wins (distance = 0), near-misses (distance = 1), full-misses 2 positions away from pay line and full-misses 3 positions away from pay line. The amplitude and the latency of the ventral striatum respond linearly to different pay line distances. The zero time point corresponds to the onset of the reward outcome presentation. The BOLD time-courses were averaged across the left and right Nacc. Shaded areas represent SEM.

line and latest for wins. It further seems that no-win events elicit higher negative peaks compared to wins and that the negative peak is symmetrical with the positive peak.

3.2.4 Comparison of groups

The group comparison on the win-no-win contrast showed a significant difference in the right anterior caudate nucleus (x,y,z = 16, 23, 0), which survived a SVC in our striatal ROI (Figure 5). The extraction of percent signal change showed that while controls showed a clear differential response to wins compared to no-wins, this difference was abolished in gamblers. The plot of percent signals change further suggested that gamblers showed both a blunted response to wins and an enhanced response to no-wins in this region. There were no other altered brain responses between PGs and HCs neither on this contrast nor on the near-miss>full-miss contrast, even not at an explorative uncorrected threshold (p<.001).

3.2.5 Behavioral ratings and brain activity

In order to determine whether the brain activity observed for each type of outcome was predictive of the motivation to continue gambling across participants, we entered the mean ratings following wins (minus no-wins) and near-misses (minus full-misses) as subject-specific covariates in multiple regression analyses. There were no significant voxels in which the brain activity correlated with the behavioral ratings.

3.2.6 Correlations with near-miss activity and gambling severity

We investigated whether the brain responses to near-misses were predicted by gambling severity in PGs. For this reason, we performed a multiple regression analysis in which the betas from the contrast near-misses>full-misses were regressed against individual SOGS scores. At an uncorrected significance threshold (p<.001) we found that gambling severity was positively associated with BOLD responses in the midbrain (x,y,z=6,-26,-12) and the bilateral insula (x,y,z=right: 41,10,-12 right, left: -42,7,-12) . The midbrain activity survived an SVC within an independent ROI. This correlation is consistent with previous results reporting similar effects in the midbrain (Chase & Clark, 2010) and we extended this association to the insula. In order to illustrate this relationship, we added plots (Figure 6) which exemplify the results of the whole-brain analysis. These plots further illustrate that the observed correlation is not merely driven by outliers.

3.2.7 Correlations with near-miss activity and gambling-related cognitive distortions

Finally, we performed a similar multiple regression analysis with the extracted betas of the near-misses>full-misses contrast and looked for a linear association with the individual GBQ scores. This analysis with an explorative significance threshold (p<.001) revealed a negative relationship between the left insula (x,y,z= -36, 6, 15) and the GBQ meaning that higher self-reported gambling-related cognitive distortions predict stronger left insula activity (GBQ indicates high cognitive distortions with low scores).

4. Discussion

The main aims of this experiment were first to replicate the main results of Clark et al. (2009) who showed that individuals exhibit reward-related brain responses to near-misses compared to full-misses and additionally reported a link between near-miss-related activity and gambling severity, as well as gambling-related cognitive distortions in PGs (Chase & Clark, 2010; Clark et al, 2009). To this aim, we compared PGs with a preference to chance games and HCs on the different slot machine outcomes and were especially interested whether PGs would elicit exacerbated brain responses to near-misses because of their known invigorating effects on gambling. Although Chase and Clark (2010) were not able to find a differential effect of near-misses in gamblers and HCs, we proposed that this effect was dependent on secondary factors as the inclusion of the gamblers with minimal to modest gambling activity and their not properly matched HCs. Second, we addressed the neurobiological signature of PG on win outcomes because of the inconsistent results in the neuroimaging literature. Based on the reward deficiency account, we hypothesized that PGs would show blunted brain responses to wins relative to HCs.

4.1 Near-miss effects on the behavioral ratings

On a behavioral level, we found a significant effect of outcome on ratings which was driven by higher ratings for wins compared to all no-wins and near-misses over full-misses. This replicates earlier

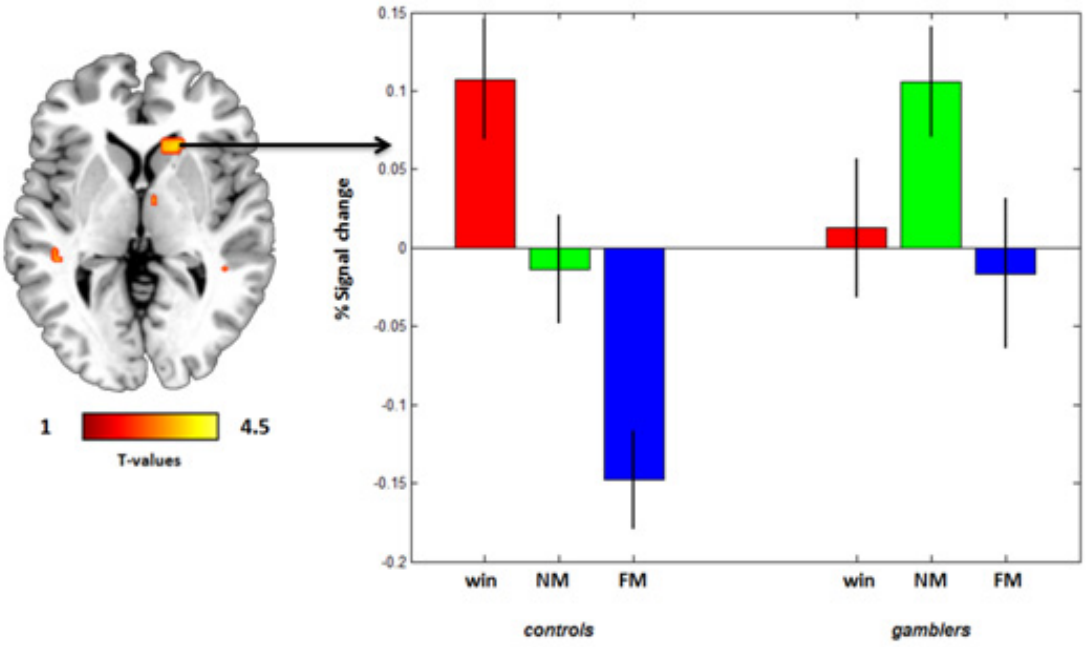

Fig. 5 Increased brain response to wins in HCs compared to pathological gamblers. The T-map contrast indicates the voxels in wins > nonwins for HCs > PGs. For display purpose, the results are shown at $p < 0.001$ uncorrected and overlaid on an anatomical image. The differential activity in the anterior caudate nucleus is significant with a small volume correction within an independent ROI. We extracted the percent signal change from the functional cluster. NM = near-miss, FM = full-miss.

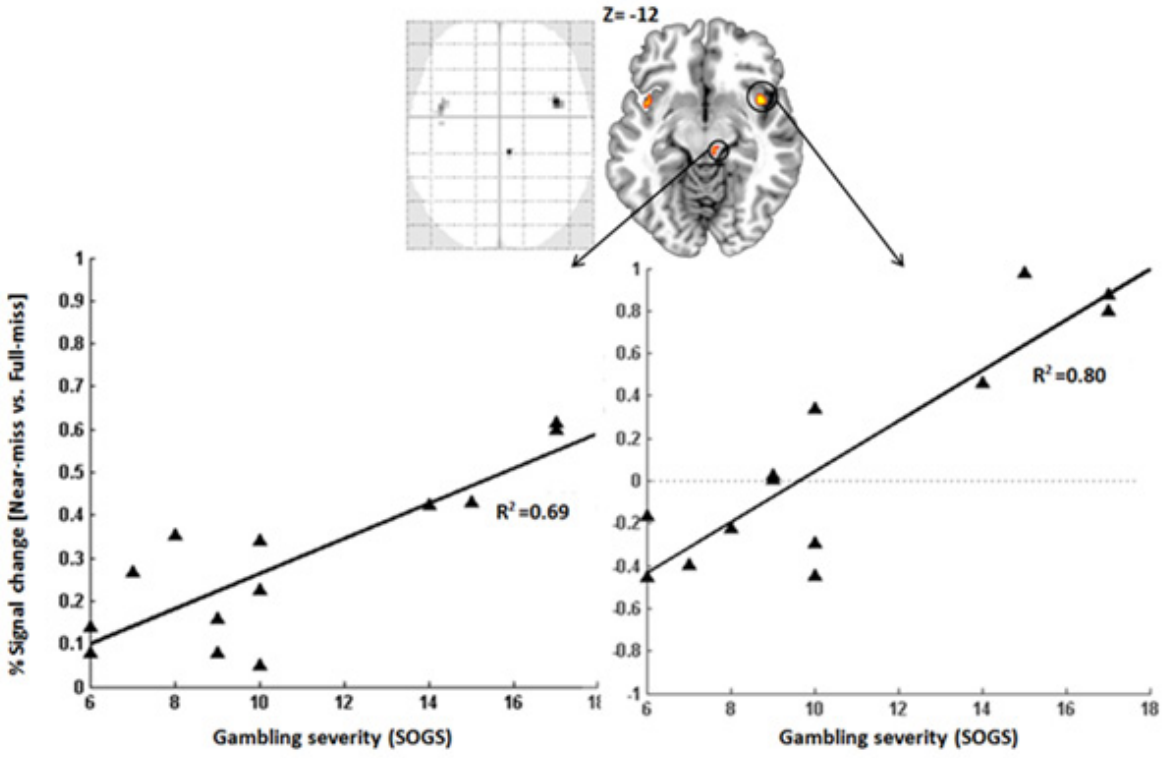

Fig. 6 Correlation between brain responses to near-misses and gambling severity in pathological gamblers. Results are displayed at $p < 0.001$ whole-brain uncorrected (note that activity in the midbrain survives a small volume correction within an independent ROI). Activations are overlaid on an average T1 scan. Percent signal change was extracted from the midbrain and left insula and plotted against SOGS scores (the plot in the right insula was similar).

findings on the near-miss effect and illustrates the invigorating effect of near-misses on gambling (Clark, Liu, McKavanagh, Garrett, Dunn, & Aitken , 2012). Unexpectedly, the analysis revealed a main effect of run reflecting a gradual decrease of the ratings across the task. This potentially indicates that participants were tired or bored after a substantial number of trials. Importantly, the group comparison revealed higher ratings in PGs relative to HCs independent of reward outcome. This suggests that PGs are intrinsically more motivated to gamble than HCs irrespective of gambling outcomes. This is consistent with the symptomatic description of PG emphasizing the persistent and compulsive nature of gambling behavior despite often negative consequences.

4.2 Brain responses to wins, near-misses and full-misses

In all participants, wins elicited a distributed pattern of reward-related activity in the ventral striatum, which is associated with reward processing (Schultz, Tremblay, & Hollerman, 2000; Pagnoni, Zink, Montague, & Berns, 2002; McClure, York, & Montague, 2004), and the anterior insula, which is known to process bodily feedback and has been linked to compulsive urges in the addiction literature (Critchley, Wiens, Rotshtein, & Oehman, 2004; Goldstein, Craig, Bechara, Garavan, Paulus, & Volkow, 2009; Naqvi & Bechara, 2009). Playing a role in decision-making (Bechara & van der Linden, 2005; Bechara, Damasio, Damasio, & Anderson, 1994), medial and superior frontal regions were also recruited, including the ACC. In the context of reward outcomes, animal lesion studies implicated the ACC in decisions related to effort costs when obtaining a reward (Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006), and integrating past decisions in the guidance of future behaviors (Behrens, Woolrich, Walton, & Rushworth, 2007; Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006). Additional activations were present in a midbrain region proximal to the VTA/SN, which is the main dopamine synthesis site (Grace & Onn, 1989), and in parietal regions, often associated with attentional and cognitive functions (Culham & Kanwisher, 2001). This circuit has been referred to the mesocorticolimbic reward system and has been previously implicated in reinforcement processing (Berns, McClure, Pagnoni, & Montague, 2001; Breiter, Aharon, Kahneman, Dale, & Shizgal, 2001; Nieuwenhuis, Slagter, von Geusau, Heslenfeld, & Holroyd, 2005b).

Despite their lack of monetary value, near-miss events elicited similar activation patterns in the ventral striatum, ACC, insula, medial and superior frontal regions, inferior frontal cortex, thalamus, and parietal regions. This is in accordance with behavioral descriptions of near-misses emphasizing its role in promoting gambling persistence by its rewarding effects. These results strengthen previous results reported by Clark et al. (2009) (Chase & Clark, 2010).

Another aim of the present study was to replicate the reported association between individual variation in gambling severity and reward-related brain activity. A regression-based analysis revealed a positive relationship between gambling severity and near-miss-related activity in the midbrain and the insula. This means that stronger disordered gambling predict higher BOLD signal changes with near-miss outcomes in both areas replicating earlier findings by Chase and Clark (2010) who reported similar midbrain responses. Our analysis extended these results to the insula which is consistent with the proposed role of the insula in drug craving behavior (Naqvi & Bechara, 2009) and is also involved in the signaling of interoceptive aspects of compulsive urges and bodily feedback (Critchley et al., 2004; Naqvi & Bechara, 2009). The associated midbrain activity may indicate an ongoing dopaminergic mechanism that may elucidate the rewarding effect of near-misses on gambling despite their nonwin status. Since gambling related cognitive distortions play an important role in PG, we were also interested whether these predicted BOLD responses in PGs. Clark et al. (2009) reported that self-reported cognitive distortions related to higher left insula activity in participants with minimal to modest gambling involvement. We replicated his results in the same area and extend this to PGs which indicates that the insula plays also an integrative role in cognitive components that are characteristics for behavioral addictions. Although, we find similar associations of gambling severity, cognitive distortions and fMRI signals as Clark et al (2009) we have to treat our findings with caution because they did not survive a multiple comparisons correction.

We further focused on characterizing the BOLD response to the different reward outcomes in the Nacc. These results suggest a linear relationship between the amplitude of the BOLD and the proximity to a win. Moreover, the positive peak seems to be followed by a negative peak for all non-win events (near-misses and different full-misses) (Figure 4).

These results are generally consistent with Clark

et al.'s (2009) hypothesis that near-misses can be decomposed into different "cognitive" time courses in which a building up of a winning expectation, akin to a positive prediction error, is followed by the omission of a reward, akin to a negative prediction error. In more detail, the time of reel spinning gives some prior information about the likely reward outcome that leads to an expectation or prediction. Whereas we have to wait until the very last moment until we know that the outcome is a win, we can already make some predictions on the likely non-win status with near-misses and even more full-misses before the reel stops. This account is also in line with animal studies that emphasize the discriminative capacity of the reward system to predict how likely a reward value will be in the future (e.g. Tobler, Fiorillo, & Schultz, 2005). The second result of the BOLD response shows an interesting pattern for no-wins in which it seems that the negative peak is symmetrical with the positive peak. This is in accordance with the negative prediction error account which states that the size of the negative prediction error is directly proportional to the size of the positive prediction error.

Generally, the proposed role of the NAcc in the computation of reward prediction errors is consistent with several animal studies highlighting that it is involved in the signaling of errors in the prediction of rewards (Schultz, Dayan & Montague., 1997). Similar suggestions have been made by human neuroimaging studies, for example in an operant conditioning study which suggested differential responses of the Nacc to events when an expected fruit juice reward was delivered compared to events when it was unexpectedly omitted (Pagnoni et al., 2002). Although there is evidence for the correlation of activity in the ventral striatum and prediction errors (D'Ardenne et al., 2008; McClure et al., 2003), the proposed relationship remains speculative.

4.3 Group differences in the slot machine task

When comparing groups, only the win-no-win contrast revealed a significant difference in a specific part of the striatum, the right anterior caudate nucleus. While controls showed a clear differential response to wins compared to no-wins, this difference was abolished in gamblers. The percent signal change in this region further suggested that gamblers showed both a blunted response to wins and an enhanced response to no-wins compared to HCs (Figure 5).

The anterior caudate nucleus has been implicated in different aspects of motivation, reward processing, reward-based learning processes as well as action initiation (Ashby, Turner, & Horvitz, 2010; Cromwell & Schultz, 2003; O'Doherty, Deichmann, Critchley, & Dolan, 2002; Knutson, Adams, Fong, & Hommer., 2001). Several fMRI studies for example demonstrated increases in BOLD responses in the striatum during anticipation of either primary, as food (O'Doherty et al., 2002) or secondary reward, like money (Knutson et al., 2001). Considering its functional role, the abolished difference to wins and nonwins in PGs may be interpreted as a blunted sensitivity to reward outcomes in general.

The anterior caudate nucleus receives dopaminergic projections from the VTA/SN (for a detailed review, see Haber, 2003). It is therefore possible that the diminished sensitivity found in PGs might relate to dopaminergic mechanisms that originate in the VTA/SN which in turn influence the reward functioning in striatal regions. Although, this is based on speculations some insights into dopaminergic mechanisms in PG have been indicated as for instance in the case of the dopamine agonist treatment of Parkinson's disease which has been found to be associated with the development of PG (Dodd, Klos, Bower, Geda, Josephs, & Ahlskog , 2005). Related to this, it would be interesting to explore whether there was an altered functional connectivity between the VTA/SN and the right anterior caudate nucleus in PGs relative to HCs. Generally, this finding would be consistent with the reward deficiency account which will be discussed below.

The direction of the reward impairment in PGs in response to wins converges with earlier reported results of e.g. Reuter et al. (2005). They similarly reported an attenuated BOLD response in the ventral striatum, and medial PFC in response to wins. Most of these findings concluded evidence for the reward deficiency account which proposes that genetic alterations initiate a cascade of changes that result into chronic hypodopaminergic levels in subcortical regions and therefore a hypoactive reward system (Blum, Sheridan, Wood, Braverman, Chen, & Comings, 1995; Comings & Blum, 2000). These changes on the brain level would further relate to compensatory mechanisms on the behavioral level, as for example sensation-seeking behavior that promote the risk for drug addiction or PG (Bowirrat & Oscar-Berman, 2005). However, we did not detect a difference in main reward processing side, the Nacc, between PGs and HCs (figure 4) which is somewhat difficult to reconcile with the reward

deficiency hypothesis.

Another debated point refers to the cause of the observed hypoactive reward system, namely whether it reflects the etiology of PG, as predicted by the reward deficiency hypothesis or whether it is the consequence of the disorder. An example that corresponds to accounts relating to the consequence of addiction is the development of tolerance. This is a key component of drug addiction in which an increasing amount of the drug is needed in order to reach the desired effect (Cami & Farre, 2003). As an analogue, it is possible that PGs built a form of tolerance after years of slot machine gambling that is compensated by higher monetary investments or a longer time to play. Ecological validity may have exerted similar reward related signal decreases. We tried to make the computerized slot machine as ecologically valid as possible. However, in real life PGs display a few typical behaviors, ranging from playing with more than one slot machine in parallel to gambling on specific time intervals. Those behaviors which are probably driven by cognitive distortions cannot be expressed in an experimental setting. This assumption is consistent with psychophysiological studies suggesting that regular players show qualitative differences when gambling in experimental settings instead of realistic gambling environments (Anderson & Brown, 1984; Meyer et al., 2004).

Even though the reward deficiency hypothesis is the currently dominant view based on a handful fmri studies, there are still some inconsistencies about the specific direction of reward processing impairments. As mentioned in the introduction, some studies showed a hyper-responsive reward system in PGs (Hewig, 2010; Oberg, 2011; Holst, 2012). For detailed discussions on these inconsistencies see the review of Hommer et al. (2011) who reviews this issue on a general base of addictions.

Limitations of this study

It is important to consider limitations of our study. HCs and PGs differed on the FTDN questionnaire indicating that the gamblers smoked more often. It is possible that this may have influenced our results especially because studies on smoking have shown attenuated BOLD responses in the reward circuit. Future studies that focus on similar group comparisons should therefore control for this possible confounding factor.

Importantly, the sensitivity of the BOLD signal and the proportion of activated voxels depend on the number of subjects per group (Thirion, Pinel, Meriaux, Roche, Dehaene & Poline,, 2007). We compared 16 subjects in the control group to 13 PGs which may be enough to detect a general trend but not to establish reliable conclusions. Furthermore, the generalizability of our study findings is restricted to males because we chose to exclude women. Another issue focuses on the nature of our task. Slot machines only include no-wins and no real losses which may also present outcomes key to the understanding of the mechanisms involved in PG. As PG is also typified by decision-making problems, this task is also limited to investigate this component of disordered gambling (Tanabe, Thompson, Claus, Dalwani, Hutchison, & Banich, 2007).

Although we do not have any direct data on dopaminergic mechanisms that may play a role in the neurobiological underpinnings of PG, we measured the listening span and found lower scores in PGs compared to HCs. The listening span has been earlier shown to predict dopamine synthesis capacity in the striatum (Cools et al., 2008). According to this, one may hypothesis that PGs and HCs differ in dopaminergic mechanisms and the lower scores may already give a hint that PGs have less DA synthesis capacity in the striatum. Our research group is currently working on a PET study that allows to investigate whether disordered gambling is directly associated with dopaminergic dysfunctions.

For understanding the underlying etiology and neurobiological signature of PG it is important to consider the heterogeneity of PGs. We tried to keep our group of PGs as homogenous as possible considering their preference for chance games. However, it is important to keep in mind that a psychiatric disorder such as PG is composed of different complex factors that may play distinct roles in different gamblers. An interesting review of Milosevic and Ledgerwood (2010) addresses this issue. A literature search demonstrated three distinct subtypes of PGs based on their motivations for gambling, psychopathological and personality factors. According to this, the first subtype would be characterized by depression and anxiety, the second typified by impulsivity who utilizes gambling to increase arousal and/or decrease boredom and the last PG subtype who gambles due to external factors and behavioral conditioning. As a consequence, it is possible that the inconsistencies in the literature may be explained by the variability in the studied PG group. The complexity of the gambling problem is also confirmed by the various psychological tests that we assessed. PGs and HCs differed on various psychological variables such as impulsivity, reward

sensitivity, false beliefs, listening span capacity, and delay discounting which all may result in different brain signatures. As human behavior is a complex multifaceted construct, behavioral addictions may be treated from a similar perspective.

Finally, neuroimaging studies investigating psychiatric disorders reported structural and functional connectivity changes in the brain compared to healthy subjects which even extend into the drug addiction literature (Ma et al., 2010). A similar approach with PG may clarify the separate role and the interaction of top-down cognitive functions and bottom-up reward mechanisms that both play an important role in behavioral addictions.

5. Conclusion

The slot machine task, with its intermediary rewarding near-miss outcome, is a perfectly suited task to study the sensitivity of the reward system. Replicating previous results, our results strengthen that near-misses elicit brain responses similar to wins despite their lack of monetary reinforcement. Further, we have shown that the insula is involved in gambling-related cognitive distortions that are key in PGs and is additionally related to gambling severity, similarly as the midbrain. The response of the nucleus accumbens has been shown to respond linearly to the distance of the jackpot. Importantly, investigations into neurobiological correlates of pathological gambling showed an abolished differential response to wins compared to no-wins in the striatum relative to controls. The results further suggested that gamblers showed both a blunted response to wins and an enhanced response to no-wins in this region. These findings provide evidence for a diminished reward sensitivity and a hypo-responsive reward system to wins in gamblers.

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Abstracts

Proceedings of the Master's Programme of Cognitive Neuroscience is committed to publishing all submitted theses. Given the number of submissions we select certain articles under the recommendation of the editors for our printed edition. To interested readers, we have provided the abstracts of all other articles of which the full versions are available on our website: www.ru.nl/master/cns/journal.

The effects of depression on the semantic processing of emotional language: an ERP study

John van Gortel, dr. Constance Vissers, and dr. Dorothee Chwilla

Nowadays, more and more evidence is accumulating in favor of an embodied perspective on language comprehension. Within this perspective, the brain, the body and the environment interact to make sense of the world. Research has shown interactions between the brain and the body in various cognitive tasks. The interactions include top-down influence of perception, action, mood and emotion on language comprehension. However, not much researchers have addressed the possible influence of mood disorders on language comprehension. We studied the semantic processing of emotional language material in patients suffering from clinical depression compared with nondepressed controls. To this aim, we recorded event-related potentials (ERPs) while participants attentively read scenarios containing either affectively positive, negative and neutral words within nonconstraining neutral contexts, after which they were required to respond to a positively or negatively valenced statement. In contrast with other studies, the present results suggest differences in the processing of emotionally affective language between depressed and nondepressed individuals, as reflected by differences in the N400 window. For affectively neutral language, no differences in N400 are found. In addition, the preliminary ERP-results suggest an increase in N400 for positive words compared to negative and neutral words for the patients. This would point to a 'negativity bias', an automatic preference for negative information, as is reported more often in depressed patients. Behavioral data suggest a negativity bias for nondepressed controls, but not for patients. Despite some caveats, present results are consistent with the embodied perspective on language processing.

Decoding Tactile Properties of Visually Presented Objects

Claudia S. Lüttke, Marcel van Gerven, Floris P. de Lange

Multimodal perception (i.e. perceiving the world around us using multiple sensory systems simultaneously) requires integration of information from different senses responsible for visual, tactile (touch) or auditory perception. It is unclear whether visual perception of an object can automatically activate a representation in a different sensory modality (e.g. tactile modality) and thereby potentially facilitate multimodal integration. In this functional magnetic resonance imaging (fMRI) study we visually presented objects that differed in the tactile property texture (smooth and rough) and used multivoxel pattern analysis (MVPA) to categorize these stimuli according to their roughness. We further asked whether multimodal representations are automatically triggered or only when task-relevant. To address this issue, we asked subjects on different blocks to either make judgments about the tactile roughness of the stimuli (tactile task) or about their semantic category (semantic task). Classification performance was above chance in two regions of interest (ROIs): First, the collateral sulcus, which is sensitive to texture; and second, the lateral occipital cortex, which is involved in visual and haptic shape processing. We did not observe a modulation of classification accuracy as a function of task. Furthermore, we could not classify texture in the primary and secondary somatosensory cortices. We conclude that a visual object can activate representations of its tactile properties in higher order areas in the occipital lobe but not in somatosensory areas. Since visual and tactile texture perception may converge in medial occipital cortex, this region might facilitate multimodal integration of vision and touch.

The interplay between emotion and attention on the processing of syntactic anomalies: evidence from P600

Martine W.F.T. Verhees, Dorothee J. Chwilla

Little is known about the relationship between language and emotion. Vissers and colleagues (2010) investigated the effects of mood on the processing of syntactic violations, as indexed by P600. An interaction was observed between mood and syntactic correctness for which three explanations were offered: one in terms of syntactic processing, one in terms of heuristic processing, and one in terms of more general factors like attention. The aim of the present article was to further determine the locus of the effects of emotional state on language comprehension by investigating whether, and if so how, more general factors like attention contribute to the mood-related modulation in P600 effect to syntactic anomalies. To this aim we compared the P600 effect to subject-verb agreement errors relative to correct sentences while ERPs were recorded and mood was manipulated by presenting happy or sad film clips. Attention was directed by using a task manipulation: participants either had to focus their attention on the syntactic features of the sentences or they had to focus their attention on the physical features of the sentences. The main findings were as follows: we effectively induced the intended mood. That is after watching happy film clips, participants were happier and after watching sad film clips participants were sadder compared to baseline. For P600, interactions with emotional state, task and correctness were obtained, reflecting that mood and attention both modulated the P600 correctness effect. The mood manipulation led to a reduction in P600 effect for the sad mood condition as compared to the happy mood condition, in the syntactic task. The task manipulation led to a reduction in P600 effect in the physical task as compared to the syntactic task, in the happy mood condition. The present data show that attention can contribute to the mood-related modulation in P600 effect to syntactic anomalies. Future studies will have to take into account that attention can play a role in the emotion-related modulation of language processing.

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