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From the Editors-in-Chief of the CNS Journal

Dear Reader,

Only two months after releasing the first issue, we are happy to present the second issue of the 9th volume of the *Proceedings of the Master's Programme Cognitive Neuroscience*. While in our first issue we focused on MRI research, in this issue you will find articles that represent a large variety of neuroscientific methods. This diversity reflects the interdisciplinary nature of cognitive neuroscience research.

This issue will be launched at the Synapsium 2014, which is a one-day conference organised by students from the Nijmegen Cognitive Neuroscience Research Master. We are glad to see that after almost 10 years of publishing our own research in this journal, students from our master's programme have created a platform that allows students from similar master's programmes in the Netherlands and neighbouring countries to present and discuss their research. We hope that this conference will continue to be organised in the years to come, and that it will become an integrated part of the programme just like this journal is now.

Since this is our last issue as Editors-In-Chief, we would like to use this opportunity to briefly reflect on what we have learned during our time in this journal. We have certainly gained insight into the process of publishing peer-reviewed scientific articles, but we have also improved our project management and leadership skills. Although in our plans for the future we might not be headed in the same direction, we both feel that this experience will be very valuable for our future careers.

We would like to express our appreciation to all the people who contributed to the journal that you are now holding in your hands. More specifically, we want to thank all the reviewers for helping us to maintain the high quality of the articles published, as well as our funders for making it possible to publish the excellent research conducted by the CNS master students. And finally, a big thank you to our hardworking team for their patience with us and our ambitious deadlines - fortunately, the current junior team members will have a nice long summer break before having to worry about the next issue. As for the current senior team members, we hope that you have enjoyed your time as a part of the journal team, and we wish you all the best.

All that is left for us to do now is to wish the next Editors-in-Chief, Dorien and Kelly, the best of luck with volume 10!

Wishing you a pleasant read,

Heidi Solberg Økland & Lorijn Zaadnoordijk

Editors-in-Chief

From the Director of the Behavioural Science Institute

Thank you for the invitation to contribute an editorial to this issue of the Proceedings of the Master's Programme Cognitive Neuroscience. I am honored by your request. These Proceedings, or the CNS Journal as it is known, is in its 9th year of consecutive publication, and is an impressive part of the many achievements of the Donders Institute for Brain, Cognition, and Behavior. Over the past few years, I have served Radboud University in various administrative roles, in addition to conducting my own research and teaching. This allows me to comment on the CNS Journal, and the current issue specifically, from various perspectives. I thank the editors, Lorijn and Heidi, for giving me the opportunity to do so.

As a former Vice Dean of the Faculty of Social Sciences, I have witnessed the growth of our three research master programmes: Cognitive Neuroscience, Behavioural Science, and Social and Cultural Science. Cognitive Neuroscience is the oldest (starting two years before Behavioural Science which celebrates its 10th anniversary this year) and has set the stage for the development of research master programmes in our Faculty. Cognitive Neuroscience has consistently received high evaluations from students and colleagues in national and international comparisons. As the former Director of the Research Master in Behavioural Science, I am very aware of the challenges involved in running such a programme. Research Master programmes at Radboud University are interdisciplinary, cutting across the boundaries of research programmes, institutes, and faculties. The current issue of the CNS Journal attests to the quality of the programme, by including six reports of rigorous state-of-the-art empirical research, written according to the highest standards of publication. I congratulate the students who made this issue possible with the continued success of the journal and I congratulate the CNS staff with the continued success of the programme.

Currently, I serve as the Director of the Behavioural Science Institute and I also consider myself a behavioural scientist. The papers in this issue address various topics that are close to the behavioural scientist's heart: imitation, psychopathology, affective processing, delay discounting, decision making, depression, neuroticism, and social information processing. The current papers address fundamental aspects of these phenomena by examining underlying neurological and hormonal processes, and in one case explicitly linking them to genetic influences. Furthermore, it is very encouraging to observe that some topics in this issue, especially those in the social and clinical domains, have points in common with research expertise in the Behavioural Science Institute. Such points offer opportunities for collaboration that may be valuable for the future growth of both cognitive neuroscience and behavioural science at Radboud University.

Toon Cillessen

Director of the Behavioural Science Institute

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Modulations of Speaking-Induced Suppression in Speech Imitation

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Recently, prediction via forward models has been at the forefront of language production research. For example, neuroimaging evidence has demonstrated that activation of the auditory cortex is suppressed to self-produced speech relative to listening without speaking (i.e. speaking-induced suppression, or SIS). This finding has been explained via a forward model that predicts the auditory consequences of speech actions. When this prediction matches incoming auditory feedback, auditory activity is suppressed; when the feedback does not match predictions, an error signal is generated that can guide changes in articulation. The present study was designed to test two critical predictions from these frameworks: First, whether the release from SIS varies as a function of the acoustic distance between feedback and prediction, and second, whether this in turn is predictive of the amount of adaptation in people's speech production. Magnetoencephalography (MEG) was recorded while subjects (N = 32) alternated between blocks of an imitation and a listening task. In the imitation task, subjects always began speaking a single vowel (/e/), and then listened to a subject-specific vowel recording. People were instructed to imitate the vowel they heard, adapting their speech online when their initial production didn't match the target. In the listening task, subjects simply listened to the same stimuli. Event-related fields time-locked to stimulus onset replicated the phenomenon of SIS for the first time in an explicit imitation task. However, we did not find clear evidence that the amount of suppression scaled with the distance between participants' speech and the speech target. Finally, and importantly, correlations across subjects revealed that subjects with less SIS showed better imitation performance. This result supports the view that a release from SIS may act as an error signal generated via predictions of acoustic targets of speech from forward models. Such error signals could potentially drive subsequent speech adaptation.

Keywords: speech production, magnetoencephalography, forward model, imitation, individual differences

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1. Introduction

Feedback plays a crucial role in speech motor control as it signals a speaker whether a speech motor action was successful or not. In order to account for an extensive number of findings related to adaptation and feedback processing in motor control, a number of theories have posited a monitoring mechanism that utilizes internal forward models which represent the kinematics, dynamics, and sensory consequences of movements (Blakemore, Frith, & Wolpert, 1999; Wolpert & Flanagan, 2001; Wolpert & Ghahramani, 2000; Wolpert, Ghahramani, & Jordan, 1995). Similar ideas have been applied to speech motor control specifically (Hickok, 2012; Houde & Nagarajan, 2011; Tian & Poeppel, 2010, 2012), where motor commands sent to the vocal tract produce an 'efference copy' that is sent through internal forward models to predict the somatosensory and/or auditory consequences of articulatory motor commands (Scott, 2012; Wolpert & Flanagan, 2001). The workings of this internal forward model for speech production are thought to be reflected in speaking-induced suppression (SIS), the reduction of auditory cortex response to self-produced speech relative to listening to recordings of the same speech. Electrophysiological studies have found SIS as a reduction of the M100 (also known as N100, N1 or N100m), a well-known auditory component that occurs roughly 100 ms after audio onset (Naatanen & Picton, 1987). Theoretical models (Guenther, Ghosh, & Tourville, 2006; Hickok, 2012; Houde & Nagarajan, 2011) explain SIS as reflecting a comparison mechanism: if the internal forward model's prediction of the auditory consequences of speech commands matches the actual auditory input, the cortical response is attenuated. When the prediction does not match the auditory feedback entirely, there is a release from SIS (i.e., the auditory cortex shows less suppression). This release from SIS then acts as an error signal, driving compensatory mechanisms that adapt motor output towards internal, auditory goals.

Over the last decade, a number of properties of SIS have emerged. Houde et al. (Houde, Nagarajan, Sekihara, & Merzenich, 2002) showed using magnetoencephalography (MEG) that masking the auditory feedback abolished SIS, which is in line with the view that SIS relates to a match between expected and actual auditory feedback. Subsequent studies (Heinks-Maldonado, Nagarajan, & Houde, 2006; Ventura, Nagarajan, & Houde, 2009) suggested that the amount of suppression can be modulated by properties of the feedback. For example, when auditory feedback was altered, there was a release from suppression (Heinks-Maldonado et al., 2006) and when feedback was masked, SIS was abolished (Houde et al., 2002). Together, these studies support a view on SIS as reflecting a match between predicted and actual auditory feedback. More recently, additional support for this view comes from direct cortical recordings (Chang, Niziolek, Knight, Nagarajan, & Houde, 2013; Flinker et al., 2010). However, few studies have directly linked SIS to behavioral output (Chang et al., 2013).

The current study was designed to more clearly establish this link by having people engage in a task in which auditory feedback is critical to performance. Speech imitation is one condition in which feedback control mechanisms are especially relevant, and in which the motor consequences of mismatch are likely to adapt in real time. Crucially, imitation first requires auditory processing of a speech sound that then serves as the target for subsequent speech production. In case of an unfamiliar speech target, feedback control is important in guiding speech production towards the correct target. Although imitation may not be frequent practice, it is an important part of language learning: foreign language students need to learn to produce novel articulation patterns. This typically involves listening to native speakers of the language, and trying to imitate them. Failure to accurately imitate native speakers is frequent in second language learning and leads to a foreign accent.

The goal of this study was to replicate SIS in an imitation task and to test two claims of the aforementioned theories of speech motor control. First, if SIS indexes the match between a prediction and the actual incoming auditory signal, then the amount of suppression should relate to the degree of (mis)match between the prediction and the auditory signal (Heinks-Maldonado et al., 2006; Houde et al., 2002). Second, if release from SIS serves as an error signal to drive motor adaptation, then individual variation in the amount of suppression should be predictive of the individual variation in imitation aptitude. Specifically, people who show more SIS should not adapt their speech as readily as those who show less SIS, as the latter would more easily release from SIS and produce error signals that would drive imitation performance.

To test these claims, the current study measured MEG during online imitation and listening tasks. In the speech imitation task, subjects were instructed to produce a neutral vowel (/e/) when a visual cue appeared. At the same moment, subjects heard a

recording of themselves producing an auditory stimulus that was the same, close, or far from the neutral vowel, and they were asked to imitate this new speech sound by adjusting their ongoing vowel production. By varying the acoustic distance between the neutral vowel and the auditory target, we were able to investigate whether this acoustic distance modulated the amount of SIS. Given the findings mentioned above, we predicted a release from SIS when the acoustic distance between prediction and auditory input is larger, and that this release would scale with the degree of mismatch. Based on the hypothesized link between SIS and changes to speech-motor behavior, we also predicted that individuals who show less SIS will be better at speech imitation.

2. Materials and methods

2.1 Participants

Thirty-two healthy participants (age: M = 21.8, SD = 5.2; 21 females) participated after providing written informed consent in accordance with the Declaration of Helsinki and the local ethics board. All subjects had normal hearing, normal or corrected-to-normal vision, were right-handed and were native speakers of Dutch. The study was approved by the local ethics committee (CMO region Arnhem / Nijmegen).

2.2 Stimuli

The auditory stimuli used were subject-specific vowel recordings made in a 20min. session that preceded the MEG experiment. Participants were recorded while producing the Dutch vowels /i/, /ɪ/, /e/, /ɛ/ and /a/. The vowels were equalized in length (to 1s), in pitch (to the subject's average pitch) and in intensity (to 82dB). All audio editing was done in Praat (Boersma & Weenink, 2013). Catch stimuli were made by shifting the pitch in the final 100 ms of the sound up by 8 mel.

2.3 Experimental design and procedure

Subjects alternated between an imitation task and a listening task in a blocked design. The imitation trials started with a fixation cross (1260-2260 ms, jittered). Participants were instructed to start saying /be/ as soon as three exclamation marks appeared. The participant's speech onset triggered playback of an auditory stimulus. The participant's task was to imitate the auditory stimulus as accurately as possible by adapting his/her pronunciation of the vowel when there was a mismatch between his/her speech and the stimulus. The exclamation marks remained visible for 1500 ms after speech onset. The inter-trial interval was 1000 ms.

The listening trials were similar, except the stimulus played directly when the exclamation marks appeared, and participants did not have to vocalize. Instead they did an auditory detection task. Per block, four catch trials were added. Participants listened to the stimuli and pressed a button when they detected a rising pitch at the end of the stimulus. Feedback for accuracy was provided for catch trials. The experimental design was implemented using a PC running Presentation® software (Version 16.2, www.neurobs.com), participants' speech was recorded with a single microphone in the magnetically shielded room with a sampling rate at 44100 Hz and all auditory stimuli were presented binaurally via MEG-compatible air tubes.

The experiment consisted of 17 blocks. The first block was an imitation block consisting of 72 baseline trials (i.e. all the trials had the same stimulus, /e/). The remaining blocks (stimuli varied between /i/, /ɪ/, /e/, /ɛ/, /a/) alternated between tasks, starting with a listening block. Imitation blocks consisted of 48 trials (9 of /i/, /ɪ/, /ɛ/ and /a/ each, and 12 of /e/), and listening blocks consisted of 52 trials (48 trials + 4 catch trials), yielding 872 trials in total. The order of trials within blocks was randomized across participants.

2.4 Data acquisition

The MEG system (VSM/CTF systems, Port Coquitlam, Canada) contained 275 axial gradiometers and was housed in a magnetically shielded room. Synchronized with the MEG, we also recorded an electro-oculogram (EOG) and electromyogram (EMG) bipolarly from eight electrodes, which were placed to record resp. horizontal EOG (electrodes lateral to left and right outer canthi), vertical EOG (electrodes at left sub- and supraorbital ridge), lip EMG (one electrode on the left side of the upper lip, and one on the right side of the lower lip) and electrocardiogram (ECG) (one electrode below the right collar bone and one below the left lower rib). In addition an electrode at the left mastoid was placed to act as a reference. Impedance on all electrodes was kept below 20 kΩ. Three localization coils, fixed to anatomical landmarks (nasion, left and right preauricular points), were used to determine head position relative to the gradiometers. Head

position was monitored online by the experimenter and if necessary corrected between the experimental blocks.All data were low-pass filtered by an anti-aliasing filter (300 Hz cut-off), digitized at 1200 Hz and stored for offline analysis. Participants were seated upright, with the head rested against the back of the helmet and touching the top of the helmet. A small cushion was used to fix the head's position so as to minimize free head movement.

2.5 Analyses

2.5.1 Behavior

In the participants' speech recordings, speech onset served as a marker for the beginning of the epoch of interest. The first 900 ms of each epoch were divided into 10 time bins of 90 ms, for which the average first formant (F1) and second formant (F2) values were calculated using an iterative Burg algorithm. The acoustic distance to the stimulus (i.e. the imitation target) was quantified as the Euclidian distance in F1/F2 space between the subject's speech and the stimulus. Two-way repeated-measures ANOVAs with factors Time Bin and Vowel were carried out on the F1 data, the F2 data and the acoustic distance separately. One participant failed to perform the task appropriately and was excluded from all further analyses.

2.5.2 MEG

All MEG data analyses were performed using the Fieldtrip toolbox for EEG/MEG-analysis (Oostenveld, Fries, Maris, & Schoffelen, 2011) in Matlab (The Mathworks Inc., Natick, MA, USA). Epochs of interest were defined from 1s before stimulus onset to 2s after and the data was resampled at 300 Hz. Trials were inspected on the range and variance of the MEG signal. Trials with a range or variance that differed at least an order of magnitude from the other trials of the same subject were discarded. Imitation trials in which subjects failed to speak or spoke too softly (therefore not triggering the stimulus playback) were discarded as well. On average, we ended up with 827 trials per subject (out of 840, excluding the catch trials). Due to time constraints, the datasets for two subjects lacked one imitation block and one participant's dataset lacked both an imitation and a listening block. Not counting these trials, we rejected on average seven trials per subject.

For artifact removal, we performed an independent components analysis (ICA; see Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997). Prior to ICA ('fastica'), a principal components analysis (PCA) was run on the data to reduce the number of dimensions to 100. Upon visual inspection of the ICA components' time courses and scalp topographies, on average about 13 components were rejected per subject (SD = 6). Components were identified as containing artifacts when they showed clear signatures of eye movement, heartbeat, or speech-related muscle artifacts. Subsequently, the data was transformed back to sensor space and band-pass filtered using a two-pass Butterworth filter (order: 4; pass-band: 1-12 Hz).

Event-related fields (ERFs) were calculated by averaging the trials time-locked to stimulus onset (baseline: 400-200 ms before stimulus onset). All further analyses were done on the planar gradients. Statistical testing was done within a time window of interest from 50 ms to 250 ms after stimulus onset. An ANOVA (factors Task and Vowel) was performed on the ERF data averaged across the time window. A data-driven channel selection was done on a subject-specific basis by selecting the channels that were among the 80 most active channels within the time window in both the listening and the speaking data. This resulted in 53 channels on average per subject (SD = 7).

2.5.3 Individual differences

The amount of SIS was calculated per condition as the difference between the ERF values for the listening and the production trials, normalized by the ERF values in the listening data (i.e., (listening - speaking) / listening). The results were averaged across conditions. Imitation performance was quantified as the normalized change in acoustic distance to the stimulus: the distance between the participant's speech and the auditory stimulus was calculated for the first and the last time bin of the subject's speech. Per condition, performance was then calculated as the difference between the first time bin and the last bin, normalized by the distance to target in the first time bin (i.e., (distance_to_targetbin1 – distance_to_targetbin10) / distance_to_targetbin1). Finally, the results were averaged across conditions. So, a value of 0 for behavioral performance would indicate the subject would end up on average just as far from the target as in the beginning of the trial, whereas a value of 1 would indicate perfect imitation. An additional analysis was run to see whether the average amount of SIS was correlated with stimulus dispersion in vowel space. The latter was quantified as the average Euclidian

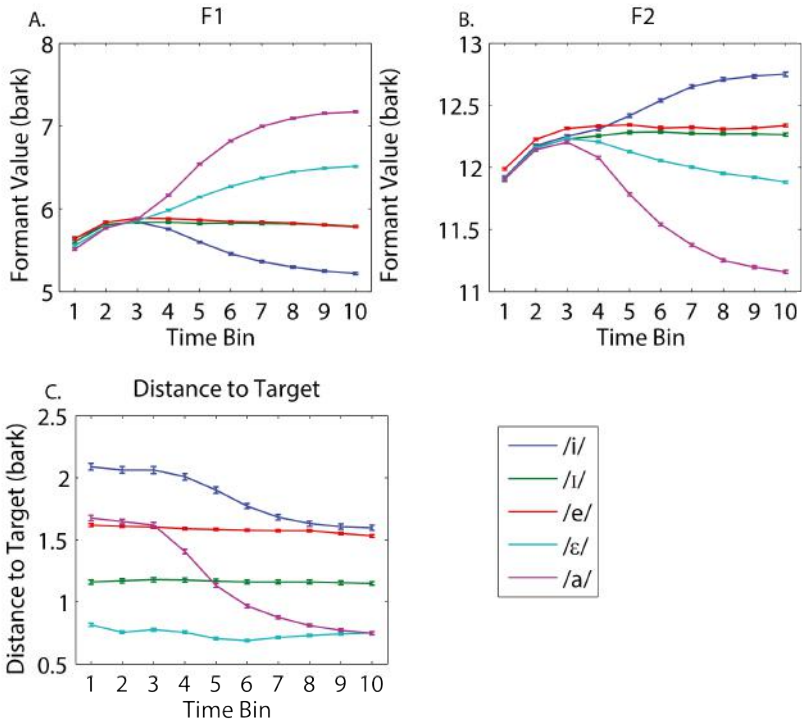

Fig. 1 Average behavioral results across participants. All error bars represent within-subject standard error of the mean (Cousineau, 2005) **A**. F1 formant values averaged per time bin and vowel across subjects. Each time bin is 90ms long. **B**. F2 formant values averaged per time bin and condition across subjects. **C**. Distance to the imitation target averaged per time bin and condition across subjects.

distance to the centroid (the point in vowel space defined by the average F1 and F2 values across all five stimuli).

3. Results

3.1 Behavioral performance

In order to assess whether participants appropriately performed the imitation task, an initial analysis focused on people's speech output as a function of time across each imitation condition (see Fig. 1). Results show that for both the first formant (F1) and second formant (F2), participants started at similar values every trial across the different conditions, and subsequently the formant values diverged depending on the condition. Results of a 9 (Time) X 5 (Vowel) repeated-measures ANOVA on F1 values (see Fig. 1A) showed significant main effects of both Time ($F(9, 270) = 23.97$; $p < .0001$) and Vowel ($F(4, 120) = 145.8$; $p < .0001$), as well as a significant Time x Vowel interaction ($F(36, 1080) = 106.9$; $p < .0001$). Similarly, for F2 (Fig. 1B) both main effects as well as the interaction were significant (Time: $F(9, 270) = 9.25$; $p < .0001$; Vowel: $F(4, 120) = 79.49$; $p < .0001$; interaction: $F(36, 1080) = 60.78$; $p < .0001$). With the exception of /ɪ/ condition, all vowels differed from the /e/ condition. A comparison of the stimuli showed that the /ɪ/ and /e/ vowels may have been too close to each other in acoustic space to differentiate in the MEG environment.

In order to quantify whether the changes in F1 and F2 brought participants closer to the imitation target, we combined the F1 and F2 data to look at the acoustic distance (Euclidian distance in F1-F2 space) between subjects' speech output and the imitation target on a given trial (Fig. 2C). Again, significant main effects of Time ($F(9, 270) = 42.1$; $p < .0001$) and Vowel ($F(4, 120) = 45.79$; $p < .0001$) were found, as well as a significant interaction ($F(36, 1080) = 34.62$; $p < .0001$). Post-hoc testing showed that for the vowels /i/ and /a/, the distance to the target significantly decreased between time bins 1 and 10 ($t(30) = 6.08$, $p < .0001$ and $t(30) = 10.99$, $p < .0001$). However, we did not find any effect for the other vowels (all $p > 0.5$). This may be due to the large individual differences, and/or to over-adaptations, i.e. when participants get closer to the target at first, but adapt too much and end up farther again from the target. Consistent with frequent over-adaptations observed in previous imitation studies (Kent, 1974), these over-adaptations were found for at least one vowel in 14 out of 31 subjects. One of these subjects showed over-adaptation for /a/, ten showed it for /ɛ/, and three showed it for both /ɛ/ and /a/. No over-adaptations were found for /i/, /ɪ/ or /e/.

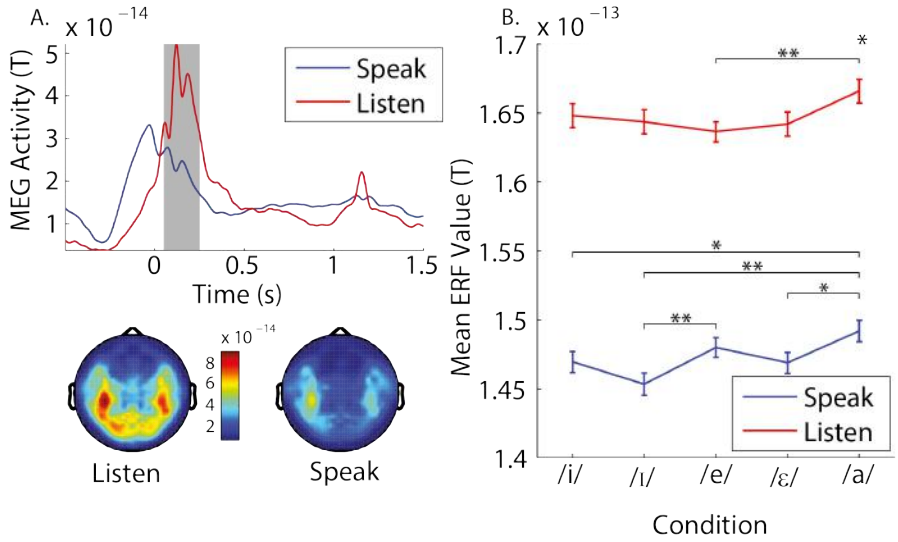

Fig. 2 ERF task effects at the group level. **A**. Group-level ERF values per task, time-locked to auditory stimulus onset (at 0ms). The time window of interest (50-250ms) is indicated by the grey shading. The scalp topographies of the listening and speaking ERF, in the time window that corresponds to the grey shading (50- 250ms) are shown as well. **B**. Group-level ERF averages by Task and Vowel. ERF values were averaged over time (50-250ms) and channels (subject-specific channel selection, see methods). (*: significant only without multiple error correction; **: significant after Bonferroni correction). Error bars represent within-subject standard error of the mean (Cousineau, 2005).

3.2 Speaking-induced suppression modulated at the group level

The comparison of the ERF in the speaking and the listening task showed a replication of SIS in this new paradigm (Fig. 2A). A clear activity peak can be seen in the listening ERF in the time window (50-250 ms) that corresponds to early auditory activity (i.e., the M100), and scalp topographies are consistent with auditory sources. In roughly the same time window, an activity peak is present in the speaking condition with similar topography. Crucially, the listening data clearly shows a larger peak compared to the speaking data. This result thus replicates the phenomenon of SIS and extends it to an explicit imitation task.

It is important to point out that activity was observed prior to stimulus onset (0 ms) in the speaking task (see Fig. 2A). This pre-stimulus activity might reflect an auditory response to early speech, or possibly speech motor activity, given that stimulus presentation in the speaking condition is triggered by the voice key (i.e., subjects started speaking already before stimulus onset; see methods). Visual inspection of the scalp topography of this early activity (not shown) showed sources that are very similar to the topography of the post-stimulus activity, thus possibly (though not necessarily) originating from a similar source. It is important to note, however, that this pre-stimulus activity peak is still lower than the post-stimulus listening peak.

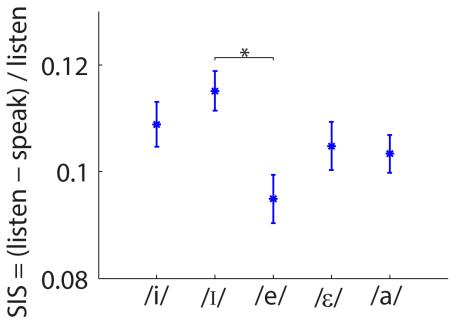

Fig. 3 SIS per vowel condition. SIS was quantified per subject as (ERF_listen – ERF_speak) / ERF_listen, and then averaged over subjects. The error bars indicate within-subject standard error of the mean (Cousineau, 2005). Only the comparison /ɪ/-/e/ was significant (see main text).

In order to look at how the different vowel conditions modulated the ERFs, we calculated the average ERF per task and per vowel. A 2 (Task) X 5 (Vowel Condition) repeated-measures ANOVA on average ERFs revealed main effects of Task ($F(1, 30) = 85.58$; $p < .0001$) and Vowel Condition ($F(4, 120) = 5.72$; $p < .0001$), and a Task x Vowel Condition interaction that approached significance ($F(4, 120) = 2.05$; $p < .1$). In order to investigate the interaction more closely, pairs of vowels were compared separately for each task condition. Figure 2B shows which comparisons came out as significant (uncorrected; speaking: /i/ vs. /a/, /ɪ/ vs. /e/, /ɪ/ vs. /a/ and /ɛ/ vs. /a/. The contrasts /i/ vs. /ɪ/ and /ɪ/ vs. /ɛ/ were close to significant ($p = .055$ and p

= .087); listening: /a/ vs. every other condition). We found only partial support for our prediction that the magnitude of SIS would scale with deviation from the initial speaking condition. Corresponding to our expectations, the production ERF in a more deviant condition (i.e. acoustically more distant from the neutral /e/ vowel) like /a/ was higher than the ERF for a less deviant condition like /ɛ/, which suggests a release from suppression for the more deviant condition. Note that when the results were corrected for multiple comparisons (Bonferroni), for production only the /ɪ/ vs. /a/ and the /ɪ/ vs. /e/ comparisons survive (p = .0001 and p = .0032). For listening, only the /e/ vs. /a/ comparison survives multiple comparison correction (p = .0004). When we looked at the amount of suppression per condition (Fig. 3), we found on both sides of the neutral /e/ condition that the less deviant condition (/ɪ/ and /ɛ/) showed numerically larger SIS than the more deviant one (/i/ and /a/), but these differences were not significant.

However, the results shown in Figure 2B do not fully correspond to our expectations. One unexpected result is that the ERF for /a/ in the listening condition was larger than the ERF for the other vowels, but maybe even more surprising is the fact that the production ERF for the 'neutral' condition /e/ was so high. Note that also that the amount of suppression for /ɪ/ was larger than for /e/ (t(30) = 2.88, p = .0072, see Fig. 3). Given that the stimulus in the /e/ condition should be acoustically closest to what participants started with on every trial, this condition should elicit the most suppression and thus the lowest ERF value in the production task. This was not observed, and is difficult to explain (see discussion).

3.3 SIS predicts participants' imitation performance

A second hypothesis we aimed to test related to individual differences in SIS. If a release from SIS reflects an error signal driving motor adaptation, variability in SIS should be reflected in behavioral variability. To explore this issue, we utilized an individual differences approach by investigating whether the average amount of SIS across subjects correlated with the subjects' performance in the imitation task (Fig. 4). We hypothesized that people will vary in their sensitivity to errors, and that this variation would be reflected in the average amount of suppression they show. Less suppression on average should then relate to a higher sensitivity to errors, which would lead to stronger and/or quicker adjustment.

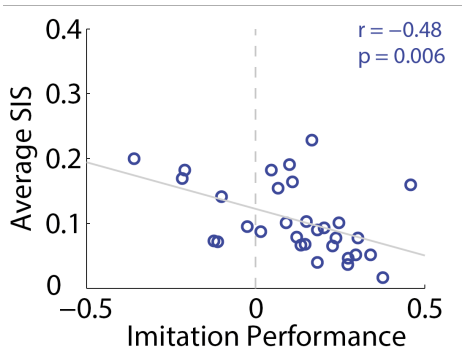

Fig. 4 Correlation between the normalized change in distance to the target ('imitation performance') and the overall SIS per subject. The dashed grey line shows an average change in distance to the target of 0. The solid grey line represents a linear fit. Pearson's r and the associated p-value are indicated in the graph.

Corresponding to this prediction, we found that participants who are more sensitive to errors were better at the imitation task (r(29) = -0.48; p < .01). However, note that participants listened to different stimuli (their own voice), so it would be possible that participants who show less SIS simply heard stimuli that were acoustically further apart. A control analysis showed that the correlation reported above was not due to subject-specific dispersion of the stimuli in vowel space (there was no correlation between the dispersion of the stimuli in vowel space for the different subjects and the average suppression: r(29) = .05, n.s.).

The negative values in the behavioral performance of some subjects might be counter-intuitive. A negative value reflects that these subjects were, on average, further from the target at the end of the trial compared to the beginning. This, however, doesn't necessarily mean they didn't imitate. As was mentioned before, a number of subjects showed 'over-adaptation': for some conditions, they did imitate the stimulus by adapting their speech in more or less the right direction, but adapted too much, ending up further from the target. Another cause for negative values could be the nonlinearities of the vowel space as mentioned before. Upon hearing a slightly different stimulus, subjects might tend to exaggerate the deviation of the stimulus and therefore the adaptation, in order to make clear they heard the difference. This thus leads to a longer distance to the target at the end of the trial. Note that the negative correlation is not only carried by these negative performance values: running the correlation only on those subjects that had a positive overall performance value still approached a significant correlation (r = -.39, p < .06).

4. Discussion

In this study, we investigated how speaking-induced suppression (SIS) was modulated in an imitation task. The behavioral data indicated that subjects did adapt their speech, and the overall task ERFs (speaking vs. listening) replicated SIS in the imitation context. Although a closer look at the ERFs of the different vowel conditions did not unequivocally confirm our hypothesis that SIS should scale with acoustic distance, individual differences in average SIS did predict individuals' performance in the imitation task, as we expected. Acoustic measures of the people's speech output during the task showed that subjects complied with the task instructions by adapting their speech to the auditory stimuli. However, the pattern for condition /ɛ/ was unexpected: while the F1 and F2 values show a clear change over time (Fig. 1A,B), the distance to target did not change a lot (Fig. 1C). This may be due to a number of different causes.

First, note that the metric 'distance to target' does not take into account the subject's trajectory or direction of movement in vowel space during imitation: only the distance to the target is taken into account. This means that if individuals did adapt in the right direction, but too much (i.e. they over-adapted), this may show up as only a small or no adaptation or even negative adaptation. In fact, upon visual inspection of single subject data, 14 out of 31 subjects indeed showed over-adaptation. This is in line with an early vowel imitation study that also noted frequent 'over-adaptations' (Kent, 1974).

Second, because subjects were instructed to start off with /e/, subjects might be inclined to exaggerate the difference between the vowel they produced initially and the perceived target (Kent, 1974). This way, an instance of the /ɛ/-phoneme could prompt subjects to produce a more extreme instance of the phoneme than was actually presented. Of course, this is all conjecture, and further studies can shed more light on nonlinearities in speech imitation[1]. The fact that also /e/ and /ɪ/ showed no change in distance to the target is congruent with the F1 and F2 data. Subjects apparently did not differentiate between these conditions. Also, the stimuli in these conditions did not consistently dissociate in vowel space across subjects[2].

One of the main questions of the present study was whether we could replicate SIS in the imitation task, and if so, whether SIS was modulated by the acoustic distance of the stimuli from the starting vowel. Comparing the overall speaking and listening ERFs showed a clear replication of speaking-induced suppression in the 50-250 ms time window (Fig. 2A). This result thus replicates the phenomenon of SIS in an explicit imitation task, and extends previous work which has observed SIS in simple speech production tasks and in altered feedback paradigms. In line with the topographies for this component and with previous findings in the literature, we take this suppression as reflective of the prediction by the internal forward model as explained in the introduction.

The aim of the present study was to test two hypotheses about variation in speaking-induced suppression. The first one concerns within-subject variation or modulation of SIS by vowel condition. The second one is concerned with between-subject variation, or with the question whether individual differences in suppression relate to behavioral performance. ERF analyses did not provide strong evidence in support of the first hypothesis. Notably, we found that the standard /e/-stimulus, which should have been closest to the participant's initial speech output and therefore elicit the most SIS, in fact showed less SIS than any other vowel condition. One post-hoc explanation is that given the explicit imitation instructions and the fact that in the majority of trials the auditory stimulus was different from the starting vowel, subjects might have expected a need to adapt. From this perspective, the standard stimulus may have been the least rather than most expected stimulus (i.e., the standard stimulus was unexpectedly close to the vowel with which participants started). Obviously, this is merely a post-hoc explanation and may need further investigation.

Despite the fact that SIS did not scale according to the deviance from people's initial speech production, we can still address the second major

[1] It should also be mentioned that the MEG hardware causes some background noise, which ends up in the speech recordings. This background noise may have had some effect on the formant value estimations, as well as on subjects' ability to perceive phonetic detail of the auditory stimuli, though it is unclear to what extent.

[2] Note that Adank, Hout & Smits (2004) argue that Dutch /e/ should be considered a semi-diphthongized vowel and is not well distinguished from /ɪ/ based on steady-state characteristics such as its average F1 or F2 value. Reilly & Dougherty (2013) show that subjects' adaptation to perturbed feedback is modulated by the importance of the perturbed feature to the vowel's perception.

hypothesis that stems from current theories of speech motor control, namely, that the amount of SIS should be predictive of how much people adapt their speech motor output. The results showed that a stronger release from suppression (i.e. lower SIS values) was associated with better imitation performance, supporting the prediction from the theoretical model. Here we take the average size of people's SIS as indicative of their sensitivity to errors. Specifically, some people might be more sensitive to small errors between their intended speech and acoustic mismatches. As a result, these participants would show less SIS on average. A higher sensitivity to mismatches would lead people to generate error signals more readily, thus driving speech motor adaptation, resulting in better imitation performance. This result is consistent with the models of speech motor control described in the introduction and is the first finding that explicitly links SIS to behavioral changes.

5. Conclusion

In the current study we investigated speaking-induced suppression (SIS) in the context of a speech imitation task. Based on current theories of speech-motor control (Guenther et al., 2006; Hickok, 2012; Houde & Nagarajan, 2011), we predicted that the magnitude of SIS would decrease with the acoustic distance from the starting vowel of people's speech production, and furthermore, that that amount of SIS would predict how much people adapted their speech in the imitation task.

The findings from the current study are the first to explicitly link individual variation in SIS to speech imitation performance. This result provides an important test of one of the main hypotheses stemming from current theories of speech motor control, and is consistent with SIS indexing an error signal that is used to adapt speech motor behavior. Despite this result, we did not find strong evidence in favor of another prediction stemming from these accounts, namely, that the amount of SIS should decrease with the distance from the original target utterance. As noted above, this may be a result of the current task conditions, and thus remains an open question for future investigation. Nevertheless, the current study highlights the importance of taking individual differences into account when studying speech production and opens up possibilities for further investigations in how inter-individual variation in speech production relates to variation in the psychological and neural mechanisms underlying speech motor control.

Although imitation may not explicitly occur in an everyday setting, it is a crucial aspect of language learning. In addition to a new vocabulary and a new grammar, the foreign language student is required to learn new articulations: he/she has to learn to pronounce unfamiliar speech sounds. Acquiring these new articulations involves imitation: the student listens to native speakers, who provide him/her with the acoustic target that is to be produced. By repeated imitation and listening, the student may gradually improve in his/her articulation of the unfamiliar speech sound. Crucially, people vary in how well they succeed in this imitation task, which is reflected in differing degrees of foreign accent. The task that subjects performed in the current study was very similar to this aspect of language learning: subjects were instructed to listen to the target and to try to imitate it to the best of their ability. Better insights in how individual differences with respect to foreign language aptitude arise might at long term lead to applications in language education, e.g. more individually-tailored learning paradigms.

6. References

Blakemore, S. J., Frith, C. D., & Wolpert, D. M. (1999). Spatio-temporal prediction modulates the perception of self-produced stimuli. *Journal of Cognitive Neuroscience, 11*(5), 551-559.

Boersma, P., & Weenink, D. (2013). Praat: doing phonetics by computer [Computer Program] (Version 5.3.52). Retrieved from http://www.praat.org

Chang, E. F., Niziolek, C. A., Knight, R. T., Nagarajan, S. S., & Houde, J. F. (2013). Human cortical sensorimotor network underlying feedback control of vocal pitch. *Proceedings of the National Academy of Sciences of the United States of America, 110*(7), 2653-2658.

Cousineau, D. (2005). Confidence intervals in within-subject designs: A simpler solution to Loftus and Masson's method. *Tutorials in Quantitative Methods for Psychology, 1*(1), 42-45.

Flinker, A., Chang, E. F., Kirsch, H. E., Barbaro, N. M., Crone, N. E., & Knight, R. T. (2010). Single-Trial Speech Suppression of Auditory Cortex Activity in Humans. *Journal of Neuroscience, 30*(49), 16643-16650.

Guenther, F. H., Ghosh, S. S., & Tourville, J. A. (2006). Neural modeling and imaging of the cortical interactions underlying syllable production. *Brain and Language, 96*(3), 280-301.

Heinks-Maldonado, T. H., Nagarajan, S. S., & Houde, J. F. (2006). Magnetoencephalographic evidence for a precise forward model in speech production. *Neuroreport, 17*(13), 1375-1379.

Hickok, G. (2012). Computational neuroanatomy of speech production. *Nature Reviews Neuroscience, 13*(2), 135-145.

Houde, J. F., & Nagarajan, S. S. (2011). Speech production as state feedback control. *Frontiers in Human Neuroscience, 5*(82), 1-14.

Houde, J. F., Nagarajan, S. S., Sekihara, K., & Merzenich, M. M. (2002). Modulation of the auditory cortex during speech: an MEG study. *Journal of Cognitive Neuroscience, 14*(8), 1125-1138.

Kent, R. D. (1974). Auditory-Motor Formant Tracking: A Study of Speech Imitation. *Journal of Speech and Hearing Research, 17*(2), 203-222.

Makeig, S., Jung, T. P., Bell, A. J., Ghahremani, D., & Sejnowski, T. J. (1997). Blind separation of auditory event-related brain responses into independent components. *Proceedings of the National Academy of Sciences, 94*(20), 10979-10984.

Naatanen, R., & Picton, T. (1987). The N1 Wave of the Human Electric and Magnetic Response to Sound - a Review and an Analysis of the Component Structure. *Psychophysiology, 24*(4), 375-425.

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J. M. (2011). Fieldtrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Computational Intelligence and Neuroscience, 2011*(2011).

Scott, M. (2012). *Speech Imagery as Corollary Discharge*. (PhD thesis), The University of British Columbia, Vancouver.

Tian, X., & Poeppel, D. (2010). Mental imagery of speech and movement implicates the dynamics of internal forward models. *Frontiers in Psychology, 1*, 166.

Tian, X., & Poeppel, D. (2012). Mental imagery of speech: linking motor and perceptual systems through internal simulation and estimation. *Frontiers in Human Neuroscience, 6*(314), 1-11.

Ventura, M., Nagarajan, S., & Houde, J. (2009). Speech target modulates speaking induced suppression in auditory cortex. *BMC Neuroscience, 10*(1), 58.

Wolpert, D., & Flanagan, J. R. (2001). Motor prediction. *Current Biology, 11*(18), R729-R732.

Wolpert, D., & Ghahramani, Z. (2000). Computational principles of movement neuroscience. *Nature Neuroscience, 3*(Suppl), 1212-1217.

Wolpert, D., Ghahramani, Z, & Jordan, M. I. (1995). An internal model for sensorimotor integration. *Science, 269*(5232), 1880-1882.

Acting Upon Objects in Multiple-Object Tracking Affects the Distribution of Attention

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Recent work by Atsma, Koning, and Van Lier (2012) has shown that, under conditions of high attentional load, attention is distributed along the direction of movement but ignores imminent changes of direction such as upcoming bounces. Nevertheless, we seem to be able to anticipate object bounces in everyday life. To further investigate this we created a new, "pong-like" multiple-object tracking (MOT) task in which a paddle had to be actively moved in order to hit targets while avoiding distractors. We combined this task with a probe detection task, which allowed us to investigate how interacting with objects influences the anticipatory distribution of attention. In addition, we compared participants that actively moved a paddle with participants that only observed recordings of the active participants' trials. We found that when the need to exert influence on objects in an MOT task is vital to successful task completion, bounce-paths are anticipated as evidenced by improved bounce-path probe detection compared to when there is no such need. Our experiment provides support for the idea that besides the tight coupling between perception and action, there is also a tight coupling between action and attention.

Keywords: attention, multiple-object tracking, probe-detection, perception, action

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1. Introduction

Our sensory systems are constantly bombarded with a great deal of stimuli, more than can be processed at once. Selecting relevant stimuli is vital to performing our daily tasks without distraction. Which stimuli are important and which are not depends on the task we are performing. For example, very precise processing of a soccer ball's movement is less crucial when you are watching that ball as a supporter than when you are watching that ball as a goalkeeper. For the goalkeeper, extrapolating the ball's path correctly is vital to winning the game. In this study, we will investigate the difference between actors and observers in distributing attention to multiple moving stimuli.

Although we have an idea of what attention entails, the exact mechanisms of attention are not yet fully understood. In the literature a distinction is usually made between space-based and object-based attention. Space-based attention means that attention can prioritise processing of signals that come from selected areas of the retina (Bengson, Lopez-Calderon, & Mangun, 2012; Golomb, Nguyen-Phuc, Mazer, McCarthy, & Chun, 2010; Posner, 1980). A well-known metaphor for space-based attention is the so-called spotlight that can select important stimuli by putting them inside the spotlight, thus filtering out irrelevant, distracting stimuli that are not within the spotlight (Oksama & Hyönä, 2004; Posner, 1980). Object-based attention refers to the fact that attention instead works at the level of objects, prioritising the processing of certain objects over others (Alvarez & Scholl, 2005; Howe, Drew, Pinto, & Horowitz, 2011; Scholl, 2001; Yantis, 1992).

Recently, it has been suggested that visual selective attention is not exclusively determined by space- or object-based properties, but rather that it depends for a large part on the type of task and stimuli whether space- or object-based effects are found (Davis, Driver, Pavani, & Shepherd, 2000; Soto & Blanco, 2004; Vecera & Farah, 1994; Weber, Kramer, & Miller, 1997). A good example of a task in which both space- and object-based effects have been demonstrated is the Multiple-Object Tracking (MOT) task (Alvarez & Scholl, 2005; Atsma, Koning, & van Lier, 2012; Fehd & Seiffert, 2008; Flombaum, Scholl, & Pylyshyn, 2008; Iordanescu, Grabowecky, & Suzuki, 2007; Pylyshyn & Storm, 1988; Scholl, 2001). Pylyshyn and Storm (1988) were the first to use this task. They tested the ability of the space-based spotlight to track multiple objects serially, by investigating a model's performance on the MOT task. They found that the model's spotlight would have to move at physiologically implausible speeds to match human performance. They therefore concluded that there cannot be a single spotlight that serially tracks multiple objects, and that it is likely that there are several loci of attention that can be divided over objects simultaneously.

The main task in MOT is to keep track of several targets among distractors (Atsma et al., 2012; Howe & Holcombe, 2012; Pylyshyn & Storm, 1988; Scholl, 2001; Scholl, 2009; Yantis, 1992). The task usually starts out with a display in which a number of identical objects are shown. Some of these objects are targets that need to be tracked. To distinguish targets from distractors, targets flash briefly at the start of a trial. The rest of the objects serve as distractors. When the targets have been designated, all objects start moving around. After this period of movement the participants have to identify the targets. This simple task requires participants to keep attention on the targets, while disregarding the distractors. It was found that participants can accomplish this and more. That is, the attentional system not only can follow targets while inhibiting the distractors (Flombaum, Scholl, & Pylyshyn, 2008; Pylyshyn, 2006), it also is able to distribute more attention to areas that put additional demands on tracking, like targets in crowded areas (Iordanescu, Grabowecky, & Suzuki, 2009). To more closely investigate how attention is distributed during an MOT, researchers have recently started combining the MOT task with probe detection tasks (Alvarez & Scholl, 2005; Flombaum et al., 2008; Pylyshyn, 2006). Probes in these tasks are small, transient stimuli that can appear anywhere in the display (on targets, distractors and in open spaces). These probes are difficult to detect when attention is not aimed at the region the probe appears in. Because of this, probe detection rates provide information about where attention was aimed at the time of the probe (e.g. Flombaum et al., 2008; Iordanescu et al., 2009).

Both in an MOT task, as well as in everyday tasks, it seems obvious that trajectory information is necessary when we want to predict where an object is going to be in the moment that follows. For example, when a ball is thrown and you want to catch it, you need information about the ball's path. Because it takes time to react and move your arms and hands into position, it is necessary for the brain to anticipate the ball's upcoming positions and to execute movements before the ball actually reaches those positions (Kistemaker, Faber, & Beek, 2009; Rushton & Wann, 1999; Wolpert & Ghahramani, 2000). It then makes sense that the

brain accomplishes this by extrapolation of the ball's trajectory information. If the brain indeed anticipates a certain path of motion, this should be reflected by increased detection of probes ahead of moving objects.

Verghese and McKee (2002) showed that the 'first segment of a trajectory acts as an automatic cue that draws attention to subsequent sections of the trajectory, leading to enhanced detectability for predictable motion trajectories' (Verghese & McKee, 2002: 413). But what is a predictable motion trajectory? Consider a person that is catching a bouncing ball. To anticipate post-bounce trajectories, the brain should know that when an object hits another object it bounces in a certain way, depending on (amongst other things) the speed, materials, and mass of the objects involved. To investigate whether bounce anticipation takes place, Atsma et al. (2012) used a multiple-object tracking paradigm in combination with a probe detection task (cf. Alvarez & Scholl, 2005; Flombaum et al., 2008; Pylyshyn, 2006). In their study, participants watched trials in which six discs (three targets and three distractors) either bounced off a bar realistically, or went straight through it. Just before a disc bounced, there was a chance that a probe would appear. This probe could be either on a linearly extrapolated path (disregarding the bounce), or on the path a disc would have post-bounce. Atsma et al. referred to these probes as through-path probes and bounce-path probes, respectively. If bounce-path probes were detected more often than through-path probes, this would show that knowledge about bounces influences where attention is directed. However, Atsma et al. found that when three discs needed to be tracked, this bounce-anticipation was not seen; through-path probes were detected significantly more often. When attentional load was lower, for example when only one target needed to be tracked, some bounce-path anticipation did occur. Even so, the detection rate of the through-path probes still remained higher than the detection rate of the bounce-path probes. This lead Atsma et al. to conclude that bottom-up linear extrapolation is more dominant, especially under conditions of high attentional load.

It seems difficult, however, to reconcile this distribution of attention along a linearly extrapolated path with the fact that we are able to anticipate the upcoming locations of a ball that is about to bounce, and catch it just after the bounce. The results by Atsma et al. (2012) may not show the entire picture. That is, the task they used was a standard MOT task combined with a probe detection task. Catching balls, however, is a more active task that relies on the interplay of both the action and perception systems. Gibson (1979) already noted that 'seeing is for doing', so this distinction may make all the difference. He proposed that perception is tuned to affordances; opportunities for action. Since then, this coupling of action and perception has been confirmed by several studies (Bekkering & Neggers, 2002; Goodale & Milner, 1992; Louveton et al., 2012; Murphy, Racicot, & Goodale, 1996; Witt & Sugovic, 2012). From this coupling it follows that adding action to the rather passive task Atsma et al. used may yield different results than they originally found.

Taken together, we predict that being actively involved may cause attention to be distributed in a different way than when merely observing a disc bouncing off a wall. Therefore, in contrast to Atsma et al. (2012), we will investigate the influence of active control on the distribution of attention in anticipation of imminent object bounces. We will use a task similar to that of Atsma et al., namely the MOT task combined with a probe detection task. To add a more active component to our set-up, we will construct an MOT task in a 'Pong'1-like fashion (see Fig. 1). That is, participants will be instructed to control a 'paddle' (cf. the wall, in the Atsma et al. study) against which they need to make the targets bounce. They also need to respond by pressing a button whenever they see a probe (Fig. 2). To control for the added task of actively moving the paddle, a second group of participants will observe a recording of the task of one of the participants in the action-group, with the task of only tracking the objects and detecting the probes. To control for differences in attentional load, the participants in the Observation group will also be instructed to press a button each time one of the target discs bounces against the paddle. This way, both the active players and the observers will have an equal number of tasks (see Fig. 1). Furthermore, this ensures that observers also keep track of the paddle, to avoid a situation in which the observers need to track less objects than the active players. Still, tracking the paddle may be inherently more difficult for the Observation group than for the Action group. We touch upon this issue in the discussion.

Overall, we hypothesize that bounce-probes (Fig. 2A) will be detected less often than through-probes (Fig. 2B), and that bounce-anticipation is used more often when participants need to exert influence on the objects' directions in an MOT setting as compared to when they can only observe. Therefore we expect that bounce-path probes will be seen more often by participants who are actively controlling the paddle,

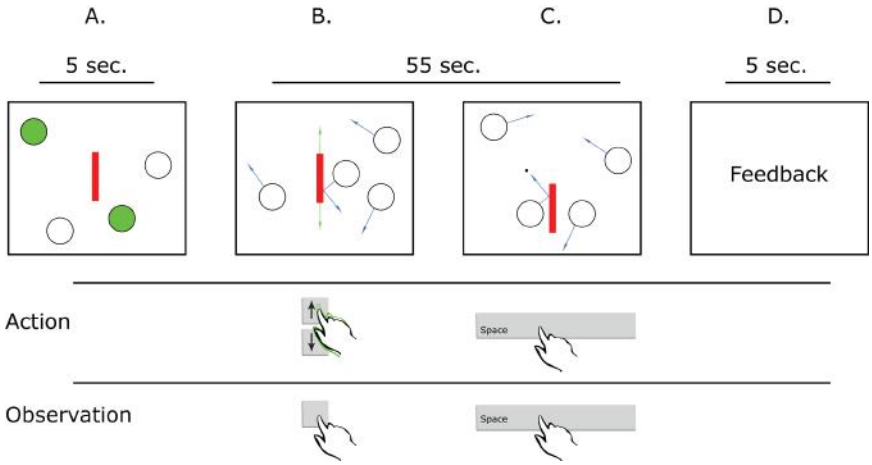

Fig. 1 The task that was used in this experiment. **A.** The target designation phase, which was identical for the Action group and the Observation group. **B.** In the Action group, the paddle had to be directed at target discs by using the arrow keys, such that the discs would bounce. In the Observation group, on the other hand, participants viewed recorded trials of participants in the Action group, and needed to press a button whenever they saw a target bounce off the paddle. **C.** Both groups of participants needed to press the spacebar whenever they saw a probe. These probes could either be bounce-probes, through-probes, or open-space probes (see Fig. 2). **D.** Finally, participants received appropriate feedback about their performance.

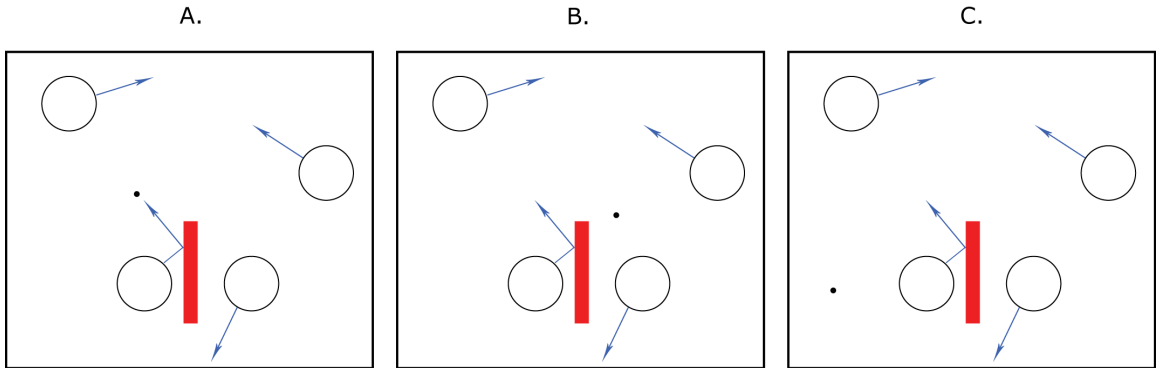

Fig. 2 The three probe-types that could appear. **A.** Bounce-probes could only appear on post-bounce paths. **B.** Through-probes could only occur on linearly extrapolated paths. **C.** Open-space probes could occur everywhere on the screen, and independent of bounces.

than by participants who have no opportunity to act upon the involved objects. Furthermore, we expect to find that through-path probes will be seen more often than bounce-path probes in the Observation group (in accordance with Atsma et al., 2012), but not in the Action group.

2. Material and methods

2.1 Participants

For this experiment 38 participants were tested (3 male, 35 female), aged between 18 and 24 years (M = 20.03, SD = 1.1). They were all first year students in either Psychology or Pedagogical Sciences at Radboud University Nijmegen. The participants were rewarded with participant-credits as a partial course fulfilment. All participants reported normal or corrected-to-normal vision. Participants were divided into two groups, an Action group (N = 19) and an Observation group (N = 19). All procedures took place in accordance with the Declaration of Helsinki.

2.2 Stimuli and materials

The experiment was programmed using Python 2.7 and PyGame, and was displayed on a 19" flat screen CRT monitor (Iiyama Vision Master Pro 454) which was set to a refresh rate of 120 Hz. The resolution was set to 1280 x 1024 pixels. The experiment was running at 60 frames per second. Distance from the participant's eyes to the monitor was roughly 30 cm. At this viewing distance, 1 pixel corresponds to about 0.03° (degrees of visual angle). On this screen a white 900 x 900 pixel (27° x 27°) square was presented centrally on a black background. Within this square, four discs could move around (see Fig. 1). The discs were bitmaps of circular black outlines on a white background, with

a diameter of 55 pixels (1.65°). At the beginning of each trial, two of the discs were filled with a green colour for 5 s, to indicate them as targets. The other discs were distractors (see Fig. 1A).

The discs moved at a speed of 5 pixels (0.15°) per frame, or 300 px/s (9°/s). Their initial direction of movement was angled to avoid pure horizontal or vertical translation. The angle of such an initial direction (relative to a purely horizontal movement) was either -66.4°, -53.1°, -36.9°, 36.9°, 53.1°, or 66.4° and could be different for each disc on the screen. Whether a disc moved along this diagonal initially in a leftward or rightward fashion was randomised for each disc as well. The paddle was a bitmap of a 20 x 150 pixel (0.6° x 4.5°) red bar placed at the centre of the square, and could move only in the y-direction (up and down) at a speed of 12 pixels (0.36°) per frame (720 px/s, or 21.6°/s). Probes were 5 x 5 pixel (0.15° x 0.15°) bitmaps of black squares, and the distance between a probe and the centre of a disc was always 200 pixels (6°). More specifically, 6° along the movement direction of a disc is the location where that disc would be 40 frames - or 670 ms - later). Finally, all parameters (object motion, probes, paddle movements) were recorded, so each trial could be played back to participants in the Observation group.

2.3 Tasks and procedure

Participants were seated in front of a monitor, in a dimly lit room. After the specific task (Action group or Observation group) was explained, the researcher showed how to 'play' one trial, and let the participants perform three practice trials themselves. Next, the actual experiment was started and participants completed 40 trials.

2.3.1 Action group

Participants in the Action group were asked to control a 'pong-paddle' using the up and down arrow keys, such that the paddle would bounce the targets discs, but not the distractor discs (Fig. 1B). In addition to this task, participants had to detect small black probes (dots) that appeared on the screen and indicate that they had seen them by pressing the spacebar within 1 second of seeing a probe (Fig. 1C). These probes could either appear on the bounce- or through-motion path of a disc at the moment that disc bounced (bounce-probes and through-probes, Fig. 2A-B), or at random locations in open space across the screen (open-space probes, Fig. 2C).

The open-space probes were added to ensure that attention was not focussed solely near the paddle. As bounce- and through-probe frequency depended on the participant's input (i.e. occurrence of object bounces depended on the movements of the paddle that was under control of the participant), these probes were configured to have a 15 percent chance each to appear when a disc bounced. Each probe lasted 50 ms (± 8 ms, taking the screen's refresh rate into account).

After each trial, the participant received feedback on-screen (Fig. 1D). The participants were shown how many times they had caused targets to bounce and how many times they had made distractors bounce. The ratio between these numbers was taken as a measure of tracking performance (and thus task performance).

2.3.2 Observation group

For the participants in this group, a similar set-up to that of the Action group was used, with the following alterations. Rather than controlling the pong-paddle themselves, these participants were shown the recordings of trials from one of the participants from the Action group, chosen randomly. Thus, what they saw was four bouncing discs, of which they needed to track the two (initially green) targets, while ignoring the distractors. Obviously, they saw a paddle moving around with discs sometimes bouncing against it. Furthermore, they saw the same probes as the corresponding participant in the Action group did, at the same time within a trial.

The task for these participants was to keep track of targets and press the spacebar within 1 second of detecting a probe, similar to the task of the Action group (Fig. 1C). Additionally, because the participants did not have to move the paddle around, they were instructed to press the up-arrow key whenever a target disc had bounced against the paddle (Fig. 1B). The number of times they had pressed the arrow key, and the actual number of target bounces was then shown to them in the feedback screen after each trial (Fig. 1D). The difference between the number of detected bounces and the actual bounces gave an indication of tracking performance in that trial.

2.4 Analysis

To analyse the results, we first conducted a Repeated Measures ANOVA (RM-ANOVA) using all trials (that is, irrespective of the actual tracking performance), with Probe-type (2 levels: Bounce-probe and Through-probe) and Disc-type (2 levels:

Target and Distractor) as within-subject factors, and Group (2 levels: Action and Observation) as a between-subject factor. The dependent variable was the probe detection rate. Open-space probes were disregarded from all analyses. In a second analysis we will also take tracking performance in the action and observation condition into account.

3. Results

The RM-ANOVA on all data, with Probe-type (2 levels: bounce-probe, through-probe) and Disc-type (2 levels: Target, Distractor) as within-subject variables, Group (2 levels: Action, Observation) as between-subject factor, and detection rate as the dependent variable, yielded the following results. There was a significant effect for Probe-type [$F(1, 36) = 18.19$, $p < .001$, $\eta p^2 = .34$], with bounce probes ($M = 36\%$, $SE = 2.7$) being detected less often than through probes ($M = 42\%$, $SE = 3.2$). Furthermore, there was a significant effect for Disc-type [$F(1, 36) = 17.38$, $p < .001$, $\eta p^2 = .33$], with probes appearing simultaneous with target bounces ($M = 43\%$, $SE = 3.2$) being detected more often than probes appearing simultaneously with distractor bounces ($M = 34\%$, $SE = 2.8$). Finally, the interaction Probe-type*Disc-type [$F(1, 36) = 4.43$, $p < .05$, $\eta p^2 = .005$] was also significant (see Table 1 for means and standard errors). The between-subjects variable Group was marginally significant at $p = .057$. All other effects were not significant.

To more closely investigate the distribution of attention in the case of acting on targets versus observing targets for both bounce- and through-probes, it is important to take the tracking performance of the participants into account. Given the different tasks and responses for both conditions we propose the following procedure to select trials with good tracking performance.

For the Action group, tracking performance can be expressed as the ratio between target bounces and distractor bounces, considering that tracking must be optimal in order to correctly bounce targets while avoiding distractors. We then determined the median of the ratio between target bounces and distractor bounces and selected the trials in which this ratio was higher than that median ratio. By doing such a median split one ensures that an equal amount of measurements are classified as 'good' or 'bad'. The median ratio was 2.33; thus all trials of participants in the Action group for which the amount of target-disc bounces was more than 2.33 times the number of distractor-disc bounces were included in this analysis.

Table 1. Mean probe detection rates (and Standard Errors, in brackets) in the overall 2x2 RM ANOVA.

Probe-type	Target-discs	Distractor-discs
Bounce-probes	40.9% (3.1)	30.1% (2.9)
Through-probes	44.4% (3.4)	38.8% (3.2)

Participants in the Observation group did not control the paddle themselves, rendering a median-split on target versus distractor bounces meaningless. Also, a ratio would not be convenient because there might be trials with zero recorded target bounces would lead to a division by zero. As an alternative, tracking performance for the participants in the Observation group was determined by subtracting the number of target bounces that a participant tallied from the actual number of target bounces in a trial. Thus, in a particular trial, a small difference between tallied and actual bounces would generally indicate a good performance in that trial. By selecting only trials that had a difference between tallied target bounces and actual target bounces of $|2|$ or less we found a similar amount of good-performance trials in both groups. More in particular, there was no significant difference ($t(74) = .99$, $p = .32$) between the amount of trials for the Observation group ($M = 24.74$, $SD = 8.85$) and Action group ($M = 26.92$, $SD = 10.23$).

The probe detection rates in the selected trials were then aggregated per participant, group (action versus observation), and probe-type (target bounce-probe versus target through-probe). Distractor-probes were not analysed, because we were only dealing with trials with good target tracking performance. Next, a RM-ANOVA on these data was performed, with Probe-type (target bounce-probes, target through-probes) as within-subject factor, and Group (Action, Observation) as between-subject factor and probe detection rate as the dependent variable, which yielded the following effects. There was a significant effect for Probe-type [$F(1, 36) = 4.64$, $p = .038$, $\eta p^2 = .11$], with bounce-probe detection ($M = 42\%$, $SE = 3.5$) being lower than through-probe detection ($M = 47\%$, $SE = 3.4$). The between-subject factor Group was also significant [$F(1, 36) = 5.02$, $p = .031$, $\eta p^2 = .12$], with probe detection being higher in the Action group ($M = 52\%$, $SE = 4.7$) than in the Observation group ($M = 37\%$, $SE = 4.7$).

Furthermore, there was a significant Probe-type * Group interaction [$F(1, 36) = 4.87$, $p = .034$, $\eta p^2 = .12$], which is shown in Figure 3. Paired sample t-tests revealed that, in the Action group, bounce-

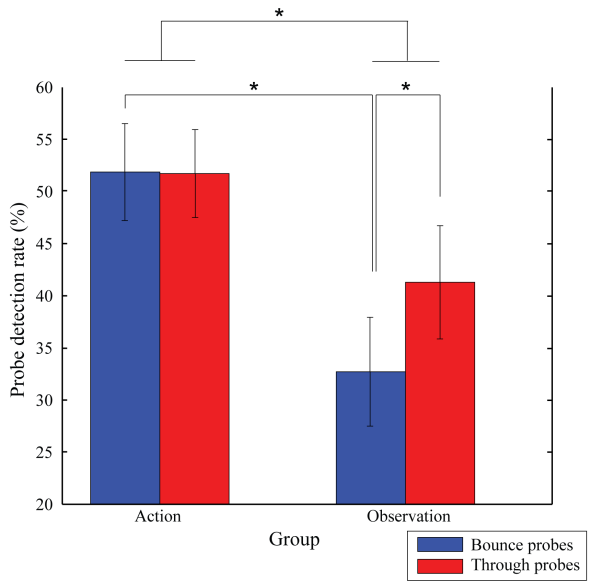

Fig. 3 The mean detection rates (%) for bounce-probes and through-probes in the Action and Observation groups, for high performance trials. Only probes belonging to target-discs are included in this graph. Relevant significant effects ($p < .05$) are marked with an asterisk. Error bars indicate standard error of the mean. Note that even though the error bars in the Observation condition overlap, a significant effect is still possible because the significant difference was analysed using a paired-samples t-test.

probe and through-probe mean detection rates did not differ significantly from each other ($p = .972$). In the Observation group, bounce-probe and through-probe mean detection rates did differ significantly [$t(18) = -3.22$, $p = .005$], with mean detection rates for bounce-probes ($M = 33\%$, $SE = 5.2$) being lower than detection rates for through-probes ($M = 41\%$, $SE = 5.4$, see Fig. 3). This mean detection rate for bounce-probes in the Observation group was also significantly lower [$t(36) = 2.75$, $p = .009$] than that for bounce-probes in the Action group ($M = 52\%$, $SE = 4.7$). Mean detection rates for through-probes did not differ significantly between the two groups ($p = .132$), nor did the detection rate of through-probes in the Observation group differ significantly from the detection rate of bounce-probes in the Action group ($p = .147$).

4. Discussion

In this study we investigated whether the default linear motion extrapolation found by Atsma et al. (2012) could be influenced by acting upon the relevant objects. Previous research has shown that attention is affected by the opportunity to act upon objects (Bekkering & Neggers, 2002; Goodale & Humphrey, 1998; Gibson, 1979; Goodale & Milner, 1992; Murphy, Racicot, & Goodale, 1996; Witt & Sugovic, 2012). To investigate whether action influences the distribution of attention in a dynamic MOT setting as well, we introduced an action component for half of the participants, while the other half observed a recording of an active participant's trials. We expected the results from the Observation group to be similar to the results in the Atsma et al. study, namely better probe detection for through-probes than for bounce probes. Additionally, we expected that in the Action group, due to the active component, bounce-probe detection would be better than that of the Observation group. Both of these expectations were confirmed in our data. More specifically, we found that in the Observation group bounce-probe detection was worse than that of through-probe detection, whereas in the Action group bounce-probe detection was equal to that of through-probe detection. Acting upon objects thus clearly influences the distribution of attention in a dynamically and demanding context such as MOT.

In the overall analysis, we found that through-probes were detected significantly better than bounce-probes. This is in line with the work by Atsma et al. (2012) and their conclusion that when attending to moving objects, we do not readily take into account upcoming bounces, but rather deploy our attention along linearly extrapolated paths. Next to this, we also found that probes near targets were detected significantly more often than probes near distractors. This too is not surprising as participants are tracking targets by distributing attention across targets but not distractors, which leads to increased probe detection near targets (Pylyshyn, 2006). Finally, there was also an interaction effect between probe-types and disc-types. This interaction shows that through-probe detection is better than bounce-probe detection for both targets and distractors, but additionally, that this difference is bigger for distractors than for targets. Thus, attending to a moving object not only leads to a higher probe detection rate as compared to not attending to a moving object, but also decreases the reliance on linear motion extrapolation. Nevertheless, through-probe detection remains better than bounce-probe detection. All of these results are in line with the conclusions by Atsma et al. (2012). Still, it is important to realize that the results of the interaction referred to here, are based on all probes of all trials, regardless of the performance of the participants. Thus, to investigate the results when attention was (correctly) focussed on the targets, we performed a second analysis. We confined our analysis to the targets (as these were the ones that needed to be acted upon or tallied, not the distractors) and took

participants' tracking performances into account.

In this second analysis, we only selected high performance trials. When taking into account performance according to the criteria mentioned earlier, a clear difference between the Action group and the Observation group emerged. That is, firstly, the results of the Observation group resembled the results in the MOT condition of Atsma et al. (2012). More specifically, participants were significantly worse at detecting bounce-probes than they were at detecting through-probes. Thus, the observation task can be assumed to be similar to the (observational) task in the Atsma et al. study. This supports the overall claim that when the attentional load is high there is a reliance on linear motion extrapolation even when the targets bounce, as can be seen by the superior detection rates of through-probes over bounce-probes.

Secondly and more crucially, we found that the through-probe detection superiority disappeared in the Action group as compared to the Observation group. That is, for high-performance trials there no longer was a significant difference between the mean probe detection rates of bounce-probes and through-probes. Furthermore, as can be seen in Figure 3, this is not due to a decrease in through-probe detection rate, but rather due to an increase of bounce-probe detection rates. These results show that when participants act upon objects to successfully complete an MOT task, attention can be directed to also anticipate post-bounce disc trajectories.

There is however an obvious limitation to our study. The way we have selected trials with good tracking is not ideal, and our experimental set-up could have been improved upon to remedy this. Specifically, our experiment would have benefitted from having a period after each trial in which participants could click on the discs they tracked. This would have been a more direct way to test tracking performance, and would have made it easier for us to compare the performance in the two groups. In our group, work is underway to replicate this study with such an improved design. For now, the current results already provide an interesting insight into how action shapes attention.

Note that the new findings mentioned above require an extension of the attentional framework to incorporate the increased bounce anticipation in conditions where participants influence objects' movements. That is, we have shown that also in a divided attention task such as MOT, there is a tight coupling between action and perception. This is in line with current theories that emphasize such a coupling of perception and action (Bekkering & Neggers, 2002; Gibson, 1979; Goodale & Milner, 1992; Louveton et al, 2012; Murphy, Racicot, & Goodale, 1996; Witt & Sugovic, 2012). We pose that the need to act upon objects shapes perception in a different way than does merely observing those objects.

As mentioned earlier, there was a main effect for Group (Action versus Observation), in the sense that participants in the Observation group detected less probes on average than participants in the Action group. This might indicate that the observation task was more difficult than the action task, which can be the result of a possible difference in the demands both tasks placed on the attention system (cf. Iordanescu et al., 2009). Attentional demands may have been higher in the Observation group because of the relative unpredictability of the paddle's movement. Because participants in the Action group could control this paddle themselves, there was less uncertainty about when and where a bounce would occur. According to recent findings by Howe and Holcombe (2012), tracking performance decreases when objects move unpredictably. This could mean that in our case, performing well in the Observation group may have been more difficult due to the unpredictable paddle movement. In the Action group, there may have been less demand to track the paddle because its motion was very predictable as it was self-initiated, and this may have enabled these participants to distribute their attention more advantageously to also detect bounce probes. However, where bounce-probe detection differed significantly between groups, through-probe detection did not differ significantly between groups. Thus, it is mainly bounce-probe detection that is affected by the difference between tasks. This interaction effect of probe-type and group provides additional evidence that differences in task difficulty (if any) cannot account for the main effect of group, because bounce-probe and through-probe detection are affected by the group effect to a different extent.

Alternatively, differences in bounce-probe detection might also be explained by more attention being distributed to the area around the paddle in the Action group. The idea behind this is that bounce-probes could be detected more often due to attention being around the paddle in the Action group more often than in the Observation group. Note that according to this explanation, through-probe detection should also benefit from this attention near the paddle. Therefore, the fact that we only found significant differences in bounce-probe detection between the two groups renders

this explanation implausible. Furthermore, if bounce-probes are detected more often in the action condition simply because probe detection is higher near the paddle, detection of probes for distractor bounces should also benefit from this effect. In our overall analysis we already found that probes shown near distractors are detected significantly less often than probes shown near targets. To investigate this more closely, we performed an additional analysis identical to the one we performed for the target probes, only this time for the distractor probes. The results showed that mean bounce-probe detection rates appeared to be somewhat lower than mean through-probe detection near distractors. However, none of these effects were significant (all p's > .1). Furthermore, the group effect was not significant ($p > .4$). Thus, it seems that target bounce-probe detection did not increase due to more attention on the paddle area in the Action group, especially when considering the notion that paddle unpredictability in the observation task might have caused a need to distribute more attention to the paddle in the Observation group than in the Action group. Finally, in both tasks we explicitly required participants to attend the paddle; in the action task because the discs needed to be bounced off the paddle, and in the observation task because target-disc bounces against the paddle needed to be tallied.

In sum, our results are in line with previous studies on motion anticipation and trajectory extrapolation (Atsma et al., 2012; Verghese & McKee, 2002), but add to these the observation that when there is a need to exert influence on object movements, attention is distributed differently. More specifically, this influence leads to more anticipatory distribution of attention. This notion fits well with current ideas on perception-action coupling. Regarding the more traditional discussion about whether attention is space-based or object-based, our results fit best to a middle-of-the-road theory in which attention uses both, depending on stimuli and task (Goldsmith & Yeari, 2012; Soto & Blanco, 2004). According to our results, such a theory combining both space- and object-based attention is especially prominent when there is an action component in the task at hand. The difference between merely observing objects and acting on objects apparently shifts attention from being mainly in front of a moving object (cf. Atsma et al., 2012) to being both in front of the object and taking into account future object positions based on the specific context (i.e. object bounces). The current setup appears more ecologically valid than previous MOT tasks, in which participants merely had to track objects moving around.

5. Conclusion

Taken together, our results support the idea that the perception and action systems are tightly coupled, also in the domain of visual selective attention. In line with previous work (Atsma et al., 2012), we showed that distribution of attention in conditions of high load is determined primarily by proximal stimulation, and not complex object behaviour. More importantly, interacting with the objects alters this distribution of attention, so that more complex object behaviour is taken into account as well.

6. Footnotes

Pong is a famous two-dimensional sports game that simulates table-tennis. It was originally created by Allan Alcorn as a training exercise, and manufactured by Atari Incorporated, who released it in 1972. (Retrieved May 7th, 2013, from the Pong Wikipedia: http://en.wikipedia.org/wiki/Pong).

7. References

Alvarez, G. A., & Scholl, B. J. (2005). How does attention select and track spatially extended objects? New effects of attentional concentration and amplification. *Journal of Experimental Psychology, 134*, 461-476.

Atsma, J., Koning, A., & van Lier, R. (2012). Multiple object tracking: Anticipatory attention doesn't "bounce". *Journal of Vision, 12*(13):1, 1-11.

Bekkering, H., & Neggers, S. F. W. (2002). Visual search is modulated by action intentions. *Psychological Science, 13*(4), 370-374.

Davis, G., Driver, J., Pavani, F., & Shepherd, A. (2000). Reappraising the apparent costs of attending to two separate visual objects. *Vision Research, 40*, 1323-1332.

Fehd, H. M., & Seiffert, A. E. (2008). Eye movements during multiple object tracking: where do participants look? *Cognition, 108*, 201-209.

Flombaum, J. I., Scholl, B. J., & Pylyshyn, Z. W. (2008). Attentional resources in visual tracking through occlusion: The high-beams effect. *Cognition, 107*, 904-931.

Gibson, J. J. (1979). *The Ecological Approach to Visual Perception*. Boston, MA: Houghton Mifflin.

Goldsmith, M., & Yeari, M. (2012). Central-cue discriminability modulates object-based attention by influencing spatial attention. *Experimental Psychology, 59*(3), 132-137

Goodale, M. A., & Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends in Neurosciences, 15(*1), 20-25.

Goodale, M. A., & Humphrey, G. K. (1998). The objects of action and perception. *Cognition, 67*, 181-207.

Howe, P. D. L., Drew, T., Pinto, Y., & Horowitz, T.

S. (2011). Remapping attention in multiple object tracking. *Vision Research, 51*, 489-495.

Howe, P. D. L., & Holcombe, A. O. (2012). Motion information is sometimes used as an aid to the visual tracking of objects. *Journal of Vision, 12*(13):10, 1-10.

Iordanescu, L., Grabowecky, M., & Suzuki, S. (2009). Demand-based dynamic distribution of attention and monitoring of velocities during multiple-object tracking. *Journal of Vision, 9*(4), 1-12.

Kistemaker, D. A., Faber, H., & Beek, P. J. (2009). Catching fly balls: a simulation study of the Chapman strategy. *Human Movement Science, 28*, 236-249.

Louveton, N., Bootsma, R. J., Guerin, P., Berthelon, C., & Montagne, G. (2012). Intersection crossing considered as intercepting a moving traffic gap: effect of task and environmental constraints. *Acta Psychologica, 141*, 287-294.

Murphy, K. J., Racicot, C. I., & Goodale, M. A. (1996). The use of visuomotor cues as a strategy for making perceptual judgments in a patient with visual form agnosia. *Neuropsychology, 10*(3), 396-401.

Oksama, L., & Hyönä, J. (2004). Is multiple object tracking carried out automatically by an early vision mechanism independent of higher-order cognition? An individual difference approach. *Visual Cognition, 11*, 631-671.

Posner, M. L. (1980). Orienting attention. *Quarterly Journal of Experimental Psychology, 32*, 3-25.

Pylyshyn, Z. W., & Storm, R. W. (1988). Tracking multiple independent targets: Evidence for a parallel tracking mechanism. *Spatial Vision, 3*, 179-197.

Pylyshyn, Z. (2006). Some puzzling findings in multiple object tracking (MOT): II. Inhibition of moving nontargets. *Visual Cognition, 14*(2), 175-198.

Scholl, B. J. (2001). Objects and attention: the state of the art. *Cognition, 80*, 1-46.

Scholl, B. J. (2009). What have we learned about attention from multiple object tracking (and vice versa)? In D. Dedrick & L. Trick (Eds.), *Computation, cognition, and Pylyshyn* (pp. 49-77). Cambridge, MA: MIT Press.

Soto, D., & Blanco, M. J. (2004). Spatial attention and object-based attention: a comparison within a single task. *Vision Research, 44*, 69-81.

Vecera, S., & Farah, M. (1994). Does visual attention select object or locations? *Journal of Experimental Psychology: Human Perception and Performance, 23*, 1-14.

Verghese, P., & McKee, S. (2002). Predicting future motion. *Journal of Vision, 2*, 413-423.

Weber, T. A., Kramer, A. F., & Miller, G. A. (1997). Selective processing of superimposed objects: an electrophysiological analysis of object-based attentional selection. *Biological Psychology, 45*, 159-182.

Witt, J. K., & Sugovic, M. (2012). Does ease to block a ball affect perceived ball speed? Examination of alternative hypotheses. *Journal of Experimental Psychology: Human Perception and Performance, 38*(5), 1202-1214.

Wolpert, D., & Ghahramani, Z. (2000). Computational principles of movement neuroscience. *Nature Neuroscience Supplement, 3*, 1212-1217.

Yantis, S. (1992). Multielement visual tracking: attention and perceptual organization. *Cognitive Psychology, 24*, 295-340.

Delay Discounting Behaviour in Dopamine D1 Mutant and Wild Type Rats

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The role of D1 receptors in cognition and behaviour has remained mysterious, despite great strides being made in the understanding of their diverse functions. A particularly interesting facet of behaviour yet to be explicated is their precise role in the economic process of delay discounting behaviour - a proxy for cognitive impulsivity and disorders of decision making. In this study, a delay discounting task was designed and administered to homozygous and heterozygous DRD1 mutant rats, as well as their wild type equivalents. Homozygous animals were unable to learn the task or acquire stable behavioural responding. Heterozygous and wild type animals did not differ in the majority of test metrics, but showed significantly different delay discounting curves, with heterozygous animals discounting less steeply and thus showing a reduced sensitivity to delay compared to wild types. This finding partially elucidates the role of the D1 receptor, as well as reiterating the importance of defining the optimal level of a particular neurotransmitter only in context - where appropriate behaviour is determined by the environmental conditions; that is, a tendency toward impulsive choice is either adaptive or not depending on the conditions in which the choice is taking place. Further study is required to completely explicate the relationship between genetic, behavioural, receptor, and environmental optima.

Keywords: delay discounting, DRD1, dopamine, inverted U, impulsivity

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1. Introduction

Brick and mortar are the constituent components of wall building in much the same way as numerous psychological and physiological features combine to constitute human and animal behaviour. That is, one must refer to the combined properties of a wide range of trait and state conditions, tendencies and dispositions, to explain action. In explicating drug addiction, compulsive behaviour, attention deficit hyperactivity disorder (ADHD), and similar disorders, the constituting features are myriad, but the substrates predicting vulnerability to these dysfunctions are, generally, shared.

One such substrate is impulsivity – broadly defined as a "predisposition towards rapid, unplanned reactions to either internal or external stimuli, without regard for negative consequences" (Grant & Potenza, 2006). High levels of impulsivity have been correlated with the likelihood of developing an impulse-control disorder (ICD), which affect approximately 5% of the worldwide population in the form of ADHD (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), 1–3% in the form of pathological gambling (Dell'Osso, Altamura, Allen, Marazziti, & Hollander, 2006) and significantly predispose individuals to a myriad of negative effects, including substance abuse (Verdejo-Garcia, Perales, & Pérez-García, 2007).

Far from being a unitary construct, however, impulsivity can be fractionated into motoric and cognitive components and assessed with different tasks (Winstanley, 2011). One cognitive facet of impulsivity is a reduced ability to defer gratification, as assessed by a measure of impulsive choice: delay discounting (DD). Delay discounting is defined by the rate at which a reward is devalued as the latency to acquire such a reward increases. A canonical example is that of a diet – in which the hedonic value of a slice of chocolate cake now is compared against the hedonic value of achieving one's long-term goal of weight-loss or healthy eating. In this situation, the former and latter values are in opposition, as the immediate reward disturbs or detracts from the ability to achieve the second. A famous study of a similar concept is the so-called "marshmallow test", in which children are promised an additional marshmallow if they do not eat one made available to them now within a certain period of time. The ability to resist temptation and wait for the reward is highly correlated with measures of success in later life, such as body mass index (BMI) and standard aptitude test (SAT) scores and ability to cope with social and other life stresses (Mischel, Ebbesen, & Zeiss, 1972; Mischel, Shoda, & Rodriguez, 1989; Schlam, Wilson, Shoda, Mischel, & Ayduk, 2013; see Mischel et al., 2011 for an extensive review). Deficits in delay discounting have frequently been observed in ADHD (Costa Dias et al., 2013; Wilson, Mitchell, Musser, Schmitt, & Nigg, 2011), compulsive gambling (Dixon, Marley, & Jacobs, 2003; Petry & Caserella, 1999), vulnerability to substance abuse and addiction (de Wit, 2009; Kreek, Nielsen, Butelman, & LaForge, 2005; Reynolds, 2006; Verdejo-Garcia, Perales, & Pérez-García, 2007), and other negative states such as failure to mitigate aggression (Colder & Stice, 1998). Delay discounting behaviour in humans and animals alike conforms to hyperbolic (Ainslie, 2001) or quasi-hyperbolic (e.g. Laibson, 1997) discounting models, in which a rapid drop-off in value in the short time immediately proximate to a reward allows for the possibility of preference reversal. A classical description of a hyperbolic discounting formula is that of Mazur (1987),

$$V = A/(1+kD)$$

where V is the computed value of the reward, A is the initial value of the reward at delay 0, D is the amount of delay, and k is the factor by which delay is multiplied. In particular, the model characterises "high impulsivity" as a steep initial fall in reward magnitude by time and is indicated by a large k value. Analytically, for large values of k, the effects of delay are amplified and thus produce smaller values of V overall, and will be particularly evident at small delays. Figure 1 shows canonical hyperbolic discount curves of varying steepness.

Numerous studies have found that dopaminergic systems are intimately involved with DD (e.g. Pine, Shiner, Seymour, & Dolan, 2010), and impulsivity more broadly. This body of evidence includes a

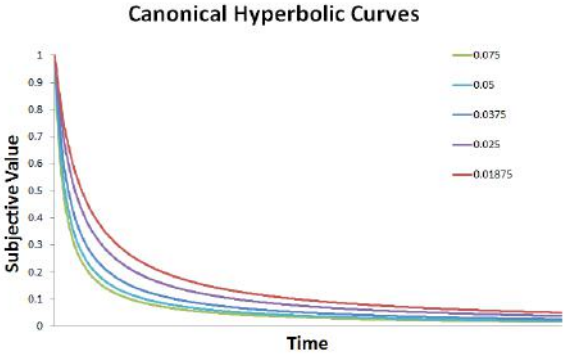

Fig. 1 Canonical hyperbolic discount curves for a variety of k rates. The steepness of the curve indicates how rapidly rewarding value falls by arbitrary units of Time/Delay.

link between high Barratt Impulsiveness Scale (BIS) scores and greater striatal dopamine transporter availability (Costa et al. 2013) and an inverted U of hyper- and hypo- activated dopamine systems in explaining impulsivity in ADHD (Williams & Dayan, 2005). Further evidence of this link are the, often complex, findings of pharmacological challenges, where dopaminergic agonists such as d-amphetamine can alternately reduce (de Wit, Crean, & Richards, 2000) or increase facets of impulsivity (Richards, Zhang, Mitchell, & de Wit, 1999) depending on acute or chronic administration, drug dose, and the type of impulsivity being assessed. For DD specifically, a previous study demonstrating that systemic administration of the dopamine precursor levodopa (L-dopa) significantly shifts DD behaviour towards increased impulsivity is particularly interesting (Pine, Shiner, Seymour, & Dolan, 2010). However, even this does not admit of an easy interpretation. The role of dopamine in impulsivity is further complicated by the differential roles identified for D1/D5 (D1-like) and D2/3 and D4 (D2-like) dopamine receptors. These two classes of receptor operate in functionally distinct ways to either increase (D1-like) or decrease (D2-like) glutamate transmission by means of cyclic adenosine monophosphate (cAMP) (Beaulieu & Gainetdinov, 2011). Due to a lack of D1 selective ligands, the role of D2-like receptors in impulsivity has been far better characterised than that of D1-like. For example, diminished D2/D3 binding has been implicated in susceptibility to cocaine use (Dalley et al., 2007), and in impulsivity generally (Buckholtz et al., 2010). Evidence such as Loos et al., (2010) suggests that increased D1-like neural transmission is predisposing towards increased impulsivity in a delay discounting task, however the evidence is mixed – as Koffarnus, Newma, Grundt, Rice, and Woods (2011) implicates the D1-like antagonist SCH23390 in increasing impulsive choice. Taken together, these findings indicate that the role of dopamine and its oppositional receptor subtypes remains complex – but can plausibly be subsumed under an explanation of dopamine, and D1 in particular, in facilitating volitional motivated behaviour (Berridge & Robinson, 1998). This is supported by studies from schizophrenia that have shown avolition and apathy as negative symptoms (Simon et al. 2010), which are not mitigated by the antagonism of D2-like receptors, associated with most antipsychotic treatments. Similarly, Pine, Shiner, Seymour, and Dolan (2010) also found that haloperidol administration did not reduce impulsive choice in comparison to placebo. Thus, it is likely that D1-like transmission itself, rather than the D2-like brake on transmission, is what is responsible for these avolitional symptoms. Additionally, Depoortere, Li, Lane, and Emmett-Oglesby (1993) showed the effect of SCH23390 in decreasing the breakpoint for responding to cocaine reward. This reduction in the reinforcing power of cocaine concords well with D1's role in volition, and with previous findings that D1 knockout rats show deficits in volition/motivation, but do not suffer from anhedonia (Homberg et al., unpublished findings).

One particularly powerful potential explanation of these mixed results is Cools and D'Esposito (2011), which predicts paradoxical effects of dopaminergic agents dependent on underlying synthesis capacity, receptor density, and/or transporter availability. Although specifically concerned with working memory, this model's inverted U concords well with that of Williams and Dayan (2005) and Zahrt, Taylor, Mathew, and Arnsten (1997), and, as such, suggests a parsimonious explanation of disparate findings in both the attention deficit hyperactivity disorder and dopamine (DA) literature. In addition, models which posit this optimum, with impairment occurring in either hyper- or hypo- activation of dopaminergic systems, allow for a particularly nuanced investigation of internal and environmental factors in behaviour, whereby the optimum for different tasks might be different, and the optimum for particular conditions in the environment might differ as well.

Given the lack of selective D1 ligands, and the complex relationship between D1 and impulsivity, the present study considers the selective function of the D1 receptor by utilising homozygous DRD1 knockout rats (HOM), heterozygous DRD1 knockouts (HET), and wild-type (WT) Wistar rats in a standard 2-lever delay discounting task for sucrose reward (Mar & Robbins, 2007).

The DRD1 knockout animal is characterised by a missense mutation in the DRD1 gene, which results in increased constitutive activity for D1 receptors, and reduces the response of these receptors to an active ligand. In particular, this is driven by a reduced expression of the receptor on the cell surface to ~50% of the norm. Previous work on these animals (Homberg et al., unpublished findings) has indicated that in addition to a loss of D1 function and reduced binding by D1 ligands, they possess deficits in spatial memory, as well as motivational and learning deficits. However, their performance and ability to learn a delay-discounting paradigm, specifically, have not been assessed before. Similarly, the heterozygous animals have not yet been phenotyped at all – and it

Table 1. Mean and SE results for variables of interest in Phase 1 responding. No significant genotype differences obtained.

Variable	Mean (standard error of the mean) wild type	Mean (standard error of the mean) heterozygous	Significance
Total lever pressing	75 (8.9)	53 (10.7)	$F(1, 15) = 2.59$, $p = .128$
Number of rewards obtained	61 (7.2)	45 (9.1)	$F(1, 15) = 1.96$, $p = .182$
Specificity of pressing	83 (2.2)	85 (2.8)	$F(3.8, 53.4) = 2.16$, $p = .089$

remains an open question whether they will resemble their WT or homozygous counterparts. Given the dopamine D1 receptor's role in the motivation of volitional action, and in the absence of any cognitive impairment, it is hypothesised that the overall responding for reward will be lower than that of WT animals due to avolition. This, however, could plausibly lead to increased impulsivity due to the amplification of delay cost and only a small appetitive desire, or to indications of reduced impulsivity in that a low response rate will yield larger payoffs for an unmotivated animal overall. That is, in the former case an animal who has only a small desire to pursue a reward may not develop a strong preference for the larger reward with even a minor obstacle (short delays). In the latter case, an animal who has a significant disincentive to respond per se might find large rewards for minimal effort particularly enticing – and be less affected by reinforcement schedule.

2. Methods

30 male rats were used in this study, 10 each of the Homozygous (HOM) and Heterozygous (HET) DRD1 animals, and 10 Wild Type (WT) animals of a Wistar background. HET and HOM animals were generated from the same Wistar background by N-ethyl-N-nitrosurea (ENU)-induced mutagenesis (Smits, Mudde, Plaserk, & Cuppen, 2004; Smits et al., 2006).

At day 21 the animals were weaned and had ear-punches taken for genotyping, which was conducted at Kbioscience (Hoddesden, UK). Animals were pair-housed individually ventilated cages due to an outbreak of a parvo-virus and maintained under a 12hr reverse day night cycle (lights off at 7:00, on at 19:00). At the start of behavioural training, (150 days after birth, Mean weight = 411 g, SD = 55 g), animals were placed on a food-restriction schedule beginning at 31 g per cage per day. Subsequently, food levels were adjusted in increments of 2 g per cage per day in order to maintain the animals at a stable weight (>90% initial Body Weight (BW)). All procedures were conducted in accordance with an approved DEC ethical clearance (2012-158) from the review board of Radboud University Nijmegen.

Animals were tested on a modified version of Mar and Robbins' (2007) delay discounting protocol in 4 identical operant conditioning boxes (TSE Systems GmbH; length/width/height = 27 × 27 × 26 cm) which were placed in sound attenuating cubicles. The boxes were attached to a custom built interface (Nonkes, Pooter, & Homberg, 2012) controlled by a home-written (C++) script. The boxes have a clear, plexiglass front wall, and two levers, approximately 13 cm above a grid floor, one each in the front and back section of the left wall, with a transparently covered receptacle for reward delivery in the bottom of the centre section of the same wall. The boxes are equipped with a red house-light and a speaker generating a 2 Khz tone at 12 dB upon lever press. The animals were trained and tested in three phases. During Phase 1 training, the animals were placed into the boxes and the computer program started, resulting in the house-light being illuminated. A press on either lever immediately yielded the delivery of a single sucrose pellet (45 mg Dustless Precision sucrose pellets, BioServ, Frenchtown U.S.A.) into the receptacle between the two levers, the extinguishing of the house light, a 0.5 s presentation of the tone, and a disconnection of the levers from their programmed consequences. Upon completion of reward dispensation, the house-light was re-illuminated and the consequences of the levers restored. Training was continued until animals developed stable responding behaviour, defined as >70% responses rewarded, >15 responses p/ session, and statistically insignificant variance in pressing by genotype, all across a minimum of 3 sessions. Next, in Phase 2 training, the reward size

Table 2. Mean and SE results for variables of interest in Phase 2 responding. No significant genotype differences obtained.

Variable	Mean (standard error of the mean) wild type	Mean (standard error of the mean) heterozygous	Significance
Total lever pressing	55 (10.5)	46.5 (10.4)	$F(1, 15) = .378$, $p = .5$
Number of rewards obtained	93 (14.8)	111 (9.7)	$F(1, 15) = .718$, $p = .410$
Specificity of pressing	87 (2.1)	88 (2.6)	$F(1, 15) = .001$, $p = .971$
% Preference for large reward	41 (11)	71 (9.0)	$F(1, 15) = 3.59$, $p = .077$

associated with one of the levers was increased to four sucrose pellets, the "large reward" lever was counterbalanced for any responding bias established in Phase 1 training, across animals, and alternately for animals with no bias. Phase 1 and Phase 2 were also characterised by a progressive increase in intertrial interval (ITI) (1 s for 5 days, 5 s for 11 days, 10 s for 1 day, and 30 s for 4 days) in order to drive motivation to respond by limiting consumption. Finally, the animals were moved onto the progressive delay schedule (Phase 3): Here, the "large" and "small" reward amounts and assignments were maintained, but a delay was introduced before dispensation of the large reward, ranging from 0–50 seconds. The delay increased progressively every 10 minutes in 10 s intervals throughout a 60-minute session. Outcomes of a lever press were the same as for the Phase 1 and 2 testing, with the exception of a delay before reward dispensation. Phases 1, 2 and 3 lasted 13, 21, and 17 days respectively.

Data were extracted from log-files into MATLAB (MathWorks Inc.) and processed to yield indications of rewarded and unrewarded presses, total number of pellets delivered, and response pattern by increment of delay in Phase 3. Statistical analyses were conducted in SPSS version 19 (IBM), with significance thresholds set at $p = .05$, for repeated measures analysis of variance (ANOVA), Mauchley's test of sphericity was applied, and where violated the Greenhouse-Geisser (GG) correction coefficient was used. Unless otherwise indicated, the reported results are for the last 9-10 days of a Phase. Where applicable, statistical significance is indicated by an asterisk in figures. The measures of interest were 'Total Lever Pressing', 'Number of Rewards Obtained', and 'Specificity of Pressing' in Phase 1, and the same with the addition of 'Preference for Large Reward' in Phase 2, and 'Delay Discounting' proper, in Phase 3.

3. Results

All but two of the homozygous DRD1 animals failed to acquire the criteria established for appropriate lever pressing, and as such this genotype was excluded from all subsequent analyses. Heterozygous and wild type animals both acquired the appropriate behaviour as a group, although 2 heterozygous animals, and 1 wild type animal were excluded from eventual analyses as they exhibited at least 3 sessions of behaviour that was 3 standard deviations outside of the behaviour of their cohort, as identified by an outlier analysis in SPSS. Therefore the final (n) for reported behaviour and statistics is 17 (8 heterozygotes (HET), 9 wild types (WT)).

3.1 Learning

Heterozygous and wild type animals did not differ in their behaviour in any measure of learning leading up to the introduction of the DD Phase 3 testing, and acquired stability at the same time, irrespective of genotype, these results are described in detail for each Phase individually, hereafter.

3.2 Phase 1 behaviour

Animals showed no statistically significant differences by genotype in any of the measures of Phase 1 behaviour, as indicated in Table 1, and Supplementary Figures 1–3*. Within subjects measures indicated significant changes in Lever Pressing $F(3.3, 50.6) = 11.02$, $p = .000$ and Number of Rewards $F(3.6, 54.8) = 10.99$, $p = .000$, over

time, indicating continued learning for both genotypes during this period. Specificity of Pressing did not change over time, but was close to ceiling performance.

3.3 Phase 2 behaviour

Animals likewise showed no statistically significant difference by genotype in any of the Phase 2 measures, including the Preference for Large Reward, as indicated in Table 2, and Supplementary Figures 4–7*. A within subjects effect in Lever Pressing and Number of Rewards was found, but this was driven by an equipment failure (day 7) and subsequent recovery to the already established baseline.

3.4 Phase 3 behaviour

The DD (Delay Discounting) measure, i.e. preference for the large lever by delay increment, became stable after 8 days and continued to be stable for the following 9. For ease of comparison across delay periods, data were collapsed into 3 blocks of 3 days each, with the average of each delay increment across 3 sessions forming the basis for Delay Discounting comparisons. Animals of the WT genotype showed steeper delay discounting, reaching reward parity (50%) preference early in the 20 s delay block. HET animals, by contrast, reached the same parity only in the 40 s delay block $F(1, 15) = 13.2$, $p = .002$. In terms of k-rate, WT animals were described by $k = 0.0375$ and HET by $k = 0.01875$, as defined by solving for Mazur (1987) where V=A. Hyperbolic discount curves and the responding by genotype are depicted in Figure 2 and Figure 3 respectively. Delay was significantly associated with decreased responding $F(2.3, 35.6) = 109.53$, $p = .000$, and was significant across all delay increments as assessed by post-hoc t-tests. A delay by genotype interaction effect was also significant $F(2.3, 35.6) = 3.76$, $p = .027$ indicating that the rate of preference change differed between genotypes. Between genotype significance was interrogated with further post-hoc t-tests, which revealed the source of the difference was increased preference for the large reward in HET animals at the 0, 10, and 20 delay intervals $t(15) = -2.76$, $p = .015$, $t(11.88) = -3.86$, $p = .002$, $t(15) = -4.11$, $p = .001$ respectively, with the 30 s delay interval showing only a trend ($p = .58$).

In addition to the primary findings related to delay, selectivity of lever pressing dropped dramatically for

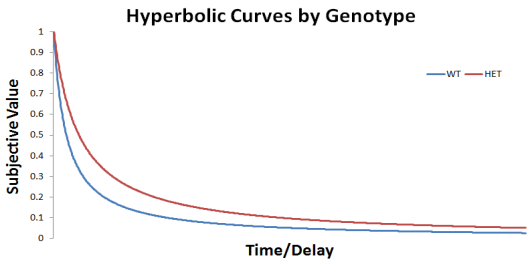

Fig. 2 Hyperbolic discount curves by the HET and WT genotypes in the study, representing a k rate of 0.0375 for the WT, and 0.01875 for the HET animals.

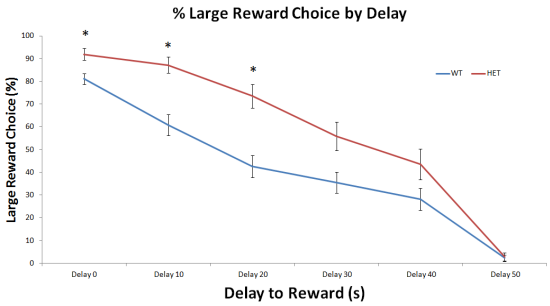

Fig. 3 Untransformed choices by delay interval, corresponding to the influence of delay on the preference for the large rewarded lever across the last 9 days of Phase 3 testing. Delay 0, 10 and 20 are significant at $p = .015$, $p = .002$, and $p = .001$ respectively.

both genotypes from Phase 2 levels (Fig. 4, compare to Supplementary Figs. 3 and 6*), but did not result in a significant selectivity difference by genotype in Phase 3 $F(1, 15) = 1.28$, $p = .275$. Lever Pressing was significantly different between genotypes, and almost twice as high for WT than HET animals $F(1, 15) = 7.34$, $p = .016$, (Fig. 5). In contrast to these findings however, the total Number of Rewards obtained by genotype did not differ significantly either within or between subjects $F(2, 3) = 1.19$, $p = .317$, $F(1, 15) = 0.61$, $p = .447$ (Fig. 6). Lever Pressing and Specificity both showed within subjects changes $F(2, 30) = 12.30$, $p = .000$, $F(1.3, 20.8) = 6.77$, $p = .01$, respectively, but with no clear directionality. These results also showed non-significant interaction ($p = .111$), and in the case of Specificity, genotype, effects $F(1, 15) = 1.28$, $p = .275$.

4. Discussion

This study sought to examine the relationship between dopaminergic genotypes and delay discounting behaviour in rats. We utilized animals with normal (100%) D1 transmission, and heterozygous and homozygous DRD1 knockouts. Homozygous animals failed to acquire the task altogether, and in the subsequent testing Wild Type

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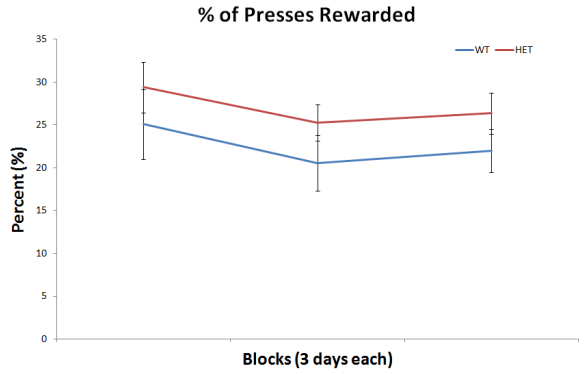

Fig. 4 Specificity of pressing during Phase 3 (DD) period, showing stable but decreased Specificity of lever pressing in both genotypes compared to Phase 1 and 2 levels. The results are non-significant between genotype in any phase individually, but Phase 3 levels represent a drop of 60+ percent each (see Supplementary Figs. 2 and 5*, for comparison)

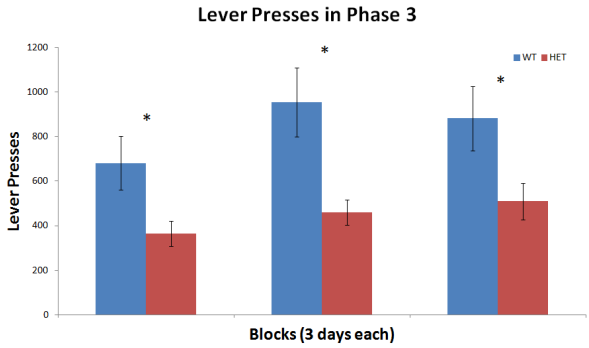

Fig. 5 Lever Press differences during Phase 3 by genotype. All three blocks show a significant difference in the mean lever pressing between genotypes. Block 1, 2, and 3 are significant at p = .03, p = .009 and p = .03, respectively.

Fig. 6 Earned Rewards by block by genotype, showing no significant differences in the amount of reward obtained during the Phase 3 testing.

and Heterozygous animals showed no significant differences in behaviour until the introduction of a delay in reward receipt. Once this occurred, Lever Pressing escalated for both genotypes, but resulted in a pronounced difference between genotypes for this measure in the final phase of the experiment. Delay Discounting measures demonstrated that HET animals were significantly more likely to wait for a large reward than switch to responding on the smaller lever compared to their WT counterparts, and as such showed less steep delay discounting.

Specificity and the Number of Rewards did not differ between genotypes, and thus Heterozygous animals showed far more efficient behaviour in attaining their rewards, by continuing to prefer the large-rewarding lever even at higher delays, and pressing less often for equal reward, as a result.

An immediate difference by genotype was obvious in the animals' initial behaviour with regards to the lever training, with none of the homozygous animals achieving the behavioural performance required in even Phase 1 training. The behavioural deficits indicated in the HOM animals closely match those reported in Homberg et al., (unpublished findings), where the animals failed to acquire pressing behaviour and did not learn/escalate their behaviour over time. In addition 2 animals that did acquire low-level pressing behaviour frequently neglected to collect their sucrose reward, indicating that the pressing was not goal-directed. This implication suggests that the animals were not motivated to press, and that awareness of the contingencies between lever pressing and the delivery of reward were not established in some animals either. Dopamine's role in motivation and in learning behaviours is well known and as such a deficit in both domains is unsurprising. As a result of this, it can be concluded that there is a 'minimum level' of D1 receptor binding density required for the successful acquisition of this kind of lever pressing task and/or learning, and the lower limit on this density is the approximately 50% density reported for homozygous DRD1-/- knockout animals in the areas relevant for reward based learning (Homberg et al., unpublished findings).

Interestingly however, the behavioural differences between WT and HET animals – with HET animals showing almost identical obtained rewards for significantly less work, and at larger delays – suggest that the optimal D1 transmission for this task lies somewhere between the 100% norm of the WT and the 50% of the HOM animals. This is especially significant in light of the D1 receptor not being implicated in time perception, unlike the D2/D3 receptor (Hass, Farkhooi, & Durstewitz, 2010). Efficiency as a result of reduced D1 transmission, in the absence of a cognitive distortion of temporality, demonstrates the importance of considering a particular trait or behavioural susceptibility as desirable or optimal only under the appropriate environmental conditions for its adaptive use.

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Namely, in our experiment, normal dopaminergic transmission is associated with increased delay discounting behaviour, and a highly increased Work vs. Reward ratio. There are, however, numerous potential reasons why such a result would obtain.

One potential explanation is the involvement of D1 in the induction and maintenance of stereotypy (Chartoff, Marck, Matsumoto, Dorsa, & Palmiter, 2001), namely, that with a prolonged period of training in an environment with non-retractable levers, various superstitious or stereotypical behaviours with regards to responding could be established. Although this is typically characterised as "excessive" dopaminergic transmission, and achieved by pharmacological challenge, it is possible that the environmental conditions under which the animals were tested facilitated this kind of behaviour. This is partly evidenced by the drop in response specificity with increasing ITI (Figs. 1, 4, 8), which demonstrates that the animals were unable to learn the significance of the timeout period effectively. This same bias might too contribute to the appearance of differential delay curves, as the motorically more active WT animals would be more likely to prefer rewards delivered on shorter schedules. The latter question is, however, impossible to answer with the current data. It is important to note, however, that stereotypy does not, in and of itself, imply that differential responding should be established. Similarly, the difference in the baseline of a difference in the preference for the large reward, by genotype, only emerges during the Phase 3 testing. Speculatively, this may indicate that the WT animals learn a more general aversion to the large lever - in predicting its future delay - nonetheless, the large lever remains the "rational" choice at all times, as the delay never exceeds 4 times the ITI.

Another potential explanation for the differences in delay discounting curves observed might be that with the increased lever pressing activity, WT animals are more rapidly satiated and therefore have a lowered incentive value for subsequent rewards. This, however, is not borne out by the data - which shows that WT and HET animals extract a similar proportion of their rewards during the "Delay 0" period as seen in Supplementary Figure 8*, and, given that their overall reward amounts do not differ significantly, that they are not differentially sated and/or prone to different response curves by time for this reason. Importantly, whilst the animals' specificity of lever pressing did not differ in any of the training conditions, the introduction of the delay was accompanied by a significant drop in pressing specificity for both genotypes. The high levels of lever pressing and reduced specificity of lever pressing in WT animals might be an indication of the D1 contribution to the initiation of action, and its role in motoric impulsivity. However, behavioural assessment of these animals previously indicated equivocal results for DRD1 effects on locomotor activity, but DRD1 knockout rats show reduced rearing (Homberg et al., unpublished findings), which may contribute to reduced lever pressing. Once again, this speaks to the environmental conditions being suitable or unsuitable for a particular genotype - in that "normal" locomotor behaviour might be maladaptive in the conditions of this test.

Avolition of the HET animals might be entertained as a potential explanation for the indication of reduced delay discounting in this genotype. However, this would not account for the preferential selection of the large reward lever by these animals - except in the presence of an additional cognitive calculation, very much in line with DD proper, that considered the trade-off between being motivated towards action and the size of the reward on offer. As a result, avolition can explain the difference in overall lever pressing, but not the maintenance of a high-rewarded choice for longer than the WT animals. On the other hand, however, a potential and more plausible explanation might be the complex working memory interaction with the task. The oppositional relationship between cognitive flexibility and stability, for example, is well known - and both supranormal and insufficient D1 receptor stimulation can induce deficits in working memory (Zahrt et al., 1997). HET animals exhibit increased constitutive activity, and with it - a reduced ability to be stimulated by a D1-agonist. Therefore, it might be the case that this generally invariant amount of D1 activity in the pre-frontal cortex (PFC) serves the function of maintaining HET animals in a state of improved cognitive stability. Namely, the middle of the inverted U of dopamine function in the PFC represents appropriate cognitive stability, with the corresponding optimal in striatal dopamine implicated in cognitive flexibility by relaxing gating requirements (Gruber, Dayan, Gutkin, & Solla, 2006). Where DA levels cannot respond by increasing D1 stimulation in the pre-frontal cortex, there might thus be expected a net shift of activation towards the striatum (and flexibility) in response to the new unpredictability (presence of delay) in the environment. This might drive WT animals towards increased exploratory behaviour, or the development of stereotypy, as their criteria for a relationship between response and reward become more lax. The relationship

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between stability and flexibility, however, has been proposed to depend on dopamine concentration in the PFC (Roberts et al., 1994), and striatum (Crofts et al., 2001), respectively, and is thus not an a priori likely explanation, as Homberg et al. (unpublished findings) suggests that the DRD1 knockout animals have equally diminished D1 receptor function in PFC and Striatum. Similarly, HET animals did not take longer to learn the contingencies of the task, or respond differently from the WT animals during any phase other than the introduction of delay. This implies that working memory only became a factor during the DD activity, and does not provide any clear reason for why WT animals should show an increased drive towards exploration except by increased frustration/impatience to delay - precisely what the test was intended to measure. As such, it again remains the most likely explanation that despite D1's multi-varied role, the development of inefficient responding, and preference for small rewards, is indicative of a decreased toleration of delay in WT animals.

5. Conclusion

The D1 receptor is vital for reward learning, as well as motivation. Its receptor density, however, follows the well established "inverted U" pattern of Cools and D'Esposito (2011), Williams and Dayan (2005) and Takahashi, Yamada, and Suhara (2012): When binding is below the 50% transmission threshold, a learning deficit prevents the acquisition of the task, but when the transmission is at wild-type levels, the level of impulsivity it conveys is maladaptive for the task. In this regard, detrimental performance can be observed with either an under-, or over-, expression of D1. This is, of course, determined by the precise nature of the task, and as such continued experimentation across a wide range of delay and effort based discounting tasks should be pursued, in order to more fully characterise the D1 receptor's role in cognition and behaviour.

6. References

Ainslie, G. (2001). *Breakdown of will.* Cambridge: Cambridge University Press.

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological reviews, 63*(1), 182-217.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews,* 28(3), 309-369.

Buckholtz, J. W., Treadway, M. T., Cowan, R. L., Woodward, N. D., Li, R., Sib Ansari, M., … Zald, D. H. (2010). Dopaminergic Network Differences in Human Impulsivity. *Science, 329*(5991), 532.

Chartoff, E. H., Marck, B. T., Matsumoto, A. M., Dorsa, D. M., & Palmiter, R. D. (2001). Induction of stereotypy in dopamine-deficient mice requires striatal D1 receptor activation. Proceedings of the National Academy of Sciences, *98*(18), 10451-10456.

Colder, C. R., & Stice, E. (1998). A longitudinal study of the interactive effects of impulsivity and anger on adolescent problem behavior. *Journal of Youth and Adolescence, 27*, 255-274.

Cools, R., & D'Esposito, M. (2011). Inverted-U–Shaped Dopamine Actions on Human Working Memory and Cognitive Control. *Biological psychiatry, 69*(12), 113-125.

Costa Dias, T. G., Wilson, V. B., Bathula, D. R., Iyer, S. P., Mills, K. L., Thurlow, B. L., … Fair, D. A. (2013). Reward circuit connectivity relates to delay discounting in children with attention-deficit/hyperactivity disorder. *European neuropsychopharmacology, 23*(1), 33-45.

Costa, A., la Fougère, C., Pogarell, O., Möller, H. J., Riedel, M., & Ettinger, U. (2013) Impulsivity is related to striatal dopamine transporter availability in healthy males. *Psychiatry Research: Neuroimaging, 211*, 251-256.

Crofts, H. S., Dalley, J. W., Collins, P., Van Denderen, J. C., Everitt, B. J., Robbins, T. W., & Roberts, A. C. (2001). Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. *Cerebral Cortex, 11*(11), 1015-1026.

Dalley, J. W., Fryer, T. D., Brichard, L., Robinson, E. S., Theobald, D. E., Lääne, K., ... Robbins, T. W. (2007). Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science, 315*(5816), 1267-1270.

Depoortere, R. Y., Li, D. H., Lane, J. D., & Emmett-Oglesby, M. W. (1993). Parameters of self-
administration of cocaine in rats under a progressive ratio schedule. *Pharmacology Biochemistry and Behavior, 45*, 539-548.

de Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction Biology, 14*(1), 22-31.

de Wit, H., Crean, J., & Richards, J. B. (2000). Effects of d-amphetamine and ethanol on a measure of behavioral inhibition in humans. *Behavioral Neuroscience, 114*, 830-837.

Dell'Osso, B., Altamura, A. C., Allen, A., Marazziti, D., & Hollander, E. (2006). Epidemiologic and clinical updates on impulse control disorders: a critical review. *European Archives of Psychiatry and Clinical Neuroscience, 256*(8), 464-475.

Dixon, M. R., Marley, J., & Jacobs, E. A. (2003). Delay Discounting by Pathological Gamblers. *Journal of Applied Behavior Analysis, 36*(4), 449-458.

Grant, J. E., & Potenza, M. N. (2006). Compulsive Aspects of Impulse-Control Disorders. *Psychiatric Clinics of North America, 29*(2), 539-551.

Gruber, A. J., Dayan, P., Gutkin, B. S., & Solla, S. A. (2006).

Dopamine modulation in the basal ganglia locks the gate to working memory. *Journal of Computational Neuroscience, 20*(2), 153-166.

Hass, J., Farkhooi, F., & Durstewitz, D. (2010). Dopaminergic modulation of time perception. Frontiers in Computational Neuroscience. Conference Abstract: Bernstein Conference on Computational Neuroscience.

Koffarnus, M. N., Newman, A. H., Grundt, P., Rice, K. C., & Woods, J. H. (2011). Effects of selective dopaminergic compounds on a delay discounting task. *Behavioural pharmacology, 22*(4), 300.

Kreek, M. J., Nielsen, D. A., Butelman, E. R., LaForge, K. S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nature Neuroscience, 8*, 1450-1457.

Laibson, D. (1997). "Golden Eggs and Hyperbolic Discounting." *The Quarterly Journal of Economics, 112*(2), 443-477.

Loos, M., Pattij, T., Janssen, M. C., Counotte, D. S., Schoffelmeer, A. N., Smit, A. B., ... & van Gaalen, M. M. (2010). Dopamine receptor D1/D5 gene expression in the medial prefrontal cortex predicts impulsive choice in rats. *Cerebral Cortex, 20*(5), 1064-1070.

Mar, A. C., & Robbins, T. W. (2007). "Delay discounting and impulsive choice in the rat." *Current Protocols in Neuroscience*, Chapter 8:Unit 8.22.

Mazur, J. E. (1987). An adjusting procedure for studying delayed reinforcement. In: Commons, M. L., J. E. Mazur, J. A. Nevin, H. Rachlin (Eds.). Quantitative Analyses of Behavior: *The Effect of Delay and of Intervening Events on Reinforcement Value, vol. 5*. (pp. 55-73). Erlbaum, Hillsdale, NJ.

Mischel, W., Ebbesen E. B., & Zeiss A. R. (1972). Cognitive and attentional mechanisms in delay of gratification. *Journal of Personality and Social Psychology, 21*, 204-218.

Mischel, W., Shoda Y., & Rodriguez M. L. (1989). Delay of gratification in children. *Science, 244*, 933-938.

Mischel, W., Ayduk, O., Berman, M. G., Casey, B. J., Gotlib, I. H., Jonides, J. … Shoda, Y. (2011). 'Willpower' over the life span: decomposing self-regulation. *Social Cognitive and Affective Neuroscience, 6*(2), 252-256.

Nonkes, L. J., de Pooter, M., & Homberg, J. R. (2012). Behavioural therapy based on distraction alleviates impaired fear extinction in male serotonin transporter knockout rats. *Journal of Psychiatry Neurosicence, 37*(4), 224-230.

Petry, N. M., & Casarella, T. (1999). Excessive discounting of delayed rewards in substance abusers with gambling problems. *Drug and Alcohol Dependence, 56*, 25–32.

Pine, A., Shiner, T., Seymour, B., & Dolan, R. J. (2010). Dopamine, Time, and Impulsivity in Humans. *The Journal of Neuroscience, 30*(26), 8888-8896.

Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis. *American Journal of Psychiatry, 164*, 942-948.

Reynolds, B. (2006). A review of delay-discounting research with humans: relations to drug use and gambling. *Behavioural Pharmacology, 17*(8), 651-667.

Richards, J.B., Zhang, L., Mitchell, S.H. and de Wit, H. (1999). Delay or probability discounting in a model of impulsive behavior: effect of alcohol. *Journal of the Experimental Analysis of Behavior, 71*, 121-143.

Roberts, A. C., De Salvia, M. A., Wilkinson, L. S., Collins, P., Muir, J. L., Everitt, B. J., & Robbins T.W. (1994). 6-Hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the Wisconsin Card Sort Test: Possible interactions with subcortical dopamine. *Journal of Neuroscience, 14*, 2531-2544.

Schlam, T.R., Wilson, N.L., Shoda, Y.S., Mischel, W., and Ayduk, O. (2013). Preschoolers' Delay of Gratification Predicts their Body Mass 30 Years Later. *The Journal of Pediatrics, 162* (1), 90-93.

Simon, J.J., Biller, A., Walther, S., Roesch-Ely, D., Stippich, C., Weisbrod, M., & Kaiser, S. (2010). Neural correlates of reward processing in schizophrenia—relationship to apathy and depression. *Schizophrenia research, 118* (1), 154-161.

Smits, B.M., Mudde, J., Plasterk, R H., & Cuppen, E. (2004). Target-selected mutagenesis of the rat. *Genomics, 83* (2), 332-334.

Smits, B.M., Mudde, J.B., van de Belt, J., Verheul, M., Olivier, J., Homberg, J., ... Cuppen, E. (2006). Generation of gene knockouts and mutant models in the laboratory rat by ENU-driven target-selected mutagenesis. *Pharmacogenetics and genomics, 16* (3), 159-169.

Takahashi, H., Yamada, M., & Suhara, T. (2012). Functional significance of central D1 receptors in cognition: beyond working memory. *Journal of Cerebral Blood Flow & Metabolism, 32*, 1248–1258.

Verdejo-García, A.J., Perales, J.C., & Pérez-García, M. (2007). Cognitive impulsivity in cocaine and heroin polysubstance abusers. *Addictive Behaviors, 32* (5), 950-966.

Williams, J., & Dayan, P. (2005). Dopamine, Learning, and Impulsivity: A Biological Account of Attention-Deficit/Hyperactivity Disorder. *Journal of Child and Adolescent Psychopharmacology, 15*, 160-179.

Wilson, V.B., Mitchell, S.H., Musser, E.D., Schmitt, C.F., & Nigg, J. T. (2011). Delay discounting of reward in ADHD: application in young children. *Journal of Child Psychology and Psychiatry, 52* (3), 256-264.

Winstanley, C. A. (2011). The utility of rat models of impulsivity in developing pharmacotherapies for impulse control disorders. *British Journal of Pharmacology, 164* (4), 1301-1321.

Zahrt, J., Taylor, J.R., Mathew, R.G., & Arnsten, A.F. (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *The Journal of Neuroscience, 17* (21), 8528-8535.

Investigating Appetitive and Aversive Associative Learning in Psychopathy Patients Using fMRI and Pupil Recordings

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Psychopathy is a personality disorder associated with affective deficits amongst others. Various studies demonstrated deficient affective processing and associative learning in psychopathy patients while evidence on the underlying neural correlates is still rather sparse, especially with respect to associative learning. Fronto-limbic areas including the amygdala, the insula and the orbitofrontal cortex seem to be hypoactive during aversive associative learning in psychopathy patients, but further investigation with larger samples is needed. Also, inter-individual differences related to the severity of psychopathy were hardly assessed in this context while appetitive associative learning, in contrast to aversive associative learning, was not yet assessed at all. This study investigated the neural and autonomic correlates of appetitive and aversive associative learning in psychopathy patients and healthy controls with abstract audio-visual stimuli and appetitive and aversive taste stimuli by means of functional magnetic resonance imaging (fMRI) and pupil recordings. Contrary to previous studies, no differences in neural or autonomic responses between psychopathy patients and healthy controls were found, though responses in the right insula and the right orbitofrontal cortex during aversive conditioning correlated with psychopathic personality traits and severity of psychopathy, respectively. This suggests that further research on affective deficits and deficient associative learning in psychopathy is needed, in particular with respect to inter-individual differences.

Keywords: psychopathy, associative learning, taste, fMRI, pupil dilation

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1. Introduction

Psychopathy is a personality disorder associated with grandiose sense of self-worth, pathological lying, shallow affect, callous and lack of empathy, impulsivity, irresponsibility, poor behavioural control and criminal versatility amongst others (Hare, 2003). These personality features can be divided into an interpersonal, an affective, a lifestyle and an antisocial factor corresponding to Factor 1 to Factor 4, respectively, of the Psychopathy Checklist-Revised (PCL-R; Hare, 2003). The affective factor is seen as a crucial component in the disorder in that a deficit in this domain constitutes an increased risk of learning antisocial behaviours and developing the disorder (Blair, Peschardt, Budhani, Mitchell & Pine, 2006). A large part of accumulating research in individuals with psychopathy indeed demonstrated affective deficits. Psycho- and neurophysiological differences in affective processing between psychopathy patients and healthy controls were commonly found, indicating an overall hypo-responsiveness to affective stimuli in psychopathy patients (Anderson & Stanford, 2012; Casey, Rogers, Burns & Yiend, 2013; Hare & Quinn, 1971; Herpertz et al., 2001; Howard & McCullagh, 2007; Kiehl, Hare, McDonald & Brink, 1999; Levenston, Patrick, Bradley & Lang, 2000; Patrick, Bradley & Lang, 1993; Sadeh & Verona, 2012; Sutton, Vitale & Newman 2002; Verona, Sprague & Sadeh, 2012; Williamson, Harpur & Hare, 1991). Specific neural correlates underlying affective deficits in psychopathy patients have been elucidated by means of functional magnetic resonance imaging (fMRI). Especially the prefrontal cortex including the orbitofrontal cortex (OFC) and limbic brain areas including the amygdala and the insula were implicated to be dysfunctional during affective processing in individuals diagnosed with psychopathy compared to control subjects in several fMRI studies (Dolan & Fullam, 2009; Harenski, Kim & Hamann, 2009; Kiehl et al., 2001; Mueller et al., 2003; Shamay-Tsoory, Harari, Aharon-Peretz & Levkovitz, 2010). All of these brain areas constitute parts of the affect-related fronto-limbic brain circuit (see e.g. Cardinal, Parkinson, Hall & Everitt, 2002 for a review).

Besides the mere processing of an affective stimulus, also the process of associative learning that involves affective stimuli seems to be deficient in psychopathy patients (Birbaumer et al., 2005; Blair et al., 2004; Flor, Birbaumer, Hermann, Ziegler & Patrick, 2002; Newman & Kosson, 1986; Rothemund et al., 2012; Veit et al., 2002). Such a deficit could be crucial for the behaviour of individuals with psychopathy because a failure to learn certain associations between stimuli, actions and outcomes might underlie some of the behavioural manifestations of psychopathy, for example antisocial behaviours which reappear in many psychopathic individuals despite adverse consequences (Blair, 2001; Herpertz & Sass, 2000). Only few studies to date assessed the specific neural correlates of deficient associative learning in psychopathy patients. Both Veit et al. (2002) as well as Birbaumer et al. (2005) detected lower activation in psychopathy patients as compared to controls during aversive associative learning in the fronto-limbic brain circuit, including the amygdala, the insula and the orbitofrontal cortex (OFC), all of which play important roles during affective associative learning (see e.g. Cardinal et al., 2002 for a review). However, both studies were limited in that their study samples were fairly small (four and nine psychopathic subjects, respectively) with moderately high mean PCL-R scores (25.3 and 24.89, respectively) and in that they investigated aversive but not appetitive associative learning. Another potential limitation of these studies were the stimuli. Both studies applied neutral faces as neutral stimuli to be associated with affective stimuli. However, it cannot be excluded that faces have their own subjective affective value possibly due to an association with a familiar face which might induce additional affective processing unrelated to the learning processes.

Whereas the studies by Birbaumer et al. (2005) and Veit et al. (2002) used painful pressure as an aversive stimulus, there is also evidence of deficient aversive associative learning with olfactory stimuli in psychopathy patients (Flor et al., 2002), but the specific neural correlates of this type of aversive associative learning in psychopathy remain elusive. Similar to olfactory stimuli, gustatory stimuli can be rewarding and punishing (O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001) and are suited to induce affective associative learning (O'Doherty, Dayan, Friston, Critchley & Dolan, 2003). An advantage of using olfactory or gustatory stimuli for associative learning is that they can act as both appetitive and aversive primary reinforcers.

In this study, we aimed to advance the current state of knowledge about affective deficits in psychopathy patients by elucidating the neural correlates of both aversive and appetitive associative learning with unpleasant and pleasant gustatory (i.e. taste) stimuli in psychopathy patients. For this we

used a Pavlovian conditioning paradigm in which the taste stimuli (i.e. the unconditioned stimuli) were paired with neutral abstract audio-visual stimuli (i.e. the conditioned stimuli) so that the latter, after repeated conditioning and when presented alone, should elicit an unconditional response that is usually elicited by the taste stimuli, thereby demonstrating successful associative learning. We investigated the neural correlates of appetitive and aversive associative learning as well as differences between these by means of fMRI in psychopathy patients with high PCL-R scores and healthy matched controls. Based on previous literature, we hypothesized that psychopathy patients, compared to healthy controls, would show hypo-activation of fronto-limbic brain areas in response to affective associative learning. Additionally, we tested whether learning-related neural activations in brain areas of interest, that is the amygdala, the insula and the OFC, would correlate with individual severity of psychopathy or psychopathic personality traits in order to draw conclusions also on an individual level. We hypothesized that the activity in these brain areas during associative learning would negatively correlate with severity of psychopathy or psychopathic personality traits. Further, we tested for differences in activation between appetitive and aversive associative learning as well as between appetitive and aversive stimuli, also on a between-group and within-group level. Besides the neural correlates, we assessed autonomic arousal as an indicator of (un-) successful associative learning. For this, we analysed the dilation of the pupil as this was shown to be a reliable physiological indicator of autonomic arousal (see e.g. Barreto, Zhai, Rishe, & Gao, 2007; Bradley, Miccoli, Escrig, & Lang, 2008; Partala & Surakka, 2003). In accordance with previous literature on affective processing in psychopathy, we hypothesized that psychopathy patients, in comparison with healthy controls, would generally show decreased arousal to affective stimuli as well as less increased arousal to stimuli associated with a taste when compared to stimuli not associated with a taste. Similarly, we expected negative correlations of the severity of psychopathy or psychopathic personality traits with arousal to affective stimuli.

2. Material and Methods

2.1 Participants

Sixteen men with a criminal history and scores of at least 26 on the Psychopathy Checklist-Revised (PCL-R; Hare, 2003) constituted the psychopathy sample (see Table 1; two additional men were recruited for the psychopathy sample but withdrew from participation). They were all in-patients from the forensic psychiatric clinic for criminal conducts of the Pompe Foundation for Forensic Psychiatry. The control sample with 20 healthy men, who were recruited among employees of the Pompe Foundation via advertisement, was matched to the psychopathy sample in terms of age and intelligence quotient (see Table 1). The healthy controls (HC) did not meet criteria for psychopathy or an antisocial personality disorder and did not have any criminal records. All participants were checked for general fMRI exclusion criteria, drug use[1], neurological history[2] and psychiatric exclusion criteria[3]. Every participant received written and oral information about the experimental procedure, filled in an informed consent and received a monetary payment for participation. The study was approved by the local medical ethical committee.

[1] Participants were asked to refrain from more than six units of alcohol during the week before the measurements, from all alcohol during 24 hours before (two healthy control participants reported having had one glass of beer or wine, respectively, within this time), from cannabis or other illicit drugs during one week before (one healthy control participant reported to have used such drugs five days before), from smoking more than five cigarettes on the day of the measurements and any cigarettes during one hour before (one healthy control participant reported to have smoked one cigarette within this time), from psychotropic medication other than oxazepam during five days before (one psychopathy patient was on a daily dose of 50 mg tryptizol) and from oxazepam during 12 hours before the measurements. Current alcohol or substance intoxication was an exclusion criterion.

[2] A history of trauma capitis, visual or auditory disorders, neurological disorders and first degree relatives with neurological disorders were exclusion criteria.

[3] Psychiatric exclusion criteria were the presence of a recurrent major depressive disorder, a single major depressive disorder within the preceding five years, a bipolar disorder, schizophrenia, a delusional disorder, a schizoaffective disorder, a schizophreniform disorder or any other psychotic disorder, any schizoid or schizotypical personality disorder, an attention-deficit-hyperactivity disorder, mental retardation, as well as first degree relatives.

Table 1. Mean characteristics of the psychopathy sample and the healthy control sample

Characteristic	Psychopathy sample	Healthy control sample	Statistics (p-value)
Sample size	16	20	—
Age	40.06 (8.78)	40.65 (10.04)	.853
Intelligence quotient	100.25 (10.23)	101.8 (8.45)	.629
Number of right-handers	13	17	—
Psychopathy checklist-revised total	30.25 (3.77)	—	—
Psychopathy checklist-revised factor 1 (interpersonal)	5.72 (1.75)	—	—
Psychopathy checklist-revised factor 2 (affective)	6.28 (1.51)	—	—
Psychopathy checklist-revised factor 3 (lifestyle)	7.5 (1.83)	—	—
Psychopathy checklist-revised factor 4 (antisocial)	7.94 (1.34)	—	—
Psychopathic personality inventory total	360.13 (40.1)	347 (35.76)	.314

Note. Standard deviations are indicated in parentheses. Statistics are based on an independent samples t-test for equality of means with the assumption of unequal variances. The intelligence quotient values are based on the Dutch Adult Reading Test (Nederlandse Leestest voor Volwassenen, NLV; Schmand, Bakker, Saan, & Louman, 1991).

2.2 Procedure

2.2.1 General procedure

Every participant attended two sessions. In the screening sessions, trained psychologists screened the participants for the psychiatric exclusion criteria. Specifically, they tested the participants for the presence of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) Axis-I disorders with the Dutch version of the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus; Sheehan et al., 1998; Dutch version: van Vliet & de Beurs, 2007) and for the presence of Axis-II disorders with the Dutch version of the Structured Clinical Interview for DSM-IV Axis-II disorders (SCID-II; First, Spitzer, Gibbon, Williams, & Benjamin, 1997; Dutch version: Weertman, Arntz, & Kerkhofs, 2000). Intelligence was assessed with the Dutch Adult Reading Test (Nederlandse Leestest voor Volwassenen, NLV; Schmand, Bakker, Saan, & Louman, 1991). For assessment of a participant's personality traits and mental state, participants filled out a Dutch version of the Psychopathic Personality Inventory (PPI; Lilienfeld & Andrews, 1996; Dutch version: Jelicic, Merckelbach, Timmermans, & Candel, 2004) and several other questionnaires that are not part of this study.

The scanning session took place a few days, but no more than two weeks, after the screening session at the Donders Centre for Cognitive Neuroimaging in Nijmegen, the Netherlands. Psychopathy patients were safely brought to the site of the scanning session by forensic transportation teams and accompanied by security guards the whole time. All participants were asked to refrain from drug use before the measurements, as described above, and further from caffeinated drinks and chocolate

on the day of the scanning session as well as from heavy meals and heavy physical exercising just before the measurements. At the scanning session, every participant performed a series of behavioural tasks inside a magnetic resonance imaging (MRI) scanner, with breaks in between. The behavioural task used for this study was divided into two blocks with a break of about 1 min in between and with each block consisting of three parts: an instrumental conditioning part, a Pavlovian conditioning part and a Pavlovian-instrumental transfer part. Only the data from the Pavlovian conditioning part was relevant for the current study. After receiving thorough instructions about the task outside the MRI scanner, participants performed this task inside the MRI scanner. In the MRI scanner, participants were equipped with earplugs and headphones. Inside the head coil of the MRI scanner, foam pads were placed for a restriction of movements. A mirror was attached to the head coil via which participants were able to see instructions and task images that were projected onto a translucent screen at the end of the scanner tube.

2.2.2 Behavioural task (Pavlovian conditioning)

Differential appetitive and aversive Pavlovian conditioning was used as a paradigm for associative learning. As conditioned stimuli (CS+), two different audio-visual stimuli were paired with an unconditioned stimulus (US), that is an appetitive (USapp) or aversive (USav) taste stimulus, respectively (see Fig. 1). An audio-visual stimulus consisted of a pure tone and a visual abstract picture. The USapp was 1 ml of sweet-tasting orange, apple or strawberry juice (depending on the participant's preferences indicated beforehand) and the USav was 1 ml of a magnesium sulphate 0.3M solution with bitter taste. Both the appetitive and aversive audio-visual CS+ (CS+app and CS+av, respectively) were presented 20 times (10 times paired with a US, 10 times unpaired, in randomized order). A third audio-visual stimulus was not paired with anything (representing a non-conditioned stimulus (CS-)) and thus served as generally unpaired neutral control stimulus. It was also presented 20 times, so that there was a total of 60 trials with three different audio-visual CS in randomized order which constituted the first of two experiment blocks. Each trial and thus each CS presentation had a length of 4.5 s with an intertrial interval of 1 s (see Fig. 1). After a randomized amount of time between 0 s and 1.5 s following CS+ onset on paired trials, the US was delivered. In order to ensure that participants were paying attention and to see whether conditioning was successful, a query trial was presented for 3.3 s (with an intertrial interval of 1 s) after every ten trials in which participants were instructed to choose the "better" of two visually presented CS. In the second experiment block, the same Pavlovian conditioning paradigm was used but with a different set of three audio-visual CS. This resulted in a total of 120 trials per participant, of which there were 40 CS+app trials (20 of them paired with a US), 40 CS+av trials (20 of them paired with a US) and 40 trials with a CS-. Before and after the Pavlovian conditioning, participants rated the pleasantness of the visually presented CS and of the US tastes on a visual analogue rating scale (VAS; 1 being very unpleasant, 4 being neutral and 7 being very pleasant).

2.3 Data Acquisition

2.3.1 Pupil recordings

During the behavioural task, the diameter and movements of the left pupil were recorded with an MRI-compatible infrared eye tracking camera and software (iView X, Tracksys Ltd., Nottingham, UK) at a sampling rate of 50 Hz.

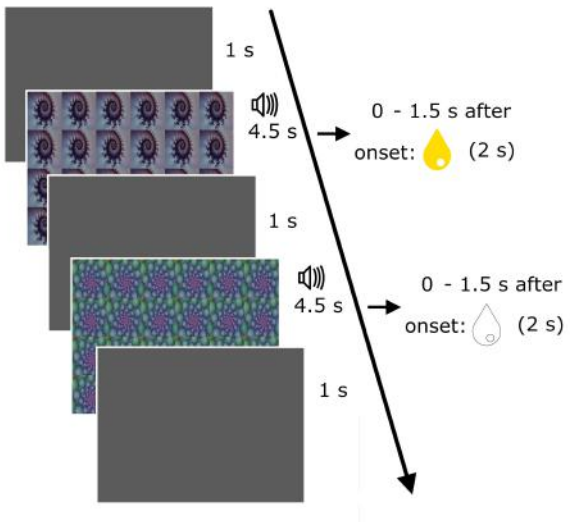

Fig. 1 Example of two (appetitive and aversive) paired trials. A trial started with the onset of an abstract visual stimulus combined with a tone (the conditioned stimulus, CS) and lasted 4.5 s. Between 0 and 1.5 s after CS onset on paired trials, 1 ml juice or bitter solution (the unconditioned stimulus, US) was delivered which lasted 2 s. In between trials, a grey screen was shown for 1 s. Appetitive and aversive unpaired trials as well as neutral trials were similar, with the only difference being the absence of the US delivery.

2.3.2 MRI data

Brain imaging data was acquired using a 3T Trio MRI scanner (Siemens, Erlangen, Germany) with a 32-channel head coil at the Donders Centre for Cognitive Neuroimaging, Nijmegen, Netherlands. For functional MRI data during the Pavlovian conditioning task, 152 gradient multi-echo T2*-weighted echo-planar images (ME-EPI; Poser, Versluis, Hoogduin, & Norris, 2006) with a blood oxygen level dependent (BOLD) contrast were acquired per participant per block. The following protocol was used: 38 axial-oblique slices; slice thickness, 2.5 mm; distance factor, 0.17; in-plane resolution, 3.3 x 3.3 mm; field of view, 211 mm; repetition time, 2320 ms; echo times, 9 ms, 19.3 ms, 30 ms and 40 ms; flip angle, 90°. For anatomical correlation, a T1-weighted magnetization-prepared rapid-acquisition gradient echo image (192 sagittal slices; slice thickness, 1 mm; in-plane resolution, 1 x 1 mm; field of view, 256 mm; repetition time, 2300 ms; echo time, 3.03 ms) was acquired from each participant in addition.

2.4 Data Analysis

2.4.1 Analysis of the stimulus ratings.

For all analyses of stimulus ratings, query trials and pre-processed pupil diameter means, SPSS Version 19 (IBM Corp., Armonk, NY, USA) was used. If data were not normally distributed, non-parametric tests were applied where appropriate.

2.4.1.1 Unconditioned stimuli (US)

Visual analogue scale (VAS) ratings of the stimuli were automatically converted from the visually presented scale that ranged from 1 to 7 into a scale from 0 to 1. The VAS ratings of the US were assessed to verify that the USapp and the USav were judged as pleasant and unpleasant, respectively, both before and after conditioning. This was an important pre-requisite for the differential conditioning to be effective. For a verification of VAS ratings being constant from before to after the conditioning, we applied Wilcoxon signed-rank tests for the two valences (appetitive and aversive) over the total sample. Potential differences between valences were also assessed with two Wilcoxon signed-rank tests, for both before and after conditioning, over the total sample. For an assessment of potential differences between healthy controls (HC) and psychopathy patients (PP), Mann-Whitney U-tests were applied to four conditions (appetitive and aversive for both before and after conditioning). One HC participant and one PP participant had to be excluded from the analyses of the VAS ratings of the US due to technical problems.

2.4.1.2 Conditioned stimuli (CS)

The VAS ratings of the visually presented CS served as an indication of whether Pavlovian conditioning was successful, that is whether CS+ became more or less pleasant over the conditioning due to the association with one of the US and whether CS differed in pleasantness between each other after conditioning. For this, the differences between the VAS ratings before and after conditioning were assessed with Wilcoxon signed-rank tests applied to the three valences (neutral, appetitive and aversive) over the total sample. To assess differences between the three valences, Friedman's analyses of variance (ANOVAs) were used for before and after conditioning. Bonferroni-corrected pairwise comparisons were conducted to determine the direction of potential differences. Group differences in VAS ratings of the CS for the six conditions (neutral, appetitive and aversive for both before and after conditioning) and the differences from before to after conditioning were assessed with Mann-Whitney U-tests. One HC participant and three PP participants had to be excluded from the analyses of the VAS ratings of the visual stimuli due to technical problems.

2.4.2 Analysis of query trials

Query trials served as an indicator of whether participants were paying attention and whether the CS were successfully conditioned with the US. Therefore, in query trials occurring after every ten conditioning trials, participants were asked to choose the "better" of two visually presented CS, that is the CS+app when compared to the CS- or the CS+av or the CS- when compared to the CS+av. Potential group differences were assessed with Mann-Whitney U-tests. Three PP participants were excluded from this analysis because they did not give an answer on more than 50% of the query trials.

2.4.3 Processing of pupil recordings

Pupil recordings served as an indicator of autonomic arousal (see e.g. Barreto et al., 2007; Bradley et al., 2008; Partala & Surakka, 2003) and

were thus used to investigate effects of conditioning (i.e. in terms of comparing the pupil diameter during the CS+ conditions to the pupil diameter during the CS- condition) as well as differences in arousal between CS+app and CS+av, between USapp and USav, and between HC and PP. Three HC participants and one PP participant had to be excluded from all processing and analyses of the pupil recordings due to technical problems.

We processed the pupil recordings with MATLAB version 7 (The MathWorks Inc., Natick, MA, USA). First, on- and offsets of eye blinks and artefacts were detected by applying a derivative threshold to the x- and y-coordinates of the pupil and to the diameter from one sample to the next. The five samples following the onset of a blink or artefact (corresponding to the next 100 ms) were marked as blink/artefact samples. Samples that were both preceded and followed by blink/artefact samples were considered as belonging to the blink or artefact, too. If a blink or artefact was longer than 1 s, the respective samples were excluded from analysis. Otherwise, the blinks/artefacts were interpolated by using the mean diameter of two samples each preceding and following the blink/artefact and by replacing the samples in-between relative to the difference of the two means and the length of the interpolation interval.

The pupil diameter data were then baseline-corrected by subtracting from all samples of a trial the mean diameter of the baseline period within 1 s preceding trial onset. Then, outliers were defined as samples differing from the mean diameter per participant per block by more than two standard deviations and were excluded from the analysis. If a trial contained at least 25% of samples that were excluded during processing (i.e. due to excessive length of a blink/artefact or due to outliers), the complete trial was excluded. Also, if a block contained at least 25% of trials that had been excluded earlier, the complete block was excluded from the analysis. For each of six HC and one PP participants, one complete block had to be excluded following this criterion. Of the remaining data, 3.48 trials per participant per block were excluded on average (corresponding to 5.8%, SD = 3.72%; HC: M = 6.89%, SD = 5.02%; PP: M = 4.94%; SD = 1.95%). Per experimental condition (CS+app, CS+av and CS- with and without US) over the remaining participants without excluded blocks, between 24 (4.8%) and 37 (7.4%) trials were discarded. Lastly, the pupil diameter means per participant per block per experimental condition of the interval of interest (i.e. starting from 2 s following trial onset after the initial light reflex, based on Bradley et al. (2008), and ending at trial offset, see Fig. 2A and 2B) were entered into statistical analyses. For the neutral condition (i.e. the CS-), the always unpaired trials were randomly and evenly distributed across the paired and unpaired condition for statistical analyses.

2.4.4 Analysis of pupil recordings

The mean pupil diameter values per participant, block, valence (neutral, appetitive and aversive) and pairing condition (with and without a US) were entered into a Block(2) x Valence(3) x Pairing(2) repeated measures omnibus ANOVA with Group(2) as a between-subjects factor. By this, we aimed to determine possible effects of the different conditions on the mean pupil diameter, also with regard to potential group differences. Since we generally expected higher mean pupil diameter on paired trials than on unpaired trials, we further split this analysis into two Block(2) x Valence(2) repeated measures ANOVAs with Group(2) as a between-subjects factor over unpaired trials and paired trials separately in order to specify possible interaction effects with Pairing.

In addition to group differences, we were also interested in within-group variations. For this, we conducted a Block(2) x Valence(3) x Pairing(2) repeated measures analysis of covariance (ANCOVA) over the HC sample with the scores on the Psychopathic Personality Inventory (PPI) questionnaires as a covariate to determine possible parametric effects of psychopathic personality traits on mean pupil diameter within the HC sample. Analogously, a Block(2) x Valence(3) x Pairing(2) repeated measures ANCOVA with the scores on the Psychopathy Checklist-Revised (PCL-R) as a covariate over the PP sample was used to investigate whether the severity of psychopathy within the PP sample had a parametric effect on mean pupil diameter in the various conditions. For all analyses of pupil recordings, Bonferroni-corrected pairwise comparisons were conducted to determine the directions of possible effects. Only the data of 11 HC participants and of 14 PP participants were included in these analyses due to the exclusion of participants beforehand and the exclusion of complete blocks of pupil recordings during processing.

2.4.5 Processing of MRI data

For pre-processing and analysis of the brain imaging data, we used statistical parametric mapping software (SPM5, Wellcome Trust Centre

for Neuroimaging, London, UK). The first five functional MRI scans of each participant were omitted from analyses in order to ensure a T1 equilibrium. Before combining the different echoes, realignment parameters relative to the first image were computed for images acquired at the first echo time and applied to the images from the other echo times. Echo summation was then conducted by a parallel-acquired inhomogeneity-desensitized fMRI algorithm that assessed the signal-to-noise ratio of 30 resting state images acquired beforehand in order to weight the echoes by their measured relative contrast-to-noise ratio (Poser et al., 2006). The combined images were slice-time corrected and coregistered before they were segmented using the tissue probability maps by SPM5 for grey matter, white matter and cerebrospinal fluid based on the brain space by the Montreal Neurological Institute (MNI). This enabled the normalization of structural and functional images based on every participant's individual structural image. Finally, an isotropic full-width half-maximum Gaussian kernel of 8 mm was applied to smooth all normalized images.

2.4.6 Analysis of MRI data

For a random-effects event-related statistical analysis of the functional fMRI data during the Pavlovian conditioning, we applied a general linear model on each participant. We modelled the three different types of stimuli (CS+app, CS+av and CS-) with three main regressors as delta (stick) functions representing the onset of the stimuli. These main regressors were convolved with a canonical hemodynamic response function. US delivery was modelled with a parametric regressor coding binarily for occurrence of the US for each of the CS+app and CS+av main regressor. To account for conditioning over time in consideration of the randomized order of CS and US, we included two additional parametric regressors for each of the CS+app and CS+av main regressor that coded for the linearly increasing number of preceding US deliveries of the respective valence. Analogous to that, we also accounted for an effect of time for the CS– main regressor by including a parametric regressor that modelled a strict linear increase, similar to the parametric regressors of the CS+app and the CS+av. Additional regressors of no interest were one coding for the onset of query trials as well as six motion regressors with parameters from the realignment procedure plus their first-order derivatives as another six regressors. Low-frequency signal drifts were removed with a high-pass filter of 128 s. During parameter estimation obtained by restricted maximum-likelihood estimation, serial correlations were accounted for with an autoregressive (AR1) model.

On the subject level, we were interested in the covariance of the fMRI data with the following t-contrasts: (a) CS+app (time) minus CS- (time) (to capture the effect of appetitive conditioning over time); (b) CS+av (time) minus CS- (time) (to capture the effect of aversive conditioning over time); (c) CS+av (time) minus CS+app (time) (to capture differences in conditioning over time between valences); (d) CS+app (US) minus CS+av (US) (to capture differences in response to the US between valences); and (e) CS+app (main) minus CS+av (main) (to capture differences in response to the CS+ between valences).

On the group level, each subject-specific mean functional image per contrast of interest was entered into the following second-level t-tests: (a) a two-sample t-test to examine differences between the HC and the PP sample (t-contrastHC minus t-contrastPP and the other way round) as well as activations in both samples (t-contrastHC plus t-contrastPP); (b) a one-sample t-test with the PPI scores as covariate over the HC sample to determine possible parametric effects of psychopathic personality traits within the HC sample; and (c) a one-sample t-test with the PCL-R scores as covariate over the PP sample to examine potential parametric effects of the severity of psychopathy within the PP sample.

For regions of interest (ROI) analyses, we used the anatomical regions of interest (amygdala, insula and orbital part of the inferior, middle and superior frontal gyrus as orbitofrontal cortex) defined in the automated anatomical labelling atlas by Tzourio-Mazoyer et al. (2002). Significance thresholds for ROI and whole-brain analyses were set to $p < .05$ family-wise error-corrected for a small volume or the whole brain, respectively. All fMRI data analyses are based on the data of 20 HC and 15 PP participants because one PP participant had to be excluded due to excessive motion.

3 Results

3.1 Stimulus ratings

3.1.1 Unconditioned (taste) stimuli (US)

The mean visual analogue scale (VAS) ratings of the US per condition and participant sample are displayed in Table 2. For the VAS ratings of the US,

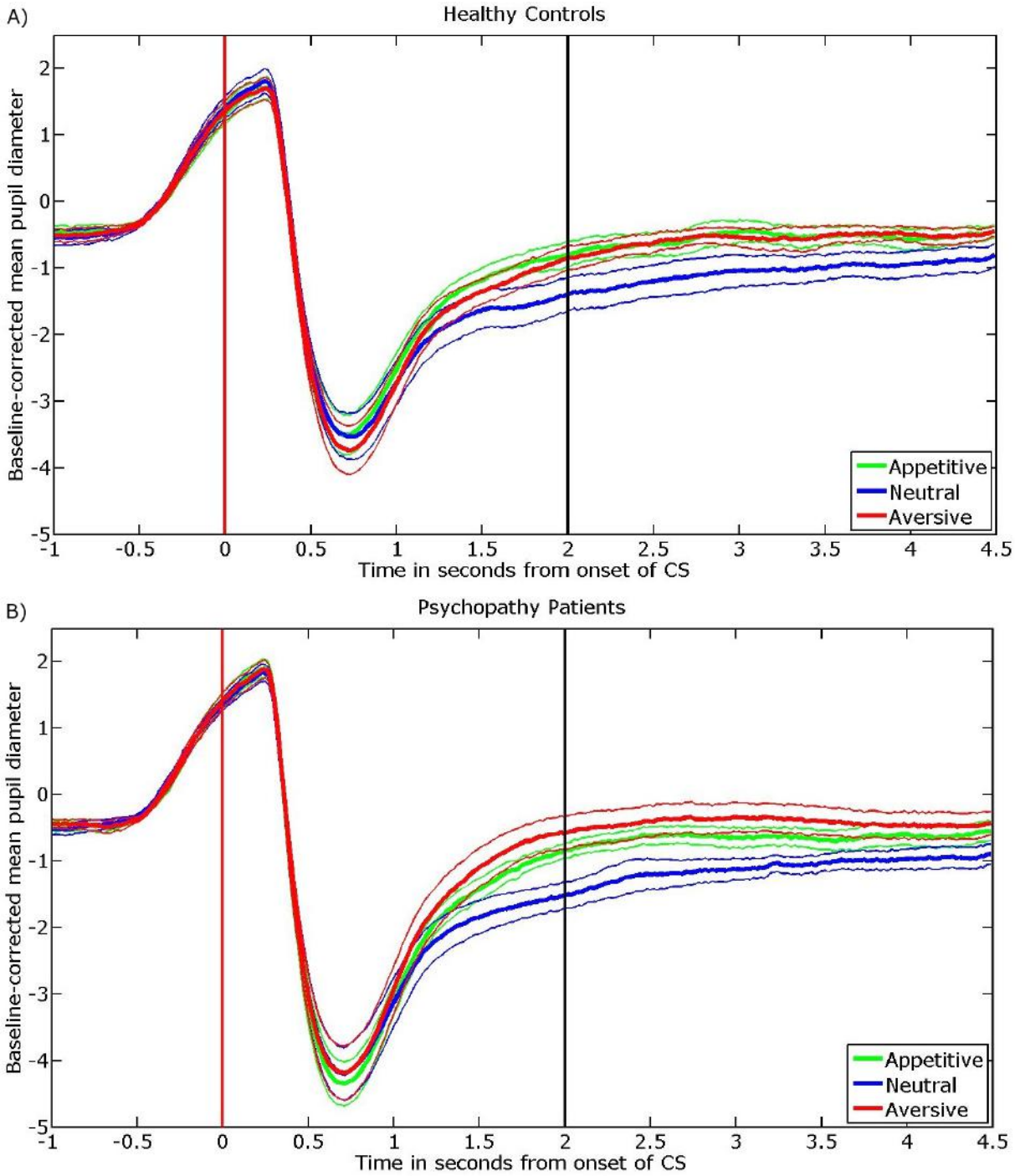

Fig. 2 Illustration of the baseline-corrected pupil diameter during a trial averaged per valence across blocks and pairing conditions for the healthy controls **A**. and the psychopathy patients **B**. The thinner lines below and above each of the three mean pupil diameter lines represent the respective standard error. The red vertical line marks the onset of the conditioned stimulus (CS) and thus the start of a trial. The black vertical line marks the start of the analysis interval. Shortly after stimulus onset, the pupil contracts in an initial light reflex and then dilates again until its width is close to baseline again. The interval from 2 s to 4.5 s after CS onset was chosen for analyses because the pupil had then roughly recovered from the initial light reflex (see Bradley, Miccoli, Escrig, & Lang, 2008) while it was still affected by the presented CS.

Wilcoxon signed-rank tests revealed no significant differences between before (pre) and after (post) conditioning over the total sample (appetitive: $p = .09$; aversive: $p = .70$). With regard to differences between valences, the appetitive US was constantly judged to be significantly more pleasant than the aversive US according to Wilcoxon signed-rank tests (pre: $z = -5.01$, $p < .001$; post: $z = -4.92$, $p < .001$). No group differences were found (ps > .59 for all Mann-Whitney U-tests). This indicates that the US were judged as expected and equally across both groups.

3.1.2 Conditioned (visual) stimuli (CS)

As for the VAS ratings of the CS, the respective means per condition and participant sample across blocks are presented in Table 3. According to Wilcoxon signed-rank tests, the VAS ratings of the appetitive CS (CS+app) significantly increased from before to after conditioning over the total sample ($z = -2.32$, $p < .05$). Aversive CS (CS+av) were rated as significantly less pleasant from before to after conditioning ($z = -3.93$, $p < .001$). No significant differences from before to after conditioning were found for the VAS ratings of the neutral CS (CS-; p=.22). This supports the assumption that conditioning was successful.

With regard to differences between valences, there were no differences in VAS ratings of the CS according to the Friedman's ANOVA ($p = .47$) before conditioning. After conditioning, there was a significant difference between valences ($\chi2(2) = 23{,}66$, $p < .001$). Post-hoc Wilcoxon signed-rank tests revealed that the VAS ratings of the CS+app were higher than those of the CS- ($z = -2.93$, $p < .01$) and higher than those of the CS+av ($z = -4.20$, $p < .001$) and that the VAS ratings of the CS+av were lower than those of the CS- ($z = -3.26$, $p < .01$), as would be expected in case of successful conditioning.

No group differences were found in VAS ratings of the CS or their differences from before to after conditioning (ps > .18 for all Mann-Whitney U-tests). Overall, this indicates that Pavlovian conditioning was successful across both groups.

3.2 Query trials

On query trials, both healthy controls (HC) and psychopathy patients (PP) mostly selected the "better" visual stimulus (HC: n = 20, M = 86%, SD = 15%; PP: n = 13, M = 86%, SD = 17%). This demonstrates that participants were paying attention and further supports the assumption that conditioning was successful. There were no group differences according to the Mann-Whitney U-test ($U = 130$, $p = 1$).

3.3 Pupil recordings

The baseline-corrected mean pupil diameter values per experimental condition (Valence x Pairing) for the two participant groups are displayed in Figures 3A and 3B. The Block(2) x Valence(3) x Pairing(2) repeated measures omnibus ANOVA with Group(2) as a between-subjects factor yielded a main effect of Valence ($F(2,46) = 8.84$, $p = .001$), with the mean pupil diameter for the neutral valence being significantly lower than for the appetitive valence ($p = .01$) and the aversive valence ($p = .001$). This supports the assumption that arousal was higher in the affective (appetitive and aversive)

Table 2. Mean visual analogue scale ratings of the unconditioned taste stimuli (from 0 to 1)

	Pre (before behavioural task)		**Post (after behavioural task)**	
Sample	Appetitive	Aversive	Appetitive	Aversive
Healthy controls	.863	.153	.764	.177
Psychopathy patients	.8	.162	.779	.143
Total	.835	.157	.771	.162

Note. Means are based on 19 of the 20 healthy control participants and on 15 of the 16 psychopathy patients.

Table 3. Mean visual analogue scale ratings of the conditioned visual stimuli (from 0 to 1)

	Pre (before behavioural task)			**Post (after behavioural task)**		
Sample	Neutral	Appetitive	Aversive	Neutral	Appetitive	Aversive
Healthy controls	.468	.518	.527	.521	.597	.336
Psychopathy patients	.491	.521	.476	.489	.608	.381
Total	.477	.519	.506	.508	.602	.354

Note. Means are based on 19 of the 20 healthy control participants and on 13 of the 16 psychopathy patients.

conditions than in the neutral condition. The analysis also revealed a main effect of Pairing ($F(1,23) = 68.41$, $p < .001$), with the mean pupil diameter for the paired condition with US being higher than for the unpaired condition without US. As would be expected for conditions of higher salience, this indicates that arousal was higher when a US was delivered compared with trials without a US (even though the trials in the neutral "paired" condition were in fact unpaired).

An interaction of Block and Pairing ($F(1,23) = 7.49$, $p = .012$) was also shown in the omnibus ANOVA, with a larger difference in mean pupil diameter between the two pairing conditions in Block 1 than in Block 2, as well as an interaction of Valence and Pairing ($F(2,46) = 17.59$, $p < .001$), with the smallest difference in mean pupil diameter between the two pairing conditions for the neutral valence and the largest difference for the appetitive valence. To specify these interactions, the Block(2) x Valence(2) repeated measures ANOVA with Group(2) as a between-subjects factor over unpaired trials only yielded a main effect of Block ($F(1,23) = 5.73$, $p = .025$) with the mean pupil diameter being higher in Block 2 than in Block 1, but no main effect of Valence ($p = .38$). Analogously, the Block(2) x Valence(2) repeated measures ANOVA with Group(2) as a between-subjects factor over paired trials revealed an effect of Block again ($F(1,23) = 4.31$, $p = .049$) with a higher mean pupil diameter in Block 1 than Block 2 this time. Therefore, the Block x Pairing interaction from the omnibus ANOVA seems to be based on a higher mean pupil diameter for paired trials and a lower mean pupil diameter for unpaired trials in Block 1 compared to Block 2. This might reflect increased arousal on unpaired trials and decreased arousal on paired trials in Block 2 as an effect of habituation to the paradigm (expectations of receiving a US might be high from the beginning and the actual delivery of a US might be less unexpected in Block 2 compared to Block 1). Moreover, a main effect of Valence was also found ($F(2,46) = 18.20$, $p < .001$) in the repeated measures ANOVA over paired trials, with a lower mean pupil diameter for the neutral valence than for the appetitive ($p < .001$) or aversive valence ($p < .001$), as would be expected for the actually unpaired neutral trials, but no differences between the appetitive and aversive valence ($p = 1$). Thus, it seems that the Valence x Pairing interaction from the omnibus ANOVA is mainly based on differences between valences over paired trials, reflecting increased arousal in salient conditions when a US was delivered, and not on differences over unpaired trials which would have

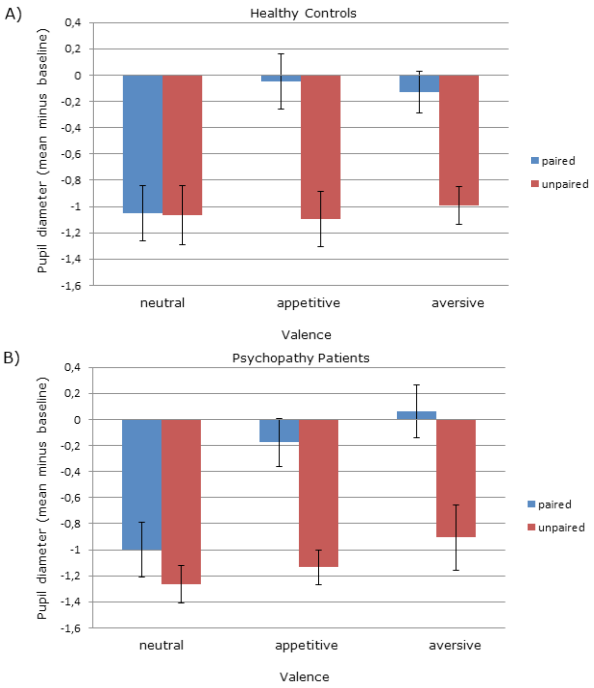

Fig. 3 Baseline-corrected pupil diameter values averaged over the interval of interest and across blocks per experimental condition (Valence x Pairing) for the healthy controls **A**. and the psychopathy patients **B**. Error bars represent the standard error.

indicated an effect of conditioning.

Regarding between-group variations, no effects of Group were found in any of these ANOVAs. With regard to within-group effects of psychopathic personality traits over the HC sample, the Block(2) x Valence(3) x Pairing(2) repeated measures ANCOVA with the PPI scores as a covariate did not yield any significant effects. Similarly, the Block(2) x Valence(3) x Pairing(2) repeated measures ANCOVA with the PCL-R scores as a covariate over the PP sample did not reveal any significant within-group effects either. Overall, this indicates that arousal in the different conditions did not differ between groups, contrary to our hypothesis, and was specific to salient conditions, that is when a US was delivered irrespective of the valence. An effect of conditioning was not found, contrary to our assumptions and to the behavioural data.

3.4 fMRI data

3.4.1 Appetitive conditioning

The contrast capturing the effects of appetitive conditioning depending on previous US exposure (CS+app (time) minus CS- (time)) did not yield any significant positive correlations with the fMRI data in the two groups and also no group differences. Whereas no correlations with the PPI score within

the HC sample were found either, a positive correlation with the PCL-R score was detected within the PP sample in the bilateral occipital cortex (see Table 4, Fig. 4A). This means that the higher the psychopathy severity was, the stronger the activation was for this contrast in this region.

3.4.2 Aversive conditioning

The contrast capturing the effects of aversive conditioning depending on previous US exposure (CS+av (time) minus CS- (time)) did also not yield any significant positive correlations with the fMRI data in the two groups and no group differences. Within the HC sample, a negative correlation of this contrast with the PPI score was found in the right insula (see Table 4, Fig. 4B). This means that the weaker the psychopathic personality traits were, the stronger the activation was for this contrast. On the other hand, a positive correlation of this contrast with the PCL-R scores was found within the PP sample in the right orbitofrontal cortex (OFC) (see Table 4, Fig. 4B). Note that this result was small-volume-corrected for the ROI and that it remained significant after correcting for multiple (i.e. 3) ROI comparisons.

3.4.3 Differences between appetitive and aversive conditioning

As for the differences between appetitive and aversive conditioning, the respective contrast (CS+av (time) minus CS+app (time)) did not result in any positive or negative correlations with the fMRI data in the two groups or any group differences. No correlations were found with the PPI scores of the HC sample or the PCL-R scores of the PP sample.

3.4.4. Differential responses to the appetitive and aversive US

With regard to differences in response to the appetitive and aversive US, both groups showed a positive correlation with the respective contrast (CS+app (US) minus CS+av (US)) in the left amygdala (see Table 4, Fig. 4C). This result seems to reflect a stronger positive correlation in response to the appetitive US than in response to the aversive US, as activation in the left amygdala correlated positively with both the CS+app (US) (MNI(x,y,z) = -28,-4,-12; k = 190, T = 8.02, pcluster = .001, ppeak < .001) and the CS+av (US) (MNI(x,y,z) = -24,-2,-12; k = 56, T = 5.3, pcluster = .01, ppeak < .001), though a definite overlap of the left amygdala clusters implicated in these correlations was not determined. Note that this result was small-volume-corrected for the ROI and that it remained significant after correcting for multiple (i.e. 3) ROI comparisons. No group differences or correlations with the PPI or PCL-R scores were found.

3.4.5 Differential responses to the appetitive and aversive CS+

The contrast capturing differences in responses to the CS+ of the two valences (CS+app (main) minus CS+av (main)) did not reveal any significant correlations with the fMRI data in the two groups and also no group differences. The contrast only yielded a positive correlation with the PPI scores of the HC sample in the right medial prefrontal cortex (mPFC) (see Table 4, Fig. 4D), but no correlations with PCL-R scores of the PP sample.

4. Discussion

In this study, we investigated the neural and physiological correlates of affective associative learning (i.e. Pavlovian conditioning) with neutral audio-visual stimuli (the conditioned stimuli, CS) and appetitive and aversive taste stimuli (the unconditioned stimuli, US) in psychopathy patients and healthy controls. Specifically, we were interested in differential neural and physiological responses between psychopathy patients and healthy controls and in differential effects between appetitive and aversive conditioning.

The behavioural results demonstrated successful conditioning that was similar in both participant groups: a CS associated with an appetitive US was rated as more pleasant and a CS associated with an aversive US was rated as less pleasant after conditioning than before. Also, differences in ratings between the appetitively and aversively conditioned (CS+app and CS+av, respectively) and non-conditioned stimuli (CS-) were absent before conditioning but were present afterwards.

Physiologically, however, successful conditioning could not be shown in either of the groups: pupil dilation was taken as an indicator of autonomic arousal and did not differ significantly between the three valences (appetitive, aversive, neutral) on unpaired trials (that is on trials when a US was omitted but should still be expected due to the learned association). Pupil dilation only differed between paired trials when a US was presented and unpaired trials when no US was presented, with

Table 4. Brain regions whose blood oxygen level dependent responses correlated with the indicated contrast

Analysis	Group contrast	Brain region	Montreal Neurological Institute coordinates (x,y,z) in millimetres	Size of cluster	T-statistic	$p_{cluster}$	p_{peak}
Appetitive conditioning (appetitively conditioned stimulus (time) minus non-conditioned stimulus (time))							
One-sample *t*-test: psychopathy patients (psychopathy checklist-revised)	Psychopathy checklist-revised positive	right occipital	30, -68, 32	360	7.66	.002	*Not significant*
		left occipital	-26, -92, 10	363	5.99	.003	*Not significant*
Aversive conditioning (aversively conditioned stimulus (time) minus non-conditioned stimulus (time))							
One-sample *t*-test: healthy controls (psychopathic personality inventory)	Psychopathic personality inventory negative	right insula	42, -24, 22	251	5.54	.018	*Not significant*
One-sample *t*-test: psychopathy patients (psychopathy checklist-revised)	Psychopathy checklist-revised positive	right orbitofrontal cortex	26, 40, -12	264	5.52	.003	*Not significant*
		right orbitofrontal cortex	26, 40, -12	171	6.13	.002*	*Not significant**
Appetitive vs. aversive unconditioned stimulus (appetitively conditioned stimulus (unconditioned stimulus) minus aversively conditioned stimulus (unconditioned stimulus))							
Two-sample *t*-test	Healthy controls and psychopathy patients positive	left amygdala	-20, 0, -20	36	3.94	.013*	.013*
Appetitive vs. aversive conditioned stimulus(appetitively conditioned stimulus (main) minus aversively conditioned stimulus (main))							
One-sample *t*-test: healthy controls (psychopathic personality inventory)	psychopathic personality inventory positive	right medial prefrontal cortex	14, 48, 2	230	6.39	.012	*Not significant*

Note. The significance threshold was set to $p < .05$, family-wise error-corrected for the whole brain volume or a small volume of interest (corrected for multiple comparisons). For activations that were significant on the cluster level, the size of the cluster, the T-statistic and the significance values on the cluster (pcluster) and peak level (ppeak) are indicated.
* corrected for small volume

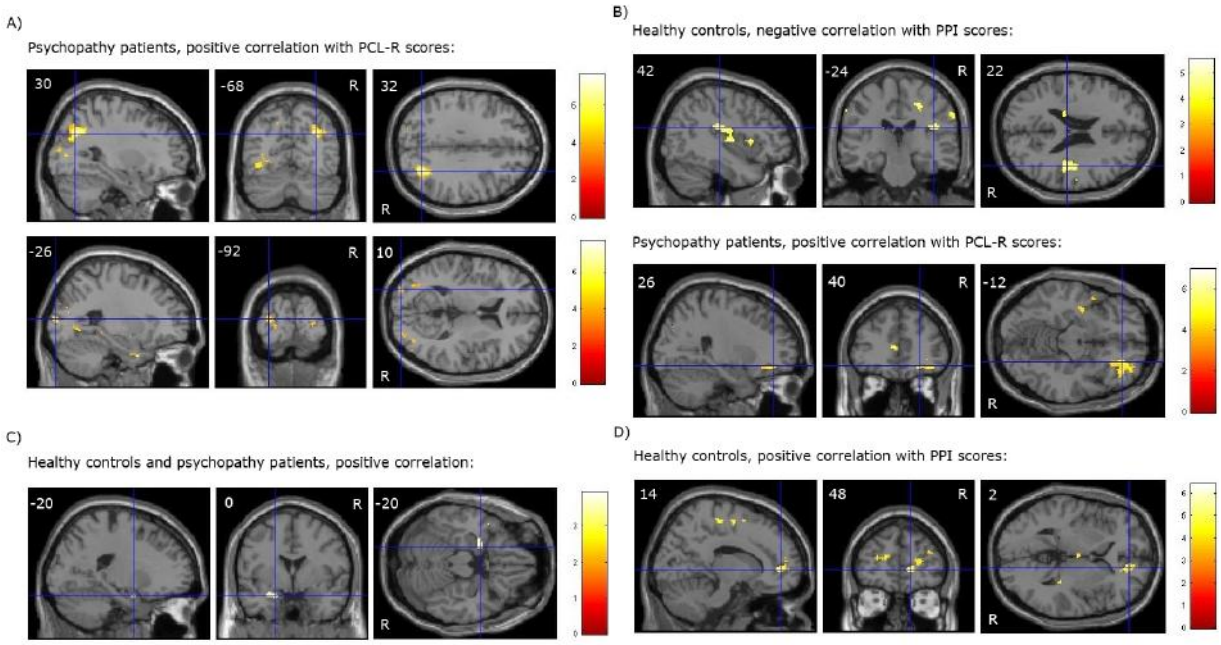

Fig. 4 fMRI signal clusters in response to appetitive conditioning **A**. aversive conditioning **B**. the appetitive minus the aversive unconditioned stimulus **C**. and the appetitive minus the aversive conditioned stimulus **D**. Signal clusters are overlaid onto a canonical T1-weighted MRI image provided by SPM at a threshold of $p < .001$, uncorrected (for visualization). The centres of the blue crosses mark the peaks of the significant signal clusters. The ranges of T-values are displayed on the right.

stronger pupil dilation on paired trials indicating higher arousal when participants experienced an appetitive or aversive taste. Therefore, the arousal indicated by pupil dilation was not valence-specific but rather salience-specific, that is specific to the experience of a taste regardless of whether it was appetitive or aversive. Importantly, there were no differences in pupil dilation between the two participant groups and no correlations of the degree of pupil dilation with the degree of severity of psychopathy within the psychopathy patient sample or with the degree of psychopathic personality traits within the healthy control sample. Thus, no effects of psychopathy or psychopathic personality traits on arousal as indicated by pupil dilation were shown, contrary to our hypotheses. With regard to the two experiment blocks, pupil dilation was generally higher in the second block than in the first block on unpaired trials and higher in the first than the second block on paired trials, possibly reflecting effects of habituation to the paradigm.

Again contrary to our hypotheses and similar to the behavioural and physiological data, the fMRI data did not reveal any differences between the two participants groups. Successful conditioning could not be demonstrated based on the fMRI data either. When contrasting the CS+app or the CS+av with the CS- with respect to an increasing effect over time (aimed to capture an effect of appetitive or aversive conditioning, respectively), only correlations with psychopathy scores within the two participants groups were found. Within the psychopathy patient sample, positive correlations with the psychopathy scores based on the Psychopathy Checklist-Revised (PCL-R; Hare, 2003) were found for appetitive conditioning in bilateral occipital areas and for aversive conditioning in the right orbitofrontal cortex (OFC). This means that the higher the PCL-R scores were, the stronger the activation within these areas correlated with appetitive or aversive conditioning over time. Within the healthy control sample, on the other hand, a negative correlation with the scores based on the Psychopathic Personality Inventory (PPI; Lilienfeld & Andrews, 1996) was found for aversive conditioning in the right insula, suggesting that the weaker the psychopathic personality traits were, the stronger the activation within the right insula correlated with aversive conditioning over time. When contrasting appetitive with aversive conditioning, no effects were found.

With regard to differential responses to the appetitive and the aversive US, we found activation in the left amygdala to be correlating positively with the contrast CS+app (US) minus CS+av (US) in both participant groups, probably reflecting a more positive correlation with the appetitive US than the aversive US. Lastly, regarding differential responses to the appetitive and aversive CS+ without

considering effects of time, there was a positive correlation with the PPI scores within the healthy control sample in the right medial prefrontal cortex (mPFC). This means that the higher the PPI scores were, the stronger the activation in the right mPFC correlated with the appetitive CS+ in contrast to the aversive CS+.

4.1 Lack of Differences Between Psychopathy Patients and Healthy Controls

The lack of between-group differences in behavioural, physiological and neural responses was contrary to our expectations. Given the evidence on affective processing deficits in psychopathy patients, we expected the psychopathy patient sample to display a different physiological and neural response to the affective (conditioned and unconditioned) stimuli when compared to the healthy control sample. For example, several studies demonstrated decreased physiological responding to affective pictures or words in psychopathy patients compared to healthy controls (Anderson & Stanford, 2012; Hare & Quinn, 1971; Herpertz et al., 2001; Sutton et al., 2002; Verona et al., 2012; Williamson et al., 1991) and some fMRI studies reported different neural responding to pictures and words with affective content (Kiehl et al., 2001; Mueller et al., 2003). However, neither the physiological nor the neural data from this study concur with this evidence on deficient affective processing in psychopathy patients in comparison to healthy controls. It is likely that the type or amount of affective processing in this study, that is experiencing or expecting a pleasant or unpleasant taste, was different from processing affective visual and linguistic content in previous studies so that deficits in affective processing in psychopathy patients detected previously were simply not pronounced or present in this study. Also the pleasantness ratings of the stimuli, being valence-specific and similar in both groups, support the assumption that affective processing of the stimuli used in this study was not deficient enough in psychopathy patients to result in between-group differences.

The pleasantness ratings of the stimuli did not demonstrate differences between the two participant groups in success of Pavlovian conditioning either, which was contrary to previous studies that found deficient conditioning in psychopathy patients in comparison with healthy controls. For example, in a study on aversive Pavlovian conditioning, psychopathy patients failed to display differential responses between the conditioned and the non-conditioned stimulus in terms of valence ratings, skin conductance rate, corrugator reactivity and startle potentiation whereas healthy controls did show such differential responses (Flor et al., 2002). Another study found a lack of differentiation between the conditioned and the non-conditioned stimulus in skin conductance rate and startle potentiation in psychopathy patients only (Rothemund et al., 2012). Similarly, two further studies on aversive Pavlovian conditioning showed deficient differentiation between the conditioned and the non-conditioned stimulus in terms of activation in the amygdala, insula, orbitofrontal cortex and anterior cingulate in psychopathy patients when compared to healthy controls (Birbaumer et al., 2005; Veit et al., 2002). Similar differences in physiological and neural responding during Pavlovian conditioning between psychopathy patients and healthy controls could not be shown in this study. Again, this might be explained by the differences in nature of the stimuli used in the present study and of those used in studies detecting differences in conditioning between psychopathy patients and healthy controls. In the studies by Birbaumer et al. (2005), Flor et al. (2002), Rothemund et al. (2012) and Veit et al. (2002), the US were either a foul odour, a painful electric shock or painful pressure. The type and strength of affective value of these US might have been different from the pleasant and unpleasant taste stimuli in the present study. Potential deficits in affective processing in psychopathy patients might thus not have been pronounced enough in the present study to affect conditioning differentially between the two groups. Additionally, the conditioned facial stimuli used in the other studies might have had a subjective affective value that the conditioned audio-visual stimuli in our study did not have, such as an emotional association with a familiar face despite the neutral facial expression of the stimulus. Overall, it is possible that the affective values of the stimuli in other studies were stronger which lead to a generally stronger affective processing when compared to the stimuli in our study so that larger differences between psychopathy patients with deficits in affective processing and healthy controls with normal affective processing occurred. Consequently, differences in affective processing between psychopathy patients and healthy controls might have been smaller in this study compared to the other studies so that this study failed to detect them.

4.2 Within-Group Differences Related to Psychopathy

Despite the lack of between-group differences, within-group differences in neural responding related to psychopathy were found within the two participant groups. Outside regions of interest, activation in bilateral occipital areas correlated positively with PCL-R scores during appetitive conditioning within the psychopathy sample, possibly reflecting visual processing similar as in another study on appetitive conditioning (Kirsch et al., 2003). Interestingly, and contrary to our hypotheses, activation in the right OFC during aversive conditioning also correlated positively with PCL-R scores. This is somewhat surprising as psychopathy was generally associated with decreased activation in the right OFC (for a recent review see Anderson & Kiehl, 2012 and for a meta-analysis see Yang & Raine, 2009). This would mean that higher degrees of severity of psychopathy would correspond to stronger aversive conditioning in the right OFC. In previous studies, the right OFC was indeed shown to be related to appetitive and aversive olfactory conditioning (Gottfried, O'Doherty, & Dolan, 2002) and to disgust- and fear-related conditioning (Klucken et al., 2012). Considering the role of the OFC in subjective value representation (see Padoa-Schioppa & Cai, 2011), this result would also suggest that higher degrees of psychopathy relate to stronger aversive value processing, also contrary to studies on affective processing in psychopathy. However, psychopathy is a rather complex personality disorder and studies with different subtypes of psychopathy can yield different results (see Koenigs, Baskin-Sommers, Zeier, & Newman, 2011 for a critical review). In this study, we included psychopathy patients solely based on total PCL-R scores without respect to subscores of the four PCL-R factors, contrary to many other fMRI studies with psychopathy patients. Also, rather few studies to date assessed inter-individual differences in neural responding based on PCL-R scores. To our knowledge, this is the first fMRI study on Pavlovian conditioning in psychopathy patients that assessed correlations with PCL-R scores. Future studies on aversive conditioning in psychopathy patients should take into account inter-individual differences as the unexpected positive correlation of right OFC activation with PCL-R scores found in this study needs further investigation.

Within the healthy control sample, a negative correlation with PPI scores was found for aversive conditioning in the right insula, in accordance with our hypothesis. Involvement of the right insula in aversive conditioning was also found previously (Büchel, Dolan, Armony, & Friston, 1999; Büchel, Morris, Dolan, & Friston, 1998; Delgado, Jou, & Phelps, 2011; Klucken et al., 2012). In psychopathy patients, the right insula was indicated to be hypoactive during aversive conditioning (Birbaumer et al., 2005; Veit et al., 2002), although it was also found to be more active during aversive processing compared to healthy controls (Mueller et al., 2003). Our results support views of decreased aversive processing in the insula in individuals with psychopathic personality traits. However, the PPI corresponds to psychopathy-typical personality traits and not to a clinical diagnosis of psychopathy like the PCL-R does, which is why the detected correlation with PPI scores cannot be generalised to clinical psychopathy. Also, the PPI and PCL-R measures of psychopathy do not necessarily correlate well with each other (Gonsalves, McLawsen, Huss, & Scalora, 2013). Together with the lack of a similar correlation in the insula with PCL-R scores of the psychopathy sample, this suggests that the PPI-related activation in the right insula during aversive conditioning needs to be interpreted with caution.

With regard to the positive correlation of medial prefrontal cortex (mPFC) activation with PPI scores of the healthy control sample in response to the appetitive relative to the aversive CS+, this result has to be interpreted with caution as well, given the limitations of the PPI scores and the lack of a similar correlation with PCL-R scores of the psychopathy sample. As for the role of the mPFC in affective processing, it was suggested that the mPFC is involved in emotion regulation and specifically in expected outcome signalling and modulation of conditioned associations (Mitchell, 2011). This view fits with the reported correlation in that the differential PPI-related mPFC activation in response to two CS+ might reflect the signalling of an outcome, that is an appetitive or aversive US, as well as the modulation of the association of the CS+ with the US. In this sense, the stronger the psychopathic personality traits were, the stronger might have been the signalling of the appetitive relative to the aversive US and the modulation of the association between the appetitive CS+ and US relative to the aversive one.

4.3 Similar Responses Across Both Groups to the US

Within both groups, the left amygdala responded to both the appetitive and the aversive US and more so to the appetitive US when contrasted with the

aversive US. Similarly, a previous study also found the left amygdala to be responsive to both appetitive and aversive stimuli (Meseguer et al., 2007). This concurs with recent views of the amygdala as a crucial site for processing both appetitive and aversive values (Murray, 2007). The amygdala was further suggested to be relevant for learning CS-US associations and for controlling arousal and response systems (Cardinal et al., 2002) which would also be in accordance with the result found in this study. It remains to be investigated more closely though whether the increased response in the left amygdala to the appetitive than the aversive US reflects a characteristic specific to the context of taste and associative learning.

4.4 Mixed Evidence on Conditioning Success

Contrary to our expectations and to the results in many other studies, we did not find any physiological or neural indicators of successful conditioning. However, the behavioural results in this study seemed to reflect successful conditioning in both participant groups, in contrast to the physiological and neural data. For the physiological data, it is possible that the stimulus timings were not suited well for pupil dilation to reflect conditioning-related arousal. As the US was delivered between 0 and 1.5 s following CS+ onset on paired trials, participants might have anticipated by the beginning of the analysis interval (i.e. at 2 s) on an unpaired trial that no US would be delivered on that trial anymore, thus possibly not displaying conditioning-related arousal during the interval of interest. In future studies, it might be more accurate to present the US a few seconds later during a CS+ trial (see O'Doherty et al., 2003), that is after the initial light reflex, so that the interval during which participants expect a US lasts longer and lies within a late interval for analysis.

Another potential confound with the pupil data analysis might be that the rather short inter-trial interval of 1 s affected the baseline correction of pupil diameter data. For the baseline correction, the mean diameter of 1 s preceding the onset of a trial was subtracted from all samples of that trial, following common procedures (see e.g. Bradley et al., 2008; Granholm, Asarnow, Sarkin, & Dykes, 1996; Partala & Surakka, 2003; Siegle, Steinhauer, & Thase, 2004). However, this baseline interval was likely to be affected by the pupil dilation from the preceding trial, which might have obscured the pupil diameter data unequally across trial types and consequently diminished the effects of arousal. In future studies, a longer inter-trial interval would thus be appropriate to ensure that the pupil diameter returns to baseline at least 1 s before onset of the next trial.

A reason for the lack of conditioning-related brain activation could be the design of the general linear model. We aimed to capture conditioning parametrically by taking into account an effect of time in terms of the number of preceding US occurrences in a simple linear fashion. This seemed appropriate as the order of US occurrences was fully randomized and thus very variable between participants and valences: While for one participant or valence the first occurrence of the US might be very early during the task, for another participant or valence the US might occur rather late so that conditioning with that US could only begin late. In contrast, other studies that found conditioning-related brain activation accounted for an effect of time in an exponential fashion by multiplying the main event regressors with an exponential function without accounting for the number of US occurrences (Büchel et al., 1998; Gottfried et al., 2002). As participants in the present study might have learned the simple associations between a CS+ and a US rather quickly with a slower learning rate towards the end, an exponential function similar to those applied in the studies by Büchel et al. (1998) and Gottfried et al. (2002) might have been more accurate than a linearly increasing function in our general linear model. Alternatively, we could have used the main event regressors and contrasts between those to model conditioning, as other studies successfully demonstrated (Delgado et al., 2011; Kirsch et al., 2003; Klucken et al., 2012). However, due to the randomization of trial types we decided that a parametric modulation would account for conditioning more accurately. Another way to model conditioning parametrically would be to use physiological indicators of arousal. For example, one study used skin conductance rate as a parametric modulation of the stimulus events (Carter, O'Doherty, Seymour, Koch, & Dolan, 2006). In the present study, we chose not to use the pupil diameter data for modelling conditioning because of the rather high rate of discarded trials and blocks of pupil diameter data.

Despite the lack of physiological and neural evidence for successful conditioning, it seems that conditioning worked in both participant groups, considering the behavioural evidence and the methodological limitations of the pupil and fMRI data analysis. The behavioural data suggest that appetitive and aversive conditioning worked in

opposite directions as expected (with an increase or decrease in pleasantness for the CS+, respectively), although this was also not reflected in the physiological or neural data.

5. Conclusion

Our main hypothesis to find differences in neural and autonomic responding during appetitive and aversive conditioning and stimulus processing between psychopathy patients and healthy controls was not confirmed. Within-group differences in aversive conditioning related to severity of psychopathy or psychopathic personality traits were found in the right OFC and the right insula, which both belong to the fronto-limbic circuit implicated to be dysfunctional in psychopathy. Considering the evidence for deficient affective processing in psychopathy from previous studies, the results from this study suggest that an affective deficit in psychopathy is not universally present and likely depends on various factors such as severity and subtypes of psychopathy or type of affective stimulation. This underlines that psychopathy is a highly complex personality disorder which will need further investigation, especially with respect to inter-individual differences, for a detailed understanding of the nature and extent of associated deficits.

As for the success of conditioning and underlying correlates, only the behavioural data demonstrated successful conditioning in the two participant samples while conditioning was not reflected on the neural or autonomic level, possibly due to stimulus- or analysis-related limitations. Differences between appetitive and aversive conditioning were restricted to the neural level where the left amygdala showed a stronger response to the appetitive than to the aversive taste stimulus in both participant samples, underlining the role of the amygdala in appetitive value processing.

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7. References

Anderson, N. E., & Kiehl, K. A. (2012). The psychopath magnetized: Insights from brain imaging. *Trends in Cognitive Sciences, 16*(1), 52–60.

Anderson, N. E., & Stanford, M. S. (2012). Demonstrating emotional processing differences in psychopathy using affective ERP modulation. *Psychophysiology, 49*(6), 792–806.

Barreto, A., Zhai, J., Rishe, N., & Gao, Y. (2007). Significance of pupil diameter measurements for the assessment of affective state in computer users. In K. Elleithy (Ed.), *Advances and innovations in systems, computing sciences and software engineering* (pp. 59-64). Dordrecht, The Netherlands: Springer.

Birbaumer, N., Veit, R., Lotze, M., Erb, M., Hermann, C., Grodd, W., & Flor, H. (2005). Deficient fear conditioning in psychopathy: A functional magnetic resonance imaging study. *Archives of General Psychiatry, 62*(7), 799–805.

Blair, R. J. R. (2001). Neurocognitive models of aggression, the antisocial personality disorders, and psychopathy. *Journal of Neurology, Neurosurgery, and Psychiatry, 71*(6), 727–731.

Blair, R. J. R., Mitchell, D. G. V., Leonard, A., Budhani, S., Peschardt, K. S., & Newman, C. (2004). Passive avoidance learning in individuals with psychopathy: Modulation by reward but not by punishment. *Personality and Individual Differences, 37*(6), 1179–1192.

Blair, R. J. R., Peschardt, K. S., Budhani, S., Mitchell, D. G. V., & Pine, D. S. (2006). The development of psychopathy. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 47*(3-4), 262–276.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology, 45*(4), 602–607.

Büchel, C., Dolan, R. J., Armony, J. L., & Friston, K. J. (1999). Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. *The Journal of Neuroscience, 19*(24), 10869–10876.

Büchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: An event-related fMRI study. *Neuron, 20*(5), 947–957.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience & Biobehavioral Reviews, 26*(3), 321–352.

Carter, R. M., O'Doherty, J. P., Seymour, B., Koch, C., & Dolan, R. J. (2006). Contingency awareness in human aversive conditioning involves the middle frontal gyrus. *NeuroImage, 29*(3), 1007–1012.

Casey, H., Rogers, R. D., Burns, T., & Yiend, J. (2013). Emotion regulation in psychopathy. *Biological Psychology, 92*(3), 541–548.

Delgado, M. R., Jou, R. L., & Phelps, E. A. (2011). Neural systems underlying aversive conditioning in humans with primary and secondary reinforcers. *Frontiers in Neuroscience, 5*(71). 1-10.

Dolan, M. C., & Fullam, R. S. (2009). Psychopathy and functional magnetic resonance imaging blood oxygenation level-dependent responses to emotional faces in violent patients with schizophrenia. *Biological*

Psychiatry, 66(6), 570–577.

First, M. B., Spitzer, R. L., Gibbon, M., Williams, G. B. W., & Benjamin, L. (1997). *Structured Clinical Interview for DSM-IV Axis II Personality Disorders* (SCID-II). Washington, DC: American Psychiatric Press.

Flor, H., Birbaumer, N., Hermann, C., Ziegler, S., & Patrick, C. J. (2002). Aversive Pavlovian conditioning in psychopaths: Peripheral and central correlates. *Psychophysiology, 39*(4), 505–518.

Gonsalves, V. M., McLawsen, J. E., Huss, M. T., & Scalora, M. J. (2013). Factor structure and construct validity of the psychopathic personality inventory in a forensic sample. *International Journal of Law and Psychiatry, 36*(2), 176–184.

Gottfried, J. A, O'Doherty, J., & Dolan, R. J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *The Journal of Neuroscience, 22*(24), 10829–10837.

Granholm, E., Asarnow, R. F., Sarkin, A. J., & Dykes, K. L. (1996). Pupillary responses index cognitive resource limitations. *Psychophysiology, 33*(4), 457–461.

Hare, R. D. (2003). *Hare Psychopathy Checklist-Revised (PCL-R)* (2nd ed.). Toronto, Canada: Multi-Health Systems Inc.

Hare, R. D., & Quinn, M. J. (1971). Psychopathy and autonomic conditioning. *Journal of Abnormal Psychology, 77*(3), 223–235.

Harenski, C. L., Kim, S. H., & Hamann, S. (2009). Neuroticism and psychopathy predict brain activation during moral and nonmoral emotion regulation. *Cognitive, Affective & Behavioral Neuroscience, 9*(1), 1–15.

Herpertz, S. C., & Sass, H. (2000). Emotional deficiency and psychopathy. *Behavioral Sciences and the Law, 18*(5), 567–580.

Herpertz, S. C., Werth, U., Lukas, G., Qunaibi, M., Schuerkens, A., Kunert, H. J., Freese, R., et al. (2001). Emotion in criminal offenders with psychopathy and borderline personality disorder. *Archives of General Psychiatry, 58*(8), 737–745.

Howard, R., & McCullagh, P. (2007). Neuroaffective processing in criminal psychopaths: Brain event-related potentials reveal task-specific anomalies. *Journal of Personality Disorders, 21*(3), 322–339.

Jelicic, M., Merckelbach, H., Timmermans, M., Candel, I. (2004). De Nederlandstalige versie van de Psychopathic Personality Inventory. Psychodiagnostisch gereedschap. *[The Dutch version of the Psychopathic Personality Inventory. Psychodiagnostic Instrument.] De Psycholoog, 39*(12), 604–608.

Kiehl, K. A., Hare, R. D., McDonald, J. J., & Brink, J. (1999). Semantic and affective processing in psychopaths: An event-related potential (ERP) study. *Psychophysiology, 36*(6), 765–774.

Kiehl, K. A., Smith, A. M., Hare, R. D., Mendrek, A., Forster, B. B., Brink, J., & Liddle, P. F. (2001). Limbic abnormalities in affective processing by criminal psychopaths as revealed by functional magnetic resonance imaging. *Biological Psychiatry, 50*(9), 677–684.

Kirsch, P., Schienle, A., Stark, R., Sammer, G., Blecker, C., Walter, B., Ott, U., et al. (2003). Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: An event-related fMRI study. *NeuroImage, 20*(2), 1086–1095.

Klucken, T., Schweckendiek, J., Koppe, G., Merz, C. J., Kagerer, S., Walter, B., Sammer, G., et al. (2012). Neural correlates of disgust- and fear-conditioned responses. *Neuroscience, 201*, 209–218.

Koenigs, M., Baskin-Sommers, A., Zeier, J., & Newman, J. P. (2011). Investigating the neural correlates of psychopathy: A critical review. *Molecular Psychiatry, 16*(8), 792–799.

Levenston, G. K., Patrick, C. J., Bradley, M. M., & Lang, P. J. (2000). The psychopath as observer: Emotion and attention in picture processing. Journal of Abnormal *Psychology, 109*(3), 373–385.

Lilienfeld, S. O., & Andrews, B. P. (1996). Development and preliminary validation of a self-report measure of psychopathic personality traits in noncriminal populations. *Journal of Personality Assessment, 66*(3), 488–524.

Meseguer, V., Romero, M. J., Barros-Loscertales, A., Belloch, V., Bosch-Morell, F., Romero, J., & Avila, C. (2007). Mapping the appetitive and aversive systems with emotional pictures using a block-design fMRI procedure. *Psicothema, 19*(3), 483–488.

Mitchell, D. G. V. (2011). The nexus between decision making and emotion regulation: A review of convergent neurocognitive substrates. *Behavioural Brain Research, 217*(1), 215–231.

Mueller, J. L., Sommer, M., Wagner, V., Lange, K., Taschler, H., Roeder, C. H., Schuierer, G., et al. (2003). Abnormalities in emotion processing within cortical and subcortical regions in criminal psychopaths: Evidence from a functional magnetic resonance imaging study using pictures with emotional content. *Biological Psychiatry, 54*(2), 152–162.

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends in Cognitive Sciences, 11*(11), 489–497.

Newman, J. P., & Kosson, D. S. (1986). Passive avoidance learning in psychopathic and nonpsychopathic offenders. *Journal of Abnormal Psychology, 95*(3), 252–256.

O'Doherty, J. P., Dayan, P., Friston, K., Critchley, H., & Dolan, R. J. (2003). Temporal difference models and reward-related learning in the human brain. *Neuron, 38*(2), 329–337.

O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., & McGlone, F. (2001). Representation of pleasant and aversive taste in the human brain. *Journal of Neurophysiology, 85*(3), 1315–1321.

Padoa-Schioppa, C., & Cai, X. (2011). The orbitofrontal cortex and the computation of subjective value: Consolidated concepts and new perspectives. *Annals of the New York Academy of Sciences, 1239*, 130–137.

Partala, T., & Surakka, V. (2003). Pupil size variation as an indication of affective processing. International *Journal of Human-Computer Studies, 59*(1-2), 185–198.

Patrick, C. J., Bradley, M. M., & Lang, P. J. (1993). Emotion in the criminal psychopath: Startle reflex modulation. *Journal of Abnormal Psychology, 102*(1), 82–92.

Poser, B. A., Versluis, M. J., Hoogduin, J. M., & Norris, D. G. (2006). BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: Parallel-acquired inhomogeneity-desensitized fMRI. *Magnetic resonance in medicine, 55*(6), 1227–1235.

Rothemund, Y., Ziegler, S., Hermann, C., Gruesser, S. M., Foell, J., Patrick, C. J., & Flor, H. (2012). Fear conditioning in psychopaths: Event-related potentials and peripheral measures. *Biological Psychology, 90*(1), 50–59.

Sadeh, N., & Verona, E. (2012). Visual complexity attenuates emotional processing in psychopathy: Implications for fear-potentiated startle deficits. *Cognitive, Affective & Behavioral Neuroscience, 12*(2), 346–360.

Schmand, B. A., Bakker, D., Saan, R. J. & Louman, J. (1991). De Nederlandse Leestest voor Volwassenen: Een maat voor het premorbide intelligentieniveau [The Dutch Adult Reading Test: A measure of premorbid intelligence]. *Tijdschrift voor Gerontologie en Geriatrie, 22*(1), 15–19.

Shamay-Tsoory, S. G., Harari, H., Aharon-Peretz, J., & Levkovitz, Y. (2010). The role of the orbitofrontal cortex in affective theory of mind deficits in criminal offenders with psychopathic tendencies. *Cortex, 46*(5), 668–677.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Thierry, H., et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry, 59*(20), 22–33.

Siegle, G. J., Steinhauer, S. R., & Thase, M. E. (2004). Pupillary assessment and computational modeling of the Stroop task in depression. *International Journal of Psychophysiology, 52*(1), 63–76.

Sutton, S. K., Vitale, J. E., & Newman, J. P. (2002). Emotion among women with psychopathy during picture perception. *Journal of Abnormal Psychology, 111*(4), 610–619.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage, 15*(1), 273–289.

Van Vliet, I. M., & de Beurs, E. (2007). Het Mini Internationaal Neuropsychiatrisch Interview (M.I.N.I.). Een kort gestructureerd diagnostisch psychiatrisch interview voor DSM-IV- en ICD-10-stoornissen. [The Mini International Neuropsychiatric Interview (M.I.N.I.). A short structured diagnostic psychiatric interview for DSM-IV and ICD-10 disorders.] *Tijdschrift Voor Psychiatrie, 49*(6), 393–397.

Veit, R., Flor, H., Erb, M., Hermann, C., Lotze, M., Grodd, W., & Birbaumer, N. (2002). Brain circuits involved in emotional learning in antisocial behavior and social phobia in humans. *Neuroscience letters, 328*(3), 233–236.

Verona, E., Sprague, J., & Sadeh, N. (2012). Inhibitory control and negative emotional processing in psychopathy and antisocial personality disorder. *Journal of Abnormal Psychology, 121*(2), 498–510.

Weertman, A., Arntz, A., & Kerkhofs, M. (2000). *Handleiding Gestructureerd Klinisch Interview voor DSM-IV As-II Persoonlijkheidsstoornissen*. Lisse, The Netherlands: Swets test publishers.

Williamson, S., Harpur, T. J., & Hare, R. D. (1991). Abnormal processing of affective words by psychopaths. *Psychophysiology, 28*(3), 260–273.

Yang, Y., & Raine, A. (2009). Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: A meta-analysis. *Psychiatry Research, 174*(2), 81–88.

Do Mental Schemas Play a Role in Negative Memory Bias? The Effect of High Risk for Depression on Memory

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Not everything is remembered equally well. Emotionally arousing information is generally better remembered than non-arousing information. Also, the presence of prior knowledge, or 'schema', facilitates memory for new information that is congruent with such schema. It has recently been shown for emotionally neutral information that this schema-related memory facilitation is mediated by the medial prefrontal cortex (mPFC) and is less dependent on the hippocampus and medial temporal lobe (MTL). Individuals at risk for or with a depressive disorder tend to remember negative information better than neutral or positive information. Using event-related functional magnetic resonance imaging (fMRI), we investigated whether this negative memory bias in people at risk for depression, defined by a high neuroticism (N) score, can be explained by a schema for sad information, defined by increased mPFC-based mnemonic processing rather than enhanced MTL-based encoding. Brain activity was measured in low-N and high-N individuals during incidental encoding of happy, sad, and negative-arousing pictures. Cued recall was assessed the next day outside the scanner to model brain activity during successful encoding. We hypothesized better memory for negative-arousing pictures independent of group and for sad pictures in the high-N group relative to the low-N group. As predicted, all subjects showed better memory for negative-arousing pictures. We found no differences between the groups in memory performance in any of the conditions, or in hippocampal or mPFC activity during successful encoding of happy or sad pictures. For subsequently remembered negative-arousing pictures, we found that low-N subjects showed more overall activity than the high-N subjects in the medial temporal lobe, the amygdala and mPFC. This study provides a fruitful starting point to investigate the potential role of mental schemas on memory biases in depressed patients.

Keywords: negative memory bias, memory, cued recall paradigm, subsequent memory effect, mental schema, medial prefrontal cortex, hippocampus, medial temporal lobe, neuroticism, depression

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1. Introduction

Whether or not something will be remembered can depend on a number of factors. On the one hand, some type of information is more likely to be remembered; on the other hand, some information is more likely to be remembered by certain types of individuals. Regarding the former, memory for information that is emotionally arousing is known to be increased compared to non-arousing neutral information (Labar & Cabeza, 2006). The amygdala modulates memory-enhancing effects of arousal by influencing the medial temporal lobe, the prime memory formation system (Eichenbaum, 2004). Also, information that is congruent with prior knowledge and attitudes, or 'schema', is remembered better than information that is incongruent with such schema. This schema-related memory facilitation is thought to be mediated by interactions between the medial prefrontal cortex (mPFC) and medial temporal lobe (MTL) (Tse et al., 2007; Van Kesteren, Ruiter, Fernández, & Henson, 2012). More specifically, Van Kesteren, Fernández, Norris and Hermans (2010) showed that schema-related encoding was associated with increased mPFC- and decreased hippocampal activity and less hippocampal-neocortical connectivity.

Memory is also highly variable between individuals. People at risk for or with a depressive disorder tend to remember negative information better than neutral or positive information. This negative memory bias is one of the most robust findings in the depression literature (Mathews & MacLeod, 2005). Depression-related memory biases have been shown for words (Matt, Vazquez, & Campbell, 1992; Gotlib et al., 2004), autobiographical memories (Williams et al., 2007; Denkova, Dolcos, & Dolcos, 2012), facial expressions (Gilboa-Schechtman et al., 2002) and self-referential recall (Williams, Watts, MacLeod, & Mathews, 1997). According to the cognitive model of depression (Beck, 1967; Beck, 2008), negative schemas cause biases in perception, attention, and memory (review by Disner, Beevers, Haigh, & Beck, 2011). The predictions of this cognitive model fit well with the neuroscientific findings of schema-facilitated memory, but have to date not been studied together.

In this study we therefore investigated whether this negative memory bias in people at risk for depression can be explained by a schema for negative, low-arousing (sad) information, which would be supported by increased mPFC activation rather than MTL-based encoding. Neuroticism has often been associated with depression and robust evidence suggests that neuroticism (N) is a key predictor for depression (Kendler, Gardner, & Prescott, 2002; Kendler, Kuhn, & Prescott, 2006; Bienvenu et al., 2004). In line with previous studies (Chan, Harmer, Goodwin, & Norbury, 2008; Kuyken & Dalgleish, 2011), risk for depression was defined as high N. Using event-related functional magnetic resonance imaging (fMRI), brain activity was measured in subjects with a high N-score and subjects with a low N-score during the incidental encoding of happy, negative low arousing (sad), and negative-arousing pictures. The next day, cued recall (Dolcos, LaBar, & Cabeza, 2004) was assessed outside the scanner. Recall performance was used to model brain activity during encoding of subsequently remembered and subsequently forgotten pictures for the three conditions. This allowed us to study the predicted memory bias for sad information in the high-N group relative to the low-N group. Crucially, if this enhanced memory is caused by a schema for sad information, we expect more mPFC activity and less MTL activity during successful encoding of sad pictures in the high-N group.

2. Materials & Methods

2.1 Subjects

Over 300 individuals were recruited to fill out the Dutch version of the Neuroticism-Extroversion-Openness Five Factor Inventory [NEO-FFI (Costa & McCrae, 1992; Hoekstra, Ormel, & De Fruyt, 1996)] to assess personality traits. The selection criterion for inclusion in the study was to obtain low neuroticism (N) scores (range 1 – 3) or high-N scores (range 7 - 9), as defined by the stanine rating scale of the NEO-FFI. Among the subjects recruited, forty-three right-handed, never-depressed volunteers were invited to participate in the study. Twenty-two were in the low-N group (M = 2.4, SD = 0.9)) and twenty-one in the high-N group (M = 7.6, SD = 0.8). Individuals who met any of the following criteria were excluded: use of psychotropic medication, any contraindications for MRI, alcohol or drug dependence, or a history of psychiatric or neurological diagnosis. All participants gave informed consent to participate in the study according to the guidelines of the local ethics committee and received a monetary reward for their participation. Two subjects were excluded from analysis for pressing the alarm button while in the scanner and thus not finishing the task. One subject was excluded because

Table 1. Demographic characteristics.

	Low N		High N	
	Mean	Standard deviation	Mean	Standard deviation
Male / female	10 / 9		6 / 10	
Age [a] *	23.9	3.1	21.8	2.0
Sleep (h)	6.8	1.0	6.8	0.9
Neuroticism-score [b] ***	2.4	0.9	7.6	0.8
Beck depression inventory-score [c] **	3.3	2.8	10.4	9.2

Notes. [a] $F(1, 33) = 3.68$, $p = .021$ [b] $F(1, 33) = .59$, $p < .001$ [c] $F(1, 33) = 4.71$, $p = .003$.
* $p < .05$, ** $p < .01$, *** $p < .001$

of a technical failure and one because of excessive head motion. Four other subjects were excluded because their memory performance did not yield a minimum of 7 trials for any of the conditions included in the fMRI data analyses. All analyses were performed on the remaining 35 participants; see Table 1 for demographic characteristics.

2.2 Stimulus Materials

One stimulus set was created for the encoding task consisting of three conditions – happy, sad and negative arousing – with each condition containing 55 pictures, supplemented with 35 null events (fixation cross). Pictures were selected from both a standard set of affective pictures [IAPS (Lang, Bradley, & Cuthbert, 1999)] and an additional set of newly rated pictures. Additional pictures were downloaded from the internet and selected on the authors' assessment. A total of 240 additional pictures were initially rated on a scale from 1 to 9 on both emotional valence and emotional arousal using the Self-Assessment Manikin (SAM) scales (Bradley & Lang, 1994) by an additional group of 21 volunteers (11 males, M = 23.9 years, SD = 9.5 years). All pictures that ended up in the final set were selected for one condition (happy, sad, negative arousing) based on their individual valence and arousal scores (see Fig. 1). All selected happy pictures were selected for their relatively high valence (M = 6,4, SD = 0,5) and low-to-moderate arousal scores (M = 2.9, SD = 0.4) Sad pictures were selected for their low-to-moderate valence (M = 3.4, SD = 0.3) and corresponding arousal scores (M = 3.5, SD = 0.6). Negative arousing pictures were selected for their low valence (M = 2.6, SD = 0.4) and high arousal scores (M = 5.5, SD = 0.5). Pictures from the three conditions were matched on content (number of faces, number of people, number of animals, scenery, in- or outdoor) and overall visual complexity.

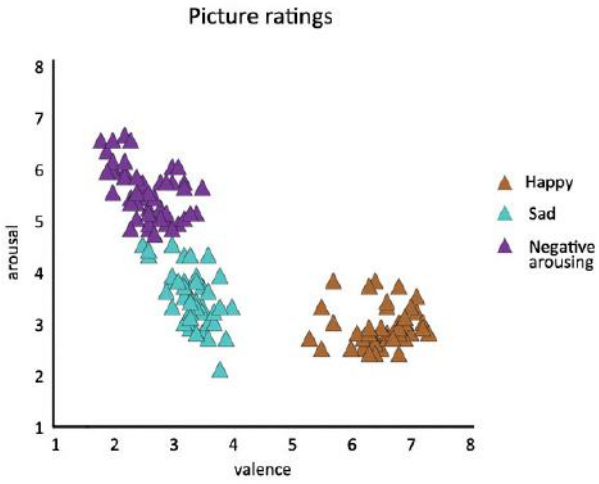

Fig. 1 Picture ratings. To create a selection of happy, sad and negative arousing stimuli, pictures were rated on valence and arousal by 21 subjects independent of the main experiment. Based on these scores, 55 pictures were selected for each condition. Mean arousal and valence scores are plotted for each selected picture.

2.3 Study design

The experiment consisted of one encoding session in the scanner on the first day and a cued recall task on the next day (see Fig. 2). On day one, participants viewed 165 pictures during incidental encoding inside the scanner. Brain activity was assessed using event-related functional magnetic resonance imaging (fMRI). The start of an encoding session was always between 17.00 h and 20.00 h to keep the interval between encoding and recall similar across subjects and to control for time-of-day effects of cortisol. Subjects returned the next day, on average 16 hours later, to perform a surprise cued recall task outside the scanner (Dolcos et al., 2004). This allowed us to investigate brain activation during memory formation as a function of subsequent memory (i.e. subsequent memory effect (SME, also known as Dm effect); greater activation during subsequently remembered trials than subsequently forgotten trials (Wagner et al., 1998)). Before subjects engaged in the cued recall task, the following

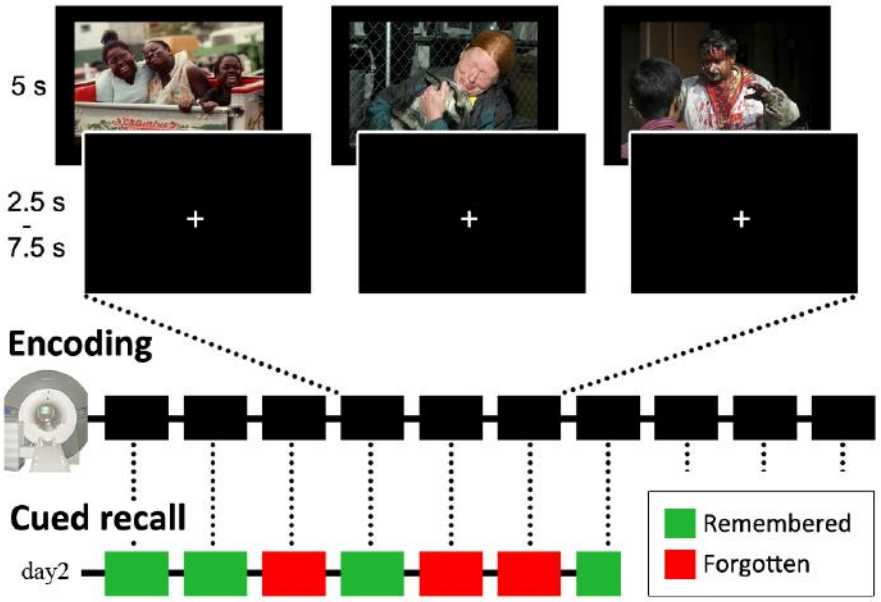

Fig. 2 Task design. Happy, sad and negative arousing pictures were encoded during fMRI scanning on the first day. Memory was tested outside the scanner the next day in a cued recall test to model brain activity during subsequently remembered and subsequently forgotten pictures.

questionnaires were completed: Positive and Negative Affect Schedule [PANAS (Watson, Clark, & Tellegen, 1988)], Beck Depression Inventory [BDI-II (Beck, Steer & Brown, 1996)], State-Trait Anxiety Inventory [STAI (Spielberger, Gorsuch, & Lushene, 1970)] and Ruminative Response Scale [RRS-NL (Nolen-Hoeksema, Morrow, & Fredrickson, 1993; Raes, Hermans, & Eelen, 2003)]. To validate that the valence and arousal scores did not differ both from the initial rating session and between the two groups (low and high N), subjects rated the set of pictures on valence and arousal after the recall task.

2.4 Procedure

Arrival. On the first day, upon arrival, subjects were asked to fill out the PANAS questionnaire to assess subjective well-being. Before going into the scanner, subjects were explicitly instructed about the task. Participants were instructed to view each picture carefully and experience any feelings the picture might elicit. Within the 5-second presentation period of the picture, subjects had to make a judgment of their emotional feeling about the picture using a 4-point scale (ranging from 1 = very sad / shocking to 4 = very happy). In order to prevent the use of encoding strategies and to enhance the effect of emotion on memory, nothing was mentioned about the subsequent memory test before or during the encoding task (incidental learning).

Scanning. Participants lay supine in the MR scanner and viewed the screen through a mirror positioned on the head coil. Respiration and heart rate were recorded throughout scanning using, respectively, a respiration belt and a 50 Hz finger pulse photoplethysmograph affixed to the left index finger. Participants were instructed to lie in the scanner as still as possible. During the encoding task, pictures were presented and subjects rated their feeling about the picture on a 4-point scale ranging from very sad/shocked to very happy. Ratings were given with right-hand button presses using the index-, middle-, ring- and pinky finger. The order of the scale and buttons was counterbalanced over subjects and groups. Pictures belonged to one of three conditions: happy, sad or negative arousing. Each picture was presented in the middle of the screen for 5 seconds, followed by a jittered intertrial interval (ITI) lasting 2.5 - 7.5 seconds during which a white fixation-cross on a black background was shown. All of the 165 pictures were presented in pseudorandom order (no more than 3 pictures of the same condition consecutively) and this order was different for every subject. In addition, 35 null-events were randomly intermixed between trials (centred fixation-cross during 5 seconds). To make sure subjects understood the task correctly, three example trials were performed inside the scanner prior to the start of data acquisition with stimuli that were not included in the cued recall the next day. After completion of the task a structural scan was performed.

Subsequent memory test. Participants came back the next morning to perform a surprise cued

recall test conducted outside the scanner. One- to four-word written cues were provided for each picture (165 cues in total) describing the gist or most salient feature of the scene present in the picture. Participants were instructed to use the cues to recall the pictures they had seen the day before and write down as many details and characteristics of all pictures as they could remember, providing enough relevant specifics (e.g., about the number of elements, colour, angle, etc.) so that an outsider could identify each picture and discriminate it from similar studied pictures (Dolcos et al., 2004). The test lasted 90 minutes or until participants could not recall any additional pictures, with a minimum of 75 minutes. A short introduction with some suggestions was provided to help the participants in listing characteristics (similar to Henckens, Hermans, Pu, Joëls, & Fernández, 2009). The written descriptions were assessed by two raters who were both blind to which N group the participant belonged. Pictures were only classified as remembered if a description allowed for both identification and discrimination of the picture. Pictures with no clear recollection of characteristics were considered forgotten. If a description could not clearly be linked to a particular picture, the picture was scored as unclear and included in the analyses as a separate regressor. Inter-rater correspondence was high (91.6%). Pictures with a non-corresponding rating were individually discussed by the two raters until agreed upon. At the end of the session participants were asked to indicate whether the memory test came as a surprise. Although six subjects answered 'no', these subjects also indicated they had not used any strategies, so none of the participants were excluded based on this criterion. Four subjects were excluded from analysis because they remembered 6 or less pictures in at least one of the conditions.

Valence and arousal rating task. After finishing the cued recall task, subjects were asked to rate the same pictures they had seen in the scanner on valence and arousal. This rating task was similar to the procedure described in the Stimulus materials section and took about 30 minutes.

2.5 Behavioural analyses

Cued Recall. A factorial 2 X 3 analyses of variance (ANOVA) (group X memory performance per condition) was conducted to reveal differences in memory scores between group and conditions. In case this revealed significant effects, additional post hoc t tests were performed.

Rating task. Two repeated-measures analyses of variance were performed with condition (happy, sad and negative arousing) as within-subject factor and group (low-N and high-N group) as between-subject factor to reveal differences in valence and arousal scores. To validate the reliability of the scores, the same ANOVAs were conducted between the initial ratings (that were assessed prior to the experiment with an independent group of subjects) and the ratings of all subjects included in the study.

2.6 Functional Magnetic Resonance Imaging

Data acquisition. Participants were scanned using a 1.5 Tesla Siemens Magnetom Avanto system equipped with a 32 channel phased-array head-coil (Siemens AG, Erlangen, Germany). T2*-weighted images were acquired covering the whole brain with ascending slice acquisition using a multi-echo planar imaging (EPI) sequence (37 axial slices; repetition time (TR) = 2320 ms; echo time (TE)1=9.4 ms, TE2=20.9 ms, TE3=33 ms, TE4=44 ms, TE5=56 ms; 3mm slice thickness; 0.49 mm slice gap, matrix size=64 X 64, field of view (FOV) = 224 X 224 X 130 mm; 90° flip angle; voxel size = 3.5 x 3.5 x 3.0 mm). Additionally, T1-weighted anatomical scans at 1 mm isotropic resolution were acquired using a MPRAGE scan with TR = 2250 ms, inversion time (TI) = 850 ms, flip angle = 15 degrees and FOV = 265 X 265 X 176 mm.

Data preprocessing. Image preprocessing and statistical analysis were done using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). To allow T1 saturation to reach equilibrium, the first six volumes were discarded. Raw multi-echo fMRI data were first processed using in-house software written in Matlab 7.9 (The Mathworks, Inc., Natick, MA, USA), which used the first 32 scans to calculate the optimal weighting of echo times for each voxel (i.e. by using a weighted measure of the contrast-to-noise ratio for each echo/scan). After realignment of the functional images, the structural scan was co-registered to the mean functional scan and normalization parameters were estimated. These EPI images were subsequently slice-time corrected to the 19th slice. Both structural and functional images were then registered to a Montreal Neurological Institute (MNI) T1-standard brain, as well as spatially smoothed by convolving them with an 8 mm full width at half maximum 3D kernel. Data from each subject was manually checked for excessive head movement (no movements bigger than 3 mm) and signal-to-noise ratio.

Data analysis. The preprocessed functional images were analyzed using a general linear model

that was individually specified for each participant. Six explanatory regressors were included in the model: for each condition (happy, sad and negative arousing) a separate regressor was created for remembered and forgotten trials based on individual performance on the cued recall task. Unclear trials were separately modelled as a regressor of no interest. For each trial a 5 s block was modelled, corresponding with the presentation time of each picture. All regressors were convolved with the canonical hemodynamic response function of SPM8. Additionally, six realignment parameters were included in the design matrix to account for translation and rotation variability. A high-pass filter was applied to the data using a cut-off period of 128 seconds to remove low frequency drifts from the signal. Subsequently, first-level contrast images were created for relevant contrasts at the single-subject level, including the SME (subsequently remembered > subsequently forgotten pictures) for each condition separately (Dolcos et al., 2004).

A whole-brain analysis was performed to investigate differences in SME between the groups for the different conditions. A factorial 3 X 2 ANOVA was used with condition [happy(remembered>forgotten), sad(remembered>forgotten) and negative arousing(remembered>forgotten)] as within-subject factor and group as between-subject factor. Whole-brain activation maps were tested for significance using cluster-inference with a cluster-defining threshold of $p < .001$ and a cluster-probability of $p < .05$ FWE-corrected critical cluster-extend of 40 voxels.

Next, region of interest (ROI) analysis were performed to test for group differences in Blood Oxygen Level Dependent (BOLD) activity in response to the different conditions in the main regions of interest: the MTL, amygdala and mPFC. The MTL (comprising hippocampus and parahippocampus) and amygdala were anatomically defined in left and right hemispheres separately based on the Anatomic Automatic Labeling atlas (Tzourio-Mazoyer et al., 2002) using the MarsBar Toolbox implemented in SPM8 (http://marsbar.sourceforge.net/). The mPFC was defined on the basis of previous studies that showed schema-learning related activations (van Kesteren, Fernández et al., 2010) and was marked by a 10 mm sphere centred on the peak voxel (MNI [2, 46, 0]). For each subject, the mean regression coefficient (β-value) over all voxels per ROI were calculated for each contrast and imported in SPSS 19.0. These subject-wise values were then submitted to factorial 2 X 5 X 3 ANOVA (group X ROI X condition) to test for group differences in activation within the five ROIs (left- and right-MTL, left- and right-amygdala and mPFC) during the viewing of subsequently remembered vs subsequently forgotten pictures for each condition (happy, sad and negative arousing). Subsequently, in case the factorial ANOVA revealed significant effect, post hoc t tests were performed. For all analyses involving the ROIs, the significance threshold was set at $p < .05$, uncorrected.

3. Results

3.1 Behavioural results

Memory performance. Overall, memory performance did not differ between the low and high neuroticism subjects [no main effect of group; $F(1,33) = 39.16$, $p = .54$], suggesting that both groups performed equally well. Memory performance differed significantly per condition [main effect of condition; $F(2, 66) = 28.75$, $p < .0005$], but this pattern did not differ between the two groups [no group by condition interaction; $F(2,66) = .73$, $p = .49$] (Fig. 3).

Valence and arousal ratings. Valence and arousal ratings of the happy, sad and negative-arousing pictures did not differ significantly between the initial rating pilot study and the ratings of the included subjects [no main effect group for valence; $F(1,54) = 2.50$, $p = .120$, or arousal; $F(1,54) = 1,31$ $p = .257$]. For the low neuroticism group, valence ratings for happy ($M = 6.8$, $SD = 1.0$), sad ($M = 3.5$, $SD = 0.8$) and negative arousing pictures ($M = 2.8$, $SD = 1.0$) and arousal ratings for happy ($M = 2.4$, $SD = 1.3$), sad ($M = 2.4$, $SD = 1.2$) and negative arousing pictures ($M = 4.7$, $SD = 1.7$) also did not differ significantly from the high neuroticism group valence ratings for happy ($M = 6.6$, $SD = 0.8$), sad

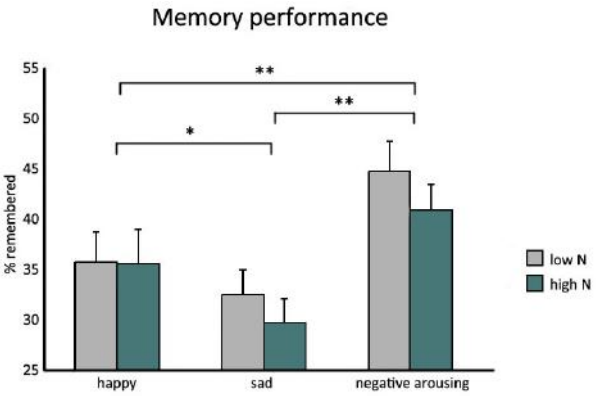

Fig. 3 Memory performance. Mean percentage remembered pictures for each group per condition. N: neuroticism. Error bars represent standard error of the mean. * $p < .01$, ** $p < .001$.

(M = 3.3, SD = 0.5) and negative arousing pictures (M = 2.6, SD = 0.6) and arousal ratings for happy (M = 2.4, SD = 0.6), sad (M = 3.3, SD = 1.2) and negative arousing pictures (M = 4.9, SD = 1.7) [no main group effect for valence; $F(1,33) = 2.17$, $p = .151$, or arousal; $F(1,33) = .11$, $p = .740$].

3.2 Imaging results

Whole brain results. To reveal those brain areas that showed increased activity for successful memory, we first contrasted brain activity of later remembered with later forgotten pictures (SME) over all subjects (main effect of memory). This contrast revealed a distributed set of brain regions including the inferior temporal gyrus, the fusiform gyrus extending in to the parahippocampal region, the middle occipital gyrus, the superior parietal gyrus and medial prefrontal cortex (see Fig. 4 & Table 2). The factorial ANOVA revealed no main effects of group, condition or interaction effects.

ROI results. To test the main hypothesis, MTL, amygdala and mPFC were defined as regions of interest. The factorial ANOVA revealed no main effects for ROI, condition and group. However, a condition by group interaction was found to be significant over all ROIs [$F(2,32) = 3.37$, $p = .047$]. This condition by group interaction was not found to be specific to one ROI [no group by ROI interaction; $F(4,30) = 1.88$, $p = .140$] or condition [no main effect of condition; $F(2,32) = 2.01$, $p = .151$]. Additionally, to test if this effect was driven by the subsequently remembered happy, sad or negative arousing pictures, a factorial 5 X 2 ANOVA was performed for each condition with ROI (L-MTL, R-MTL, L-Amygdala, R-Amygdala and mPFC) as within-subject and group (low and high-N) as between-subject factors. In the negative-arousing condition, increased activation was observed in the low-N group compared to the high-N group during successful encoding [main effect of group; $F(1,33) = 4.58$, $p = .04$] (see Fig. 5), but this effect was not restricted to specific ROIs [no group by ROI interaction; $F(2.589, 85.41) = .96$, $p = .406$)]. No significant main effect of group was found for the happy condition [$F(1,33) = .15$, $p = .701$] or sad condition [$F(1,33) = .45$, $p = .507$].

To specifically test the presence of a schema effect for sad subsequently remembered pictures in the high-N group, two additional t-tests were performed. First, we tested whether there was differential activity for successfully remembered sad pictures between the two groups in the left MTL, right MTL and mPFC. The groups did not differ significantly in any of the ROIs, suggesting that encoding of sad information was not facilitated by a schema in the high-N group. Paired sampled t tests were performed within the high-N group to investigate differences in activation between successful encoding of happy and sad pictures. This contrast also did not yield any significant differences in of the ROIs.

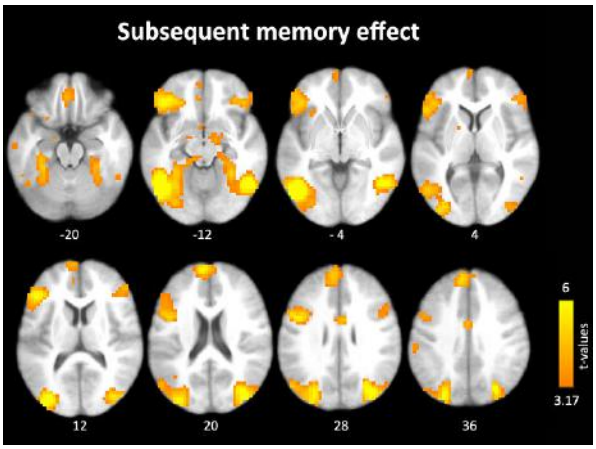

Fig. 4 Main effect of subsequent memory (remembered > forgotten). Areas showing significantly increased activity during subsequently remembered pictures versus subsequently forgotten pictures. Activation is overlaid on a mean brain-extracted anatomical image, left = left. Cluster-defining threshold of $p < .001$ and a $p = .05$ family-wise error-corrected critical cluster extend of 40 voxels.

4. Discussion

The present study investigated whether individuals with a high risk for depression show a memory bias for negative information and whether this bias can be explained by a negative emotional schema, which would be supported by increased mPFC activation rather than MTL-based encoding. Surprisingly, our results do not indicate a negative memory bias in the high neuroticism group, nor the expected enhanced activation within the mPFC and reduced activity within the medial temporal lobe during the encoding of sad information.

Consistent with the literature, we found enhanced memory for negative arousing pictures compared to sad and happy pictures (see Fig. 3) over all subjects (Bradley, Greenwald, Petry, & Lang, 1992; Kensinger & Corkin, 2004; Labar & Cabeza, 2006). Moreover, happy pictures were better remembered than sad pictures. This effect might be due to the fact that two-third of the pictures was of negative valence (sad, negative arousing) whereas only one third was positive, making them more distinct. In

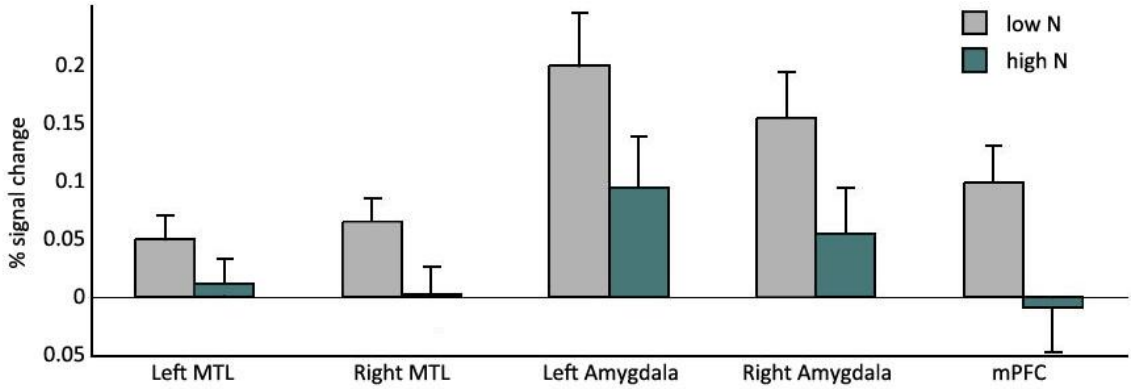

Fig. 5 Activity within the regions of interest. Mean percentage of signal change (b-values) per region of interest during subsequently remembered versus subsequently forgotten negative arousing pictures. Overall, activity was greater in subjects with low neuroticism scores relative to subjects with high neuroticism scores [main effect of group; $F(1, 33) = 4.58$, $p = .04$]. No post hoc t tests were performed because no significant main or interaction effects of region of interest were observed. Error bars represent standard error of the mean. MTL: medial temporal lobe, mPFC: medial prefrontal cortex, N: neuroticism.

line with previous studies, subsequent memory was mediated by regions including the parahippocampus, the fusiform gyrus, the inferior temporal gyrus, the middle occipital gyrus, the superior parietal gyrus and medial prefrontal cortex (see Fig. 4 & Table 2). Similar activations have often been reported in paradigms testing subsequent memory effects (Wagner et al., 1998; Dolcos, LaBar, & Cabeza, 2005; Henckens et al., 2009) and are generally thought to reflect memory formation (Fernández & Tendolkar, 2001). These findings indicate that the subsequent memory paradigm in general did work.

Surprisingly, we did not find a difference in memory performance between the two groups in any of the three conditions. Based on previous research (Lishman, 1972; Mayo, 1983; Bradley & Mogg, 1994; Chan, Goodwin, & Harmer, 2007; Denkova et al., 2012), we did expect to find increased memory for the sad pictures in the high-N group. Furthermore, our results do not show the presence of a schema in terms of neural activity for sad information both within the high-N group and between the two groups. For emotionally neutral information, previous studies have shown that facilitated encoding of schema-congruent stimuli is mediated by increased mPFC and decreased hippocampal activations (van Kesteren, Fernández et al., 2010; Tse et al., 2011). However, in this study we did not find increased mPFC and decreased hippocampal activations indicating the presence of a schema for remembered sad pictures in the high-N group compared to the low-N group, or within the high-N group for sad compared to happy pictures.

Overall, compared with the high-N group, the low-N group showed more activity during successful encoding of negative arousing pictures within the MTL, amygdala and mPFC (Fig. 5), suggesting differences in the way the negative arousing pictures were encoded between the two groups. Prior work has reported significant correlations between neuroticism and increased activity in medial temporal, caudal cingulate and prefrontal areas using affective (Canli, Zhao, & Desmond, 2001), cognitive (Eisenberger, Lieberman & Satpute, 2005) and fear learning (Hooker, Verosky, Miyakawa, Knight, & D'Esposito, 2008) paradigms. One explanation could be that the high-N subjects perceive being inside the scanner as more stressful or frightening, resulting in elevated amygdala activity already during baseline. Indeed, empirical evidence suggests that those with high neuroticism experience more intense emotional reactions to even minor stressors (Reynaud, El Khoury-Malhame, Rossier, Blin, & Khalfa 2012). Moreover, neuroticism is correlated with alterations in body arousal and the arousal-regulating circuitry, including the hippocampus, amygdala and PFC (Gatt et al., 2009). As a consequence, the relative activity increase for remembered arousing items in these regions might be diminished in the high-N group.

There are a few reasons that can potentially explain that the high-N group did not show the expected negative memory bias. First, it might be that the hypothesized schema in those at high risk for depression was not well activated. According to Beck's theory, depressed patients have dysfunctional attitudes or beliefs about themselves that cause the negative cognitive bias, which leads to the symptoms of depression (Beck, 1967; 1976; 2008). Moreover, studies that found negative memory biases in high-N subjects have mainly focused on self-referential processing and autobiographical memories (Ruiz-Caballero & Bermúdez, 1995; Dolcos et al., 2012). According to a recent theoretical framework for schema-driven learning termed SLIMM (schema-

Table 2. Main effect of subsequent memory. Clusters showing significant group differences in activation during the subsequently remembered pictures relative to subsequently forgotten pictures. MNI coordinates represent the location of the peak voxels. In bold are the peak voxels of each cluster followed by separate (>8 mm apart, max. 5) maxima within the cluster in plain text. Cluster-defining threshold of $p < .001$ and a $p = .05$ family-wise error–corrected critical cluster size of 40 voxels. L : left, R: right.

	Montreal Neurological Institute Coordinates				
Brain region	**X**	**Y**	**Z**	**Z score**	**Voxels**
Remembered > Forgotten					
L inferior temporal gyrus	**-46**	**-52**	**-14**	**7.55**	**1206**
L inferior temporal gyrus	-49	-63	-7	7.42	
L middle occipital gyrus	-38	-88	14	6.25	
L middle occipital gyrus	-28	-84	32	6.01	
L fusiform gyrus	-32	-52	-14	5.38	
R inferior temporal gyrus	**49**	**-56**	**-10**	**6.97**	**382**
R fusiform gyrus	32	-49	-14	5.35	
R parahippocampal gyrus	24	-32	-14	4.3	
R middle occipital gyrus	**32**	**-70**	**32**	**6.27**	**385**
R middle occipital gyrus	38	-77	24	6.05	
R superior parietal gyrus	28	-56	52	4.75	
L inferior frontal gyrus	**-46**	**32**	**14**	**5.84**	**589**
L inferior frontal gyrus	-46	7	24	5.69	
L lateral orbitofrontal gyrus	-38	35	-10	5.61	
L inferior frontal gyrus	-52	24	4	4.76	
L superior frontal gyrus	**-14**	**38**	**46**	**5.58**	**467**
Dorsal medial prefrontal cortex	-7	60	21	5.55	
Dorsal medial prefrontal cortex	-7	32	49	5.44	
Dorsal medial prefrontal cortex	-7	49	35	5.3	
Ventral medial prefrontal cortex	-4	35	-18	4.56	
R inferior frontal gyrus	**49**	**35**	**7**	**4.51**	**104**
R lateral orbitofrontal gyrus	46	32	-10	4.49	
R lateral orbitofrontal gyrus	35	35	-10	4.47	
R middle orbitofrontal gyrus	24	28	-18	3.5	
Cerebellum	**38**	**-70**	**-42**	**4.44**	**104**
Cerebellum	24	-74	-28	3.93	
Cerebellum	10	-77	-28	3.68	
Cerebellum	24	-77	-46	3.61	
L supramarginal gyrus	**-52**	**-32**	**46**	**4.02**	**55**
L superior parietal gyrus	-38	-35	42	3.56	

linked interactions between medial prefrontal and medial temporal regions; van Kesteren et al., 2012), only information that is related to the dominant (active) schema is effectively selected for fast, schema-related learning. Due to the self-referential nature of the schema, the stimuli might not have been congruent thus not eliciting facilitation. In line with Beck's idea of self-referential schemas, there might also be a different explanation for not finding differential activation of the mPFC during subsequently remembered and forgotten sad pictures. Neuroticism has been associated with an increased focus on internal states, self-rumination and introspection (Lam, Smith, Checkley, Rijsdijk, & Sham, 2003) and the mPFC is a key region for self-referential processing (Gusnard, Akbudak, Shulman, & Raichle, 2001). This concurs with findings that report correlations between neuroticism scores and mPFC activity during processing of emotional faces and stimuli (Britton, Ho, Taylor, & Liberzon,

2007; Haas, Constable, & Canli, 2008; Dolcos et al., 2012). High-N subjects might have had increased self-referential processing and mPFC activations resulting in a ceiling effect, thereby diluting specific differences in mPFC activity related to a schema.

Although neuroticism has been shown to be strongly correlated with depressive symptoms (r = .71) (Jylhä & Isometsä, 2006) and to be highly predictive of depression onset later in life, it is merely a risk factor. The relatively low prevalence rates of depression may imply that only a small proportion of the subjects included in the high-N group will go on to develop depression. In those who will not develop depression, the negative schema might still be weak or even not (yet) present. Therefore they might have a weaker bias towards the negative pictures and will only add noise to the potential negative schema effects. This is also highlighted by the fact that even though many studies in the field of cognitive neuroscience have tried to link neuroticism scores to differences in brain activation patterns, so far few findings have been replicated across studies and activation tasks (Britton et al., 2007). A longitudinal study might help to reveal differences between those subjects that end up having a depressive episode and those that do not. Another option would be to investigate differences in memory scores and according brain activity of individuals suffering a depressive episode and healthy controls as the hypothesized negative schema might be more prominently present. In sum, future work should test memory for self-relevant information (autobiographical memories) and depressed individuals.

Several limitations of this study should be noted. First, the number of subjects included is relatively low. Indeed, we will test more participants for both groups in the near future and reanalyze the data subsequently. And although we did not find activation differences, additional analysis could test differences in connectivity, which have previously been reported during encoding of schema-congruent information (van Kesteren, Fernández et al., 2010). Empirical evidence suggests that encoding of schema congruent information is associated with less mPFC-hippocampal connectivity (van Kesteren, Fernández et al., 2010). Moreover, subjects were scanned during encoding. The highly emotional visual stimuli cause neural activations that are memory-irrelevant in the regions of our interest. A retrieval task, during which similar activations have been found for schema-congruent information (van Kesteren , Rijpkema et al., 2010), would bypass these effects and interfere less with potential differences in activity. Another limitation in this study is that we included both males and females, casting heterogeneity in the sample. There is, however, no consensus in the literature as negative memory biases have been reported in either sex exclusively. A recent study found a bias for negatively affected autobiographical events to be only present in males, not women (Denkova et al., 2012). On the other hand, Canli et al. (2001) show a correlation between prefrontal activity and N score in women only and Kendler, Kuhn, & Prescot (2004) reported the impact of neuroticism on risk for depression to be greater in females than in males. Also, a lack of an emotionally neutral baseline (i.e. pictures with neutral valence and low arousal) made it hard to dissociate the effects of valance and arousal on the brain. In this study, we used the happy condition to match the arousal scores with the sad condition. Therefore these effects might not be specific to positive or negative, but to emotionality or arousal in general. Related to this point, and as discussed earlier, positive and negative pictures were not evenly balanced. This might have had effect on general mood of the subjects and biased their memory performance.

5. Conclusion

In conclusion, this study investigated whether the negative memory bias in people with a high risk for depression can be explained by a schema for negative, low-arousing (sad) information, which would be supported by increased mPFC activation rather than MTL-based encoding. The results do not directly indicate such an effect. We do show that risk for depression is associated with differential activation in areas important for memory during successful encoding of negative arousing pictures. Future research might use different ways to probe the negative schema in risk for depression. This is relevant and important, because it may provide an explanation for the negative memory bias in individuals with depression. Moreover, the development of a negative schema could represent an important vulnerability marker or endophenotype for depression. The idea of negative schema to explain the negative memory bias in (high risk for) depression individuals should be further investigated in future research.

6. References

Beck AT, Steer R, Brown G (1996) *Beck Depression Inventory-Second Edition manual*. San Antonio, TX: The Psychological Corporation.

Beck, A. T. (1967). *Depression: clinical, experimental, and theoretical aspects.* Hoeber Medical Division, Harper & Row.

Beck, A. T. (1976). *Cognitive therapy and the emotional disorders.* Oxford, England: International Universities Press.

Beck, A. T. (2008). The Evolution of the Cognitive Model of Depression and Its Neurobiological Correlates. *American Journal of Psychiatry, 165*, 969–977.

Beck, A. T., Steer, R., & Brown, G. (1996). *Beck Depression Inventory-Second Edition manual.* San Antonio, TX: The Psychological Corporation.

Bienvenu, O. J., Samuels, J. F., Costa, P. T., Reti, I. M., Eaton, W. W., & Nestadt, G. (2004). Anxiety and depressive disorders and the five-factor model of personality: a higher- and lower-order personality trait investigation in a community sample. *Depression and anxiety, 20*, 92–97.

Bradley, M., Greenwald, M., Petry, M., & Lang, P. (1992). Remembering pictures: pleasure and arousal in memory. *Journal of experimental psychology Learning, memory, and cognition, 18*, 379–390.

Bradley, M. & Lang, P. J. (1994). Measuring emotion: the self-assessment manikin and the semantic differental. *Journal of Behavioural Therapy & Experimental Psychiatry, 25*, 49–59.

Bradley, P. & Mogg, K. (1994). Mood and personality in recall of positive and negative information. *Behavioural Research Therapy, 32*, 137–141.

Britton, J. C., Ho, S-H., Taylor, S. F., & Liberzon, I. (2007). Neuroticism associated with neural activation patterns to positive stimuli. *Psychiatry research, 156*, 263–267.

Canli, T., Zhao, Z., & Desmond, J. (2001). An fMRI study of personality influences on brain reactivity to emotional stimuli. *Behavioral neuroscience, 115*, 33–42.

Chan, S. W. Y., Goodwin, G. U. Y. M., & Harmer, C. J. (2007). Highly neurotic never ¬ depressed students have negative biases in information processing. *Psychological medicine, 37*, 1281–1291.

Chan, S. W. Y., Harmer, C. J., Goodwin, G. M., & Norbury, R. (2008). Risk for depression is associated with neural biases in emotional categorisation. *Neuropsychologia, 46*, 2896–2903.

Costa, P. T., & McCrae, R. R. (1992). Normal personality assessment in clinical practice: The NEO Personality Inventory. *Psychological assessment 4*, 5–13.

Denkova, E., Dolcos, S., & Dolcos, F. (2012). Reliving emotional personal memories: affective biases linked to personality and sex-related differences. *Emotion, 12*, 515–528.

Disner, S. G., Beevers, C. G., Haigh, E. P., & Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nature Reviews Neuroscience, 12*, 467–477.

Dolcos, F., Denkova, E., & Dolcos, S. (2012). Neural Correlates of Emotional Memories: a Review of Evidence From Brain Imaging Studies. *Psychologia, 55*, 80–111.

Dolcos, F., LaBar, K. S., & Cabeza, R. (2004). Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron, 42*, 855–863.

Dolcos, F., LaBar, K., & Cabeza, R. (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 2626–2631.

Eichenbaum, H. (2004). Hippocampus: Cognitive Processes and Neural Representations that Underlie Declarative Memory. *Neuron, 44*, 109–120.

Eisenberger, N. I., Lieberman, M. D., & Satpute, A. B. (2005). Personality from a controlled processing perspective: an fMRI study of neuroticism, extraversion, and self-consciousness. *Cognitive, affective & behavioral neuroscience, 5*, 169–181.

Fernández, G., & Tendolkar, I. (2001). Integrated brain activity in medial temporal and prefrontal areas predicts subsequent memory performance: Human declarative memory formation at the system level. *Brain Research Bulletin, 55*, 1–9.

Gatt, J. M., Nemeroff, C. B., Dobson-Stone, C., Paul, R. H., Bryant, R. A., Schofield, P. R., ... & Williams, L. M. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Molecular psychiatry, 14*, 681–695.

Gilboa-Schechtman, E., Erhard-Weiss, D., & Jeczemien, P. (2002). Interpersonal deficits meet cognitive biases: memory for facial expressions in depressed and anxious men and women. *Psychiatry research, 113*, 279–293.

Gotlib, I. H., Kasch, K. L., Traill, S., Joormann, J., Arnow, B., & Johnson, S. L. (2004). Coherence and specificity of information-processing biases in depression and social phobia. *Journal of abnormal psychology, 113*, 386–398.

Gusnard, D. A., Akbudak, E., Shulman, G. L., & Raichle, M. E. (2001). Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 4259–4264.

Haas, B., Constable, R. T., & Canli, T. (2008). Stop the sadness: Neuroticism is associated with sustained medial prefrontal cortex response to emotional facial expressions. *NeuroImage, 42*, 385–392.

Henckens, M. J. A. G., Hermans, E. J., Pu, Z., Joëls, M., & Fernández, G. (2009). Stressed memories: how acute stress affects memory formation in humans. *Journal of Neuroscience, 29*, 10111–10119.

Hoekstra, H., Ormel, J., & De Fruyt, F. (1996) *NEO Personality Questionnaires NEO-PI-R, NEO-FFI: Manual.* Lisse, Netherlands: Swets & Zeitlinger BV.

Hooker, C. I., Verosky, S. C., Miyakawa, A., Knight, R. T., & D'Esposito, M. (2008). The influence of personality on neural mechanisms of observational fear and reward learning. *Neuropsychologia, 46,* 2709–2724.

Jylhä, P., & Isometsä, E. (2006). The relationship of neuroticism and extraversion to symptoms of anxiety and depression in the general population. *Depression and anxiety, 23*, 281–289.

Kendler, K. S., Gardner, C. O., & Prescott, C. A. (2002). Toward a comprehensive developmental model for major depression in women. *The American Journal of Psychiatry, 159*, 1133–1145.

Kendler K. S., Gardner C. O., & Prescott C. A. (2006). Toward a comprehensive developmental model for major depression in men. *The American Journal of Psychiatry, 163*, 115–124.

Kendler, K. S., Kuhn, J., & Prescott, C. A. (2004). The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *The American Journal of Psychiatry, 161*, 631–636.

Kensinger, E. A., & Corkin, S. (2004). Two routes to emotional memory: distinct neural processes for valence and arousal. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 3310–3315.

Kuyken, W., & Dalgleish, T. (2011). Overgeneral autobiographical memory in adolescents at risk for depression. *Memory, 19*, 241–250.

Labar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience, 7*, 54–64.

Lam, D., Smith, N., Checkley, S., Rijsdijk, F., & Sham, P. (2003). Effect of neuroticism, response style and information processing on depression severity in a clinically depressed sample. *Psychological Medicine, 33*, 469–479.

Lang, P. J., Bradley, M. M., & Cuthbert, S. (1999). *International Affective Picture System (IAPS): technical manual and affective ratings.* Gainesville, FL: University of Florida.

Lishman, W. A. (1972). Selective factors in memory. Part 1: age, sex and personality attributes. *Psychological Medicine, 2*, 121–138.

Mathews, A., & MacLeod, C. (2005). Cognitive vulnerability to emotional disorders. *Annual review of clinical psychology, 1*, 167–195.

Matt, G. E., Vazquez, C., & Campbell, W. K. (1992). Mood-congruent recall of affectively toned stimuli: a meta-analytic review. *Clinical Psychology Review, 12*, 227–255.

Mayo, R. (1983). Personality traits and the retrieval of positive and negative memories. *Personality and Individual Differences, 4*, 456–471.

Nolen-Hoeksema, S., Morrow, J., & Fredrickson, B. (1993). Response styles and the duration of episodes of depressed mood. *Journal of Abnormal Psychology, 102*, 20–28.

Raes, F., Hermans, D., & Eelen, P. (2003). De Nederlandstalige versie van de Ruminative Response Scale (RRS-NL) en de Rumination on Sadness Scale (RSS-NL). *Gedragstherapie, 36*, 97 – 104.

Reynaud, E., El Khoury-Malhame, M., Rossier, J., Blin, O., & Khalfa, S. (2012). Neuroticism Modifies Psychophysiological Responses to Fearful Films. *PlosOne, 7*, 3–9.

Ruiz-Caballero, J., & Bermúdez, J. (1995). Neuroticism, mood, and retrieval of negative personal memories. *The Journal of general psychology, 122*, 29–35.

Spielberger, C., Gorsuch, R., & Lushene, R. (1970). *STAI Manual.* Palo Alto, California: Consulting Psychologists Press.

Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P., Wood, E. R., ... & Morris, R. G. M. (2007). Schemas and memory consolidation. *Science, 316*, 76–82.

Tse, D., Takeuchi, T., Kakeyama, M., Kajii, Y., Okuno, H., Tohyama, C., ... & Morris, R. G. M. (2011). Schema-dependent gene activation and memory encoding in neocortex. *Science, 333*, 891–895.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., ... & Joliot, M. (2002). Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain, *Neuroimage, 15*, 273–289.

Van Kesteren, M. T. R., Fernández, G., Norris, D. G., & Hermans, E. J. (2010). Persistent schema-dependent hippocampal-neocortical connectivity during memory encoding and postencoding rest in humans. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 7550–7555.

Van Kesteren, M. T. R., Rijpkema, M., Ruiter, D. J., & Fernández, G. (2010). Retrieval of associative information congruent with prior knowledge is related to increased medial prefrontal activity and connectivity. *Journal of Neuroscience, 30*, 15888–15894.

Van Kesteren, M. T. R., Ruiter, D. J., Fernández, G., & Henson, R. N. (2012). How schema and novelty augment memory formation. *Trends in neurosciences, 35*, 211–219.

Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, A. M., ... & Buckner R. L. (1998). Building Memories: Remembering and Forgetting of Verbal Experiences as Predicted by Brain Activity. *Science, 281*, 1188–1191.

Watson, D., Clark, L., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Personality and Social Psychology, 54*(6), 1063-1070.

Williams, J. M. G., Barnhofer, T., Crane, C., Herman, D., Raes, F., Watkins, E., & Dalgleish, T. (2007). Autobiographical memory specificity and emotional disorder. *Psychological bulletin, 133*, 122–148.

Williams, J. M. G., Watts, F. N., MacLeod, C., & Mathews, A. (1997). *Cognitive Psychology and the Emotional Disorders.* New York: Wiley.

A Potential Role for MicroRNA-137 in Long-Term Depression

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Non-coding regulatory microRNAs (miRNAs) are important post-transcriptional regulators of gene expression that are abundantly expressed in the mammalian brain. In neurons, the functions of individual miRNAs are beginning to emerge and previous studies identified miRNAs to be involved in synaptic plasticity and development. In the present study, we investigated the role of miRNA-137 (miR-137) at the synapse by inhibiting miR-137 functionally in isolated cortical synaptoneurosomes. Furthermore, we monitor the expression of miR-137 and its target Mind bomb-1 (Mib1) following long-term depression (mGluR-LTD) by stimulating group I metabotropic glutamate receptors. Our results demonstrate that miR-137 has the capacity to modulate target mRNA expression of nuclear receptor coactivator 1 (NCOA1), Kruppel-like factor 4 (KLF4) and Heterogeneous nuclear ribonucleoprotein U (HNRNPU) at the synapse. Furthermore, induction of mGluR-LTD using the group I mGluR agonist (RS)-3,5-diydroxyphenylglycine (DHPG) in rat hippocampal slices revealed significantly increased miR-137 levels, while decreased precursor miR-137 levels were detected. Importantly, individual inhibition of group I mGluRs subtypes (consisting of mGluR1 and mGluR5) indicated that miR-137 expression upon mGluR-LTD is mediated through mGluR5 stimulation. In addition, monitoring Mib1 expression revealed a significant upregulation at the protein level. Further, we delineate the possible role of miR-137 during synapse development in cultured hippocampal neurons. This investigation revealed no significant difference between synaptic density in mature hippocampal neurons overexpressing miR-137 and the control condition. Together, our data suggests a role of miR-137 in mGluR-LTD and points to a possible mechanism by which this miRNA is involved in the modulation of synaptic protein synthesis.

Keywords: microRNA-137, group I metabotropic glutamate receptors, long-term depression, synaptogenesis

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1. Introduction

The capacity of the brain to convert experience into memories relies on synaptic communication. The underlying mechanism refers to the ability to modify neural circuits through environmental input. The key cellular process for learning and memory is synaptic plasticity that is the alteration of synaptic transmission between synapses. These changes in strength or efficacy of transmission within neuronal networks are activity dependent and require newly formed proteins (Citri & Malenka, 2008). Previous studies indicated that post-synaptic protein synthesis following synaptic plasticity is under tight control of a number of different processes, such as translation initiation and elongation (Costa-Mattioli, Sossin, Klann & Sonenberg, 2009) as well as microRNAs (miRNAs; Manakov, Grant & Enright, 2009). miRNAs are involved in a specific mechanism of translation regulation. These 19-25 nucleotides long single stranded non-coding RNAs are involved in post-transcriptional gene-silencing processes through base pairing with sequences located on the 3' untranslated region (3' UTR) of messenger RNAs (mRNAs; Kim, 2005). By miRNA binding to sequences on 3' UTR of mRNAs, gene expression is controlled via mRNA degradation or translational repression, with both possibilities exerting inhibitory effects on protein synthesis (Petersen, Bordeleau, Pelleties & Sharp, 2006; Mathonnet et al., 2007). Only a small sequence match is required for miRNA-mRNA association. The seed region in miRNAs determines the association with mRNAs. This region is six to eight nucleotides long (position 2-8) and it is complementary to sequences located at the 3' UTR. Therefore, miRNAs are expected to have the potential to target multiple mRNAs and vice versa: individual target mRNAs can be bound by different miRNAs, because a given mRNA can have several miRNA binding sites (Gunaratne, Creighton, Watson & Tennakoon, 2010). This feature makes the miRNA a complex gene regulation system that is associated with many biological processes such as synaptic development and morphogenesis (Schratt, 2009; Olde Loohuis et al., 2012). In humans, the recent estimates of known miRNAs are 1000 or more (Ruberti, Barbato & Cogoni, 2011). While 70% of the identified miRNAs are also detected in the central nervous system, only 35% of them are exclusively expressed in the brain (Smrt et al., 2010). Most of the brain specific miRNAs have been found to be enriched at the synapse, which suggests that miRNAs are ideally positioned for regulating local protein translation in response to synaptic activity (Wienholds et al., 2005; Kindler & Kreienkamp, 2012). In neurons, selective mRNAs are transported to sites that are distant from the cell body (Schratt et al., 2006). These pre-existing mRNAs at the synaptic sites are hypothesized to be important for synaptic plasticity. Therefore, these synaptically located small non-coding RNAs might be important regulators of the local translation system and involved in maintaining synaptic function (Beaumont et al., 2001). While little is known on the functions of individual miRNAs in neurons multiple lines of evidence point to the involvement of miRNAs in various neurological disorders (reviewed in Olde Loohuis et al., 2012).

1.1 miRNAs and synaptic plasticity

The ultimate goal in neuroscience is to identify the molecular mechanisms underlying mental brain functions and how alterations in these processes can contribute to cognitive disorders. What is known is that the cellular basis of learning and memory is determined through alterations in synaptic strength; therefore synaptic plasticity is an important feature for properly functional neurons (Hunt & Castillo, 2012). In order to consolidate newly formed memories, proteins have to be synthesized at the synapse (Guzowski et al., 2000). Synapses are dynamic structures and their activity can trigger long-lasting changes at synaptic sites. To ensure the stability of the plastic changes, local protein synthesis is necessary for weakening or strengthen the synapse (Siegel, Saba & Schratt, 2011). One intensively studied model in mammals that measures alterations in synaptic strength is long-term depression (LTD). LTD is characterized by reduced synaptic connectivity by internalizing the major ionotropic glutamate receptor called α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPA-Rs; Anggono & Huganir, 2012). Several forms of LTD exist that are relying on different signalling pathways. One form of LTD is mediated through group I metabotropic glutamate receptors (mGluR), consisting of mGluR1 and mGluR5 (Collingridge, Peineau, Howland, & Wang, 2010; Izumi & Zorumski, 2012). Stimulation of group I mGluRs results in rapid dendritic protein synthesis leading to postsynaptic receptor changes thereby inducing mGluR-LTD (Huber, Kayser & Bear, 2000; Moult et al., 2006). However, unravelling the mechanism by which mGluR-LTD regulates protein synthesis remains challenging.

Since miRNAs control post-transcriptional processes and are increased at the synapse, their altered expression might be a potential cause for cognitive disorders (Hunsberger, Austin, Chen & Manji, 2009). Fundamental observations in neurodevelopmental disorders are modified neuronal plasticity and morphology, which may result from post-transcriptional dysfunction through miRNAs (Feng & Feng, 2011). Notably, modifications of the processes involved in synaptic plasticity may contribute to intellectual disability (ID) syndromes. Enhanced mGluR-LTD has been linked to fragile X syndrome (FXS) due to loss of the translational repressor fragile X mental retardation protein (FMRP; Bear et al., 2004; Bassell & Warren, 2008). Moreover, several ID phenotypes are associated with miRNA pathway dysfunction (Nomura et al., 2008; Kuhn et al., 2010). Previous studies identified that the brain specific miR-137 is increased at the synapse and that its dysfunction might be a potential cause for ID syndromes. Expression studies showed a decreased level of precursor as well as mature miR-137 level in ID patients when compared to healthy controls. Consequently, in ID patients increased levels of downstream targets of this miRNA were found as well, suggesting that reduced miR-137 expression might be related to the ID phenotype (Willemsen et al., 2011). This miRNA further regulates neuronal maturation and morphogenesis during development. Interestingly, this maturation function of miR-137 is controlled by translational repression of Mind bomb-1 (Mib1), an ubiquitin ligase known to be important in neurodevelopment (Choe et al., 2007; Smrt et al., 2010).

Given that the brain-enriched miR-137 has a significant role in synapse maturation and morphogenesis of young neurons, we reasoned that it might act as a local translation regulator of synaptically enriched target mRNAs. In the present study we investigated the role of miR-137 as a regulator of local translation in neuronal processes. We report that mRNAs encoding NCOA1, KLF4 and HNRNPU, that are located in the synaptic compartments, are targeted by miR-137. The expression of these target mRNAs can be regulated locally at the synapse. Further, we investigate miR-137 during synaptic plasticity. We profile the temporal expression pattern of this miRNA after chemical LTD induction on rat hippocampal brain slices. Another aim in this study is to monitor the role of this miRNA during synapse maturation by overexpressing miR-137 in growing hippocampal neurons. The outcome of our study suggests that the synaptically enriched miR-137 has the capacity to act as a local translation regulator of mRNAs. Further, the temporal expression profile following mGluR-LTD induction by applying group I mGluR agonists and simultaneous application of group I antagonists, suggests that mGluR5 receptors are of specific importance for endogenous miR-137 increase during LTD. The assessment of miR-137 role during synaptogenesis revealed no involvement of miR-137 influencing the gene circuitry that is essential for synaptogenesis.

2. Materials and Methods

2.1 Animals

In the present study, all experimental procedures were performed on wistar rats.

2.2 Hippocampal slice preparation and pharmacological stimulation of hippocampal slices

Hippocampal slices were prepared from postnatal day (PND) 24-31 old rats for acute hippocampal slices, whereas 6-8 day old rats were sacrificed for organotypic hippocampal slice preparations.

Animals were anesthetized with isoflurane and decapitated. Brains were quickly removed and acute hippocampal slices (350μm) were prepared in cold dissection buffer (4°C) using VT-1000S vibratome tissue slicer (Leica). Prior to use for experiments, acute hippocampal slices were transferred into a storage chamber containing dissection buffer to recover for 2 hours at room temperature. Then hippocampal slices were replaced into artificial cerebrospinal fluid (ACSF; containing 119mM NaCl, 11mM glucose, 2.5mM KCl, 1mM NaH_2PO_4, 26mM $NaHCO_3$, 2mM $CaCl_2$, and 1mM $MgCl_2$) saturated with 95% O2, 5% CO2 and used for pharmacological stimulation (described below).

For organotypic slices, hippocampi were dissected in low Na ACSF (1M $CaCl_2$, 10mM D-Glucose, 4mM KCl, 1M $MgCl_2$, 26mM $NaHCO_3$, 233mM sucrose, 0.1% Phenol Red solution) and cut into 300μm slices. The slices were grown on 0.4 μm membranes (Millipore) in culture at 37°C, 5% CO_2 (medium contained: 10,639 g/L MEM; 1mM L-Glutamine, 1mM $CaCl_2$, 2 mM $MgSO_4$, 1g/L insulin, 25% L-ascorbic acid, 2.32 g/L D-Glucose, 0.44g/L $NaHCO_3$, 7.16 g/L Hepes, 200ml /L heat inactivated horse serum, pH was adjusted to 7.27-7.28 with 1M NaOH). Medium was refreshed every 2-3 days. At 14 days in vitro (DIV 14) organotypic

slices were pharmacologically stimulated as described below.

Acute and organotypic hippocampal slices were pharmacologically stimulated with 15 minutes bath application of 50μM group I mGluR agonist (RS)-3,5-diydroxyphenylglycine (DHPG) at 37°C thereby inducing mGluR-LTD. Individual inhibition of mGluR1 and mGluR5 was achieved by applying 100μM of mGluR1 antagonist LY367368 and 10μM of mGluR5 antagonist 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP). Drug exposure to Ly367368 and MPEP occurred 30 minutes prior DHPG induced mGluR-LTD in acute hippocampal slices and 60 minutes in organotypic hippocampal slices.

2.3 Synaptoneurosomal preparation and functional inhibition of miR-137

Based on the method of Gray and Whittaker synaptoneurosomes were isolated (Gray & Whittaker, 1962). Briefly, rat cortical tissue was homogenized using Potter Homogenizer (B. Braun, Melsungen, Germany) in ice cold homogenization buffer (containing 1.75M sucrose, 10mM HEPES, 1mM EDTA, 0.25mM DTT, pH adjusted to 7.4 with 1M NaOH) supplemented with protease inhibitor cocktail (Roche) and 40U/μl RNAse inhibitor (Fermentas). The homogenized tissue was centrifuged at 1000x g for 10 minutes. The supernatant S1 was passed through 80μm, 40μm and 10μm pore size filters and then centrifuged at 2000x g for 15 minutes. Supernatant S2 contained the cytosolic fraction, whereas the obtained pellet contained the synaptoneurosomal fraction. The pellet was resuspended in homogenization buffer and aliquoted (7 x 100μl) for further experimental procedures described below. All steps of the synaptoneurosomal isolation were performed at 4°C. Previously described protocols provide evidence that this experimental procedure isolates pure synaptoneurosomes by using western blot assays for synaptically enriched markers (e.g. PSD 95) and markers that are not enriched at the synapse (e.g. tubulin) (Willemsen et al., 2011).

Locked nucleic acid (LNA)-based miR-137 inhibitor or non-targeting control (Exiqon) with a final end concentration of 50nM were introduced into the purified synaptoneurosomes for functional silencing of miR-137 by sequestering endogenous miR-137 molecules. Utilization of LNA probes with high binding specificity for miR-137 ensured antisense inhibition of this miRNA. Subsequently, time course dependent incubation at 37°C took place for 0 min, 15 min, 30 min and 90 min. Thereafter, total synaptoneurosomal RNA was isolated using TRIzol® Reagent (Life Technologies) according to the manufacturer's protocol.

2.4 Reverse transcription and semi-quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from above mentioned tissues either by using TRIzol® Reagent (Life Technologies) or NucleoSpin ® RNA II (Macharey-Nagel) according to the protocols provided by the manufacturers. RNA purity of all isolated samples was determined by NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific) through ultraviolet (UV) absorbance at 260nm and 280nm (A260/280 ratio) and by agarose gel electrophoresis. First-strand cDNA was synthesized from 100-500ng RNA using the miScript Reverse Transcription Kit (Qiagen) according to manufacturer's protocol. Triplicate qRT-PCR reactions were performed with 1:10 diluted cDNA uitilizing the Maxima SYBR Green/ROX qPCR master mix (Fermentas) for mRNA or miScript SYBR Green PCR Kit (Qiagen) for mature miR-137 detection. All qRT-PCR reactions were performed on Rotor Gene 6000 (Corbett Life Science) using standard cycling conditions of the qRT-PCR master mix manufacturer's protocol described above. Determination of product specificity was assessed by performing a melting curve analysis. Relative gene expression differences were calculated either by applying the delta CT method (Schmittgen & Livak, 2008) or by utilizing GeNorm software and calculating a gene expression normalization factor based on geometric means of two internal control genes (Vandesompele et al., 2002). The acquired qRT-PCR data was normalized against the endogenous unregulated reference genes U6 and β-actin. The gene specific primers used are listed in Table 1.

2.5 Western Blot analysis

After drug exposure for the indicated times (as described above), hippocampal slices were also lysed in NP-40 lysis buffer (containing 25mM NaF, 1mM NapyroP, 0.1mM NaOrthovanadate, 1mM PMSF, 150mM NaCl dissolved in 1xTBS, 1% NP-40, supplemented with protease inhibitor cocktail obtained from Roche). Protein concentrations were measured using BCA protein assay (Thermo

Table 1. Primers used for qRT-PCR.

Gene	Species	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
Precursor miR-137	Rat	GTGACGGGTATTCTTGGGT	GACTACGCGTATTCTTAAGCAA
Mature miR-137	Rat	GTGACGGGTATTCTTGGGT	Universal primers provided by miScript SYBR GREEN PCR KIT (Qiagen)
U6	Rat	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
β-actin	Rat	CGTGAAAAGATGACCCAGATCA	AGAGGCATACAGGGACAACACA
KLF4	Rat	AGGGCCTTTTCCAGGTCGGAC	TCCCCTCGTGGGAAGACAGTGT
NCOA1	Rat	GACGGCCCCTTTGCCTAGCC	GACAGATCTCAGAAGCCCTAGTCCCA
HNRNPU	Rat	AGTGCCTGCGGGTTCCTTTTT	TCTTATCGTGCACAATGCTGAGGTTC
Mib1	Rat	CAGCAGCAGCTTCAGGACATT	AGAGCTGGCATGTTCCGTG

Scientific) and subjected to western blotting using standard methods.

Briefly, samples were incubated at 100°C with sample buffer and 5mM DTT for 4 minutes. Proteins were separated on a 10% SDS-PAGE gel (run at 100V) and transferred on nitrocellulose membranes (run overnight at 10V). Membranes were washed in 1x PBS and then incubated with primary antibody anti-Mib1 at a 1:1000 (M20, obtained from Choe et al., 2007) in 5% BSA blocking buffer, followed by incubation with the secondary antibody goat anti-rabbit, 1:2000. Mib1 protein was visualized by utilizing chemiluminescent detection by applying Lumi-light western-blotting substrate in a 1:1 ratio.

2.6 Neuronal cell cultures and transfections with miR-137 mimics

Primary cultures of hippocampal neurons were prepared from embryonic day 18 (E18) rats. After hippocampal dissection, neurons were washed 2 x in ice-cold Hank's Balanced Salt solution (HBSS; Gibco-Invitrogen), then trypsinized in HBSS for 15 minutes at 37°C and again washed 3x in ice–cold HBSS before plated on poly- D- lysine coated 24 well plates. Cultures were maintained at 37°C and 5% CO2 in neurobasal medium (GIBCO-Invitrogen) supplemented with B 27 (Invitrogen) and 2mmol/L glutamine. For synapse quantification, miR-137 mimics and non-targeting control (both obtained from Qiagen) were introduced into the cell line between DIV 3-6 either by using siPORT NeoFX (Life Technologies) or Lipofectamine 2000 reagent (Life Technologies). Transfections were performed according to manufacturer's protocol, except that siPORT NeoFX transfection time window was modified from DIV 0 to DIV 3-6. Final end concentration per condition was 30 nM of miRNA mimic or non-targeting control mimic.

2.7 Live–labelling surface GluA1 in transfected hippocampal neurons

Twelve to thirteen days post-transfection with siPORT NeoFX or Lipofectamine2000 as described above, hippocampal cultures were live labelled for synaptic protein GluA1 (Calbiochem PC246, rabbit, 1:50). Fixation occurred with 16% paraformaldehyde-sucrose, followed by blocking the aldehyde function with 50mM NH_4Cl in PBS. Aspecific labelling was blocked through incubation with 1xPBS-1%BSA. The secondary antibody used was goat anti-rabbit Alexa Fluor Dye 488 or goat anti-rabbit Alexa Fluor Dye 568 (Life Technology), when co-transfected with GFP. Cells were mounted in prolong gold. Imaging and quantification of synapse densities were performed with fluorescent microscopy Leica AF and Image J software, respectively.

3. Results

3.1 Functional inhibition of synaptic miR-137 results in elevated target mRNA levels of KLF4, HNRNPU, and NCOA1

The brain specific miR-137 is highly expressed in neuronal tissue (Wienholds et al., 2005). However, little is known on how this miRNA influences neuronal function and development. Recent findings demonstrate that miRNAs are ideally positioned to quickly regulate protein synthesis during neuronal activity (Muddashetty et al., 2011). Due to our previous finding on the potential regulatory role impact of miR-137 based on the technique described in Poon et al. (2006) revealed putative miR-137 mRNA binding sites within a list of a selection of genes that are abundantly present and translated locally within

the synapse (Poon, Choi, Jamieson, Geschwind & Martin, 2006). A significant correlation has been found between the expression level of miR-137 and its putative target genes that are synaptically located, namely KLF4, HNRNPU and NCOA1. Based on our findings that overexpression of miR-137 in rat cortical neurons led to a significant decrease of three dendritically localized target mRNAs NCOA1, KLF4 and HNRNPU (unpublished data), we reasoned that miR-137 might act as a local translation regulator at the synapse. To investigate whether miR-137 acts on synaptic translation, we monitored target mRNA levels in isolated synaptoneurosomes representing the dendritic spines. Time dependent inhibition of miR-137 was performed by applying locked nucleic acid (LNA) based miR-137 inhibitor to the biochemically active synpatoneurosomes. By qRT-PCR we examined the relative quantity of three target mRNAs NCOA1, KLF4 and HNRNPU at four different time points (t=0 min, 15 min, 30 min, 90 min). MiR-137 silencing resulted in an increase of the three target mRNAs NCOA1, KLF4 and HNRNPU when compared to non-targeting control LNA (Fig. 1). The mRNA levels of NCOA1, KLF4 and HNRNPU display a similar time-dependent pattern of upregulation. During miR-137 inhibition at t=30 min target mRNA levels reached maximum ($*p < 0.05$) and at t=90 min a decrease can be identified when compared to t=30 min. Together, the data demonstrates that the inhibition of miR-137 in pure synaptic fractions resulted in distinct elevation of the target mRNAs encoding KLF4, NCOA1 and HNRNPU, suggesting a role of miR-137 in local translation regulation.

3.2 Endogenous miR-137 increase during synaptic plasticity depends on mGluR5 activation

The findings that miR-137 is highly enriched at the synapse and that its dysregulation might be involved in intellectual disability (ID) phenotypes (Bear, Huber & Warren, 2004; Willemsen et al., 2011), led us to investigate the involvement of miR-137 in synaptic plasticity. Since mGluR-LTD is initiated through activation of group I metabotropic glutamate receptors in the hippocampus and that this form of synaptic plasticity requires protein translation near the synapse (Waung & Huber, 2009), we reasoned that the endogenous level of miR-137 during mGluR-LTD could be regulated through either one of the group I mGluR receptors, being mGluR1 or mGluR5. The miR-137 expression level was examined with qRT-PCR in acute and organotypic hippocampal slices treated with (RS)-3,5-diydroxyphenylglycine (DHPG), which is a selective mGluR group I agonist. We show that treatment with DHPG led to a rapid increase in miR-137 levels within 15 minutes when compared to control hippocampal slices (Fig. 2A $*p < 0.05$; 2B

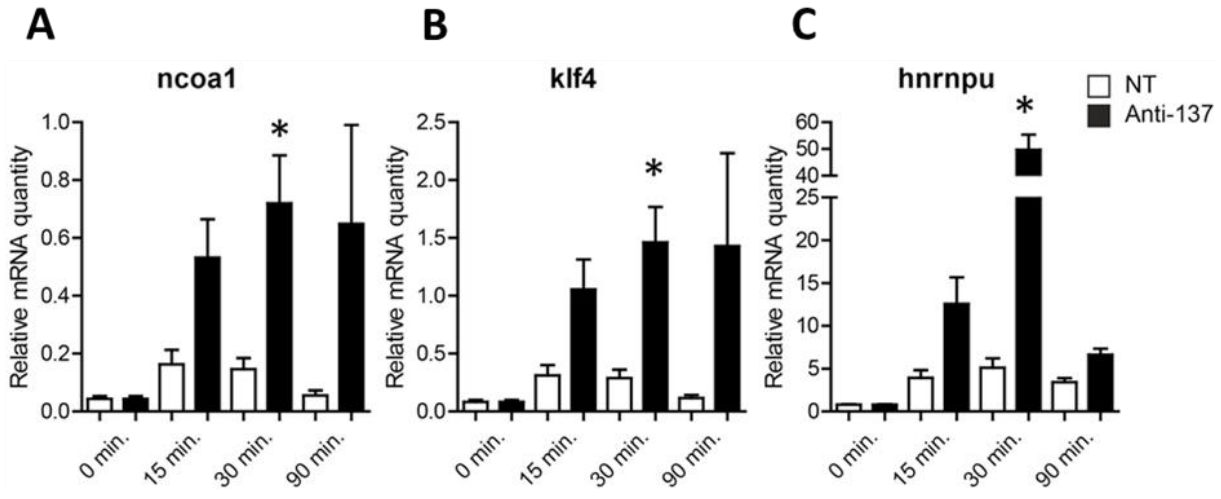

Fig. 1 Local actions of miR-137 at the synapse. Rat cortical synaptoneurosomes were prepared from postnatal day 21 and LNA-based miR-137 inhibitor was added. Following a time-course dependent incubation at 37°C, total RNA was isolated and qRT-PCR based quantification of synaptic target mRNA levels was performed. Levels of miR-137 target mRNAs were normalized to the internal control U6. The three graphs reveal upregulated mRNA levels in synaptoneurosomes treated with LNA-based miR-137 inhibitor when compared to non-targeting LNA, which was used as a negative control. Significant mRNA upregulation was detected at t=30 min, $*p < 0.05$ for **A.** NCOA1, **B.** KLF4 and **C.** HNRNPU, whereas those three target mRNAs at time points t=15 min and t=90 min show a non-significant trend of upregulation in the LNA-based miR-137 inhibitor condition ($p > 0.05$).

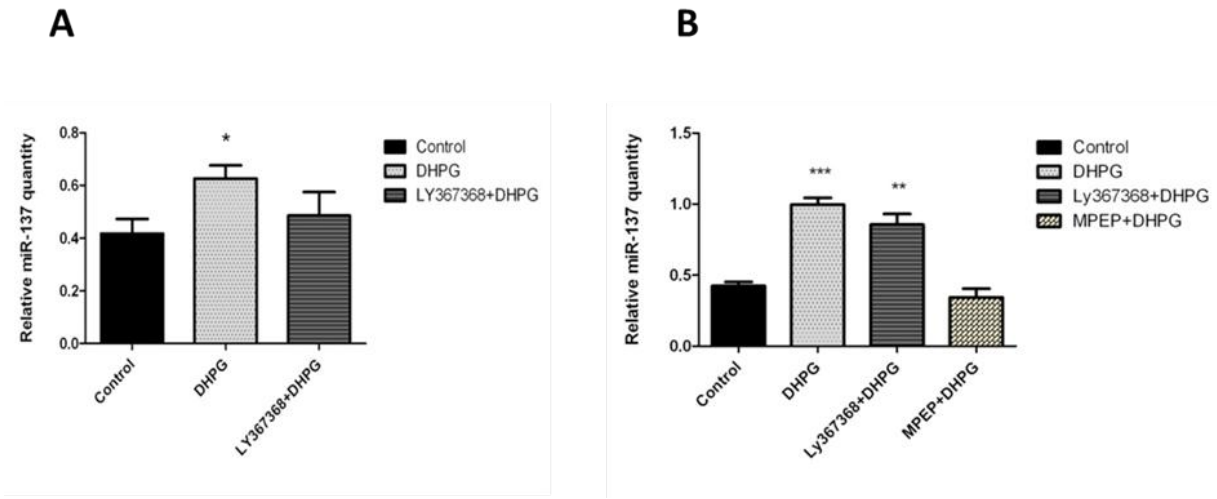

Fig. 2 Increase of endogenous miR-137 during synaptic plasticity. mGluR-LTD using DHPG was induced in rat hippocampal slices (PND 24-31). Following mGluR-LTD induction, miR-137 levels were assessed to determine the group I mGluR receptor subtype involved in the regulation of miR-137 levels. Hippocampal slices were pre-incubated with selective mGluR1 antagonist LY367368 or mGluR5 antagonist MPEP prior to DHPG application. Significance is stated in comparison with the control condition, unless stated otherwise. **A.** Mature miR-137 levels in acute hippocampal slices upon mGluR-LTD induction by DHPG application (*$p < 0.05$). Acute hippocampal slices are obtained from the rodent brain and are used for experimentation that same day. Presence of LY367368 did not significantly decrease endogenous miR-137 expression during LTD ($p > 0.05$) when compared to DHPG condition. Comparison of miR-137 levels between control and LY367368 plus DHPG revealed a non-significant tendency of miR-137 increase upon mGluR-LTD induction ($p > 0.05$). **B.** Mature miR-137 levels in organotypic hippocampal slices, which are an explant of nervous tissue. Endogenous miR-137 expression significantly increased when DHPG was applied alone (***$p < 0.001$). mGluR1 antagonist LY367368 did not affect the DHPG induced miR-137 increase (**$p > 0.01$), whereas presence of mGluR5 antagonist MPEP blocked endogenous mature miR-137 expression during mGluR-LTD (*$p < 0.05$). Comparison of DHPG condition alone with MPEP plus DHPG revealed also a significant difference in miR-137 expression (**$p < 0.01$). Significance not indicated in graph.

***$p < 0.001$). To determine through which group I mGluR subtype this endogenous increase was induced, a selective mGluR1 antagonist LY367368 was applied 30 minutes prior to DHPG treatment. Presence of LY367368 did not significantly reduce the miR-137 increase when compared to DHPG applied alone (Fig. 2A, $p > 0.05$). Comparison of miR-137 levels between control and LY367368 plus DHPG revealed a non-significant tendency of miR-137 increase upon mGluR-LTD induction (Fig. 2A, $p > 0.05$).

Based on our finding that miR-137 expression was rapidly increased during DHPG induced mGluR-LTD and that mGluR1 antagonist LY367368 did not affect the DHPG induced increase in miR-137, we next investigated whether mGluR5 contributes to the endogenous increase of miR-137. We applied LY367368 and the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP) to organotypic hippocampal slices 60 minutes prior to DHPG treatment. As expected, LY367368 did not prevent the rapid increase in miR-137. This elevation was blocked when MPEP was present (Fig. 2B). Together, these data suggests that miR-137 might be a critical regulator at activity-dependent mRNA translation in mGluR-LTD and that this endogenous upregulation could be dependent on activation of mGluR5, rather than mGluR1.

3.3 Investigation of precursor miR-137 levels following mGluR-LTD

Maturation of miRNA is necessary for the incorporation of the short RNA oligonucleotide into the RNA induced silencing (RISC) complex. We showed that upon mGluR-LTD induction mature miR-137 levels are significantly elevated. We further investigated whether precursor miR-137 (pre-miR-137) levels were also altered upon DHPG application. Our data suggest an mGluR-LTD mediated decrease in pre-miR-137 levels, indicating increased processing of pre-miR-137 into the mature form following mGluR-LTD. However, decreased pre-miR-137 levels in DHPG treated slices was not significant (Fig. 3A, $p > 0.05$).

Investigation of pre-miR-137 level in organotypic hippocampal slices revealed a non-significant tendency for decreased pre-miR-137 levels (Fig. 3B, $p > 0.05$). No increase in pre-miR-137 levels were detected, providing evidence for increased mature

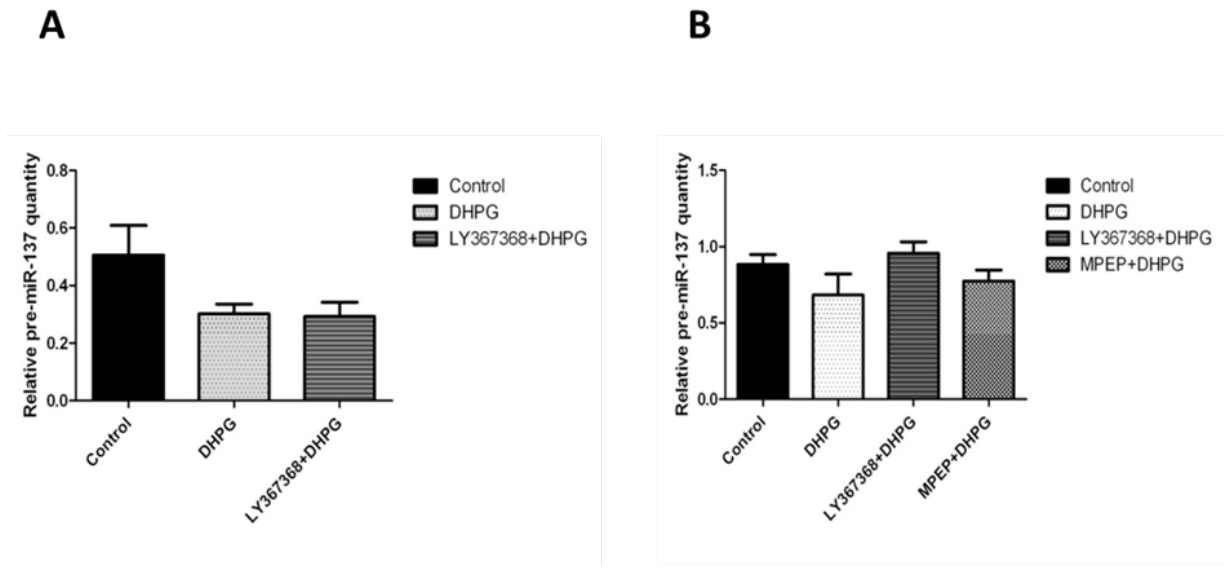

Fig. 3 Precursor miR-137 processing following mGluR-LTD. Pre-miR-137 expression in acute and organotypic slices treated with DHPG and selective group I mGluR antagonists LY367368 and MPEP. **A.** Quantification of pre-miR-137 in acute hippocampal showed a tendency for decreased pre-miR-137 level ($p > 0.05$) following mGluR-LTD, indicating that this precursor miRNA processing might be increased upon group I mGluR stimulation. **B.** Quantification of pre-miR-137 in organotypic slices displayed a non-significant tendency for reduced pre-miR-137 levels ($p > 0.05$). In the presence of LY367368 no decrease in pre-miR-137 level was detected, pre-miR-137 level remained at the control condition level. Together, the data suggests a reduction in pre-miR-137 level, indicating increased precursor miRNA processing. Error bars represent the variability between n = 3 qRT-PCR reactions. Levels of pre-miR-137 are expressed relative to the internal controls U6 and β-actin.

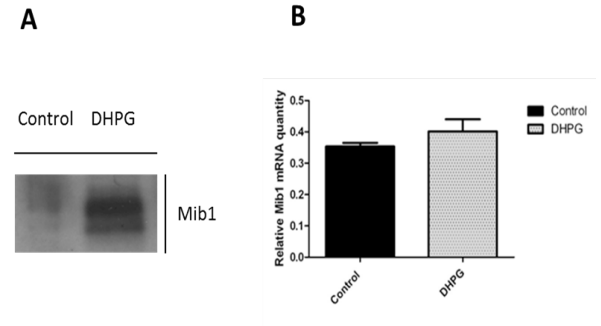

Fig. 4 Mib1 upregulation after DHPG stimulation. The expression of miR -137 target Mib1 following mGluR-LTD induction was examined on protein and mRNA level. **A.** Western blot analysis using antibodies against mouse Mib1 (~105kDa) revealed an upregulation of Mib1 after mGluR-LTD induction when compared to control. **B.** qRT-PCR quantification of miR-137 target mRNA Mib1. DHPG treatment of organotypic hippocampal slices did not induce a significant change in Mib1 mRNA level ($p > 0.05$). All mRNA levels are expressed relative to the internal controls U6 and β-actin. Error bars represent the variability between n = 3 qRT-PCR reactions.

miR-137 processing. The elevation of mature miR-137 and a concomitant reduction of pre-miR-137 levels suggest that increased mature miR-137 levels are based upon increased maturation process, but not due to enhanced transcription.

3.4 Mind bomb 1 upregulation during mGluR-LTD

The discovery that the ubiquitin ligase Mind bomb-1 (Mib1) is targeted by miR-137 (Smrt et al., 2010), motivated us to explore whether Mib1 expression is influenced during mGluR-LTD either on protein or mRNA level. Western blotting identified a significant upregulation of Mib1 protein expression in organotypic hippocampal slices treated with DHPG (Fig. 4A). Examined Mib1 mRNA levels following mGluR-LTD revealed a slight but non- significant increase in mRNA levels when compared to control (Fig. 4B). These data suggest that activation of group I mGluR triggers new synthesis of Mib1 protein following synaptic plasticity.

3.5 Investigating miR-137 involvement during synaptogenesis

Recently, miRNAs were found to be expressed at the synapse and this position enables them ideally to act on neuronal development (Schratt, 2009). Proper function requires perfect communication with other neurons. Therefore neuronal maturation, which includes among other things synaptogenesis, is a crucial step during development (Waites, Craig

& Garner, 2005). To determine whether miR-137 affects neuronal maturation, we overexpressed miR-137 levels in hippocampal neurons. Transfections were done at DIV 3 or DIV 6. For transfection efficiency identification miR-137 mimics were co-transfected with transfection indicators siGlo Red or green fluorescent protein (GFP). Both sets of cells were immunostained between DIV 16-18, a time window in which neurons are believed to exhibit the morphology of mature neurons. Synapses were stained for the AMPA receptor subunit GluA1 and imaged using fluorescent microscopy (Leica AF). To quantify synaptic density, images were analyzed with Image J. Synaptogenesis in primary hippocampal neurons transfected at DIV 3 and immunostained for surface GluA1 thirteen days post-transfection did not significantly differ from control condition (Fig. 5A, $p > 0.05$). We obtained similar results when transfection time point was varied. MiR-137 mimic and non-targeting (nt) control were introduced into primary hippocampal neurons at DIV 6. Twelve days post-transfection synapses were stained for surface GluA1. Synaptic density was not significantly altered in hippocampal neurons that overexpressed miR-137 or the non-targeting control (Fig. 5B, $p > 0.05$). Our results suggest that miR-137 is not involved in regulating the gene circuitry involved in synaptogenesis.

4. Discussion

4.1 Local role of miR-137 at the synapse

The mechanism of activity dependent mRNA translation regulation, especially in dendrites is poorly understood. Here we suggest that miR-137 is most likely involved in the fine regulation of gene expression following synaptic plasticity. We found that miR-137 is upregulated by mGluR-LTD induction. One potential explanation for this increase could be that mGluR-LTD stimulates miRNA maturation. Consistent with this idea is our data on the pre-miR-137 expression level. Upon DHPG stimulation of hippocampal slices pre-miR-137 levels decreased, indicating that mature miR-137 upregulation is not due to enhanced transcription, but rather through increased maturation of pre-existing pre-miR-137. Evidence for miRNA processing in dendrites has been suggested by a previous study. Dicer, a key enzyme for miRNA maturation, was shown to be enriched in the synaptic compartment and appears to be activated by synaptic activity (Lugli et al., 2005). However, future studies will have to address whether increased processing of pre-miR-137 is due to dicer activation upon mGluR-LTD induction.

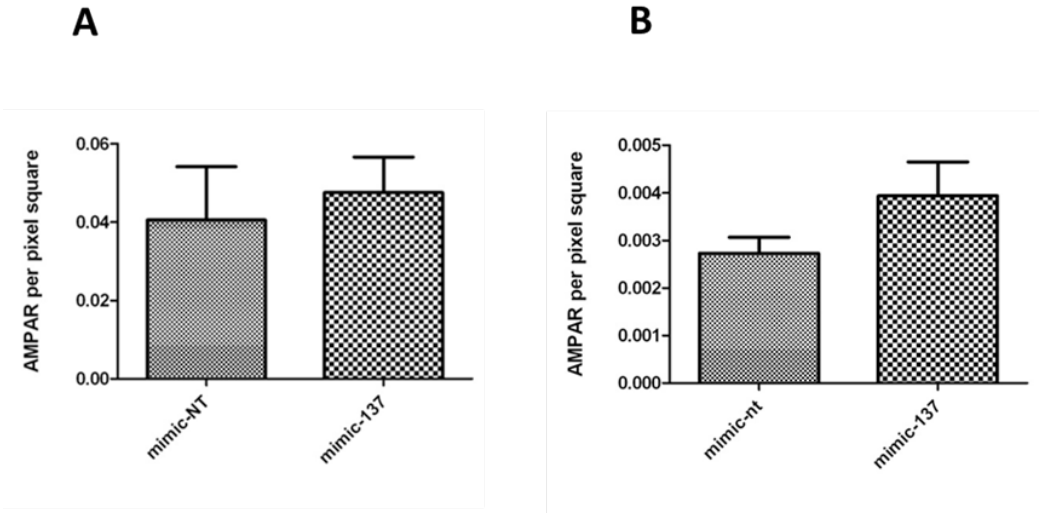

Fig. 5 MiR-137 overexpression does not affect gene circuitry involved in synaptogenesis. The role of miR-137 in dendritic development was investigated by overexpressing miR-137 in growing hippocampal neurons. Mature neurons were fixed between day 16-18 in culture, a window were synaptogenesis took place. Synapses were identified by staining for synaptic protein GluA1 and imaging using fluorescent microscopy (Leica AF). Synaptic density was assessed by Image J measurement and the release sites were compared between primary hippocampal neurons overexpressing miR-137 or non-targeting (nt) control. The analysis using Image J software displayed no significant difference between the two conditions. **A.** Synaptic density of neurons transfected on DIV 3 and stained at DIV 16. Error bars represent the variability between $n = 14$ analyzed dendrites. Student t test did not reveal a significant difference, $p > 0.05$. **B.** Synaptic density of neurons transfected on DIV 6 and immunostained at DIV 18. Error bars represent the variability between $n = 17$ analyzed dendrites per condition. Student t test did not identify a significant difference, $p > 0.05$.Quantification of the number of surface GluA1 is represented as the amount of synapses per pixel square meter.

Our results also demonstrate that miR-137 has the capacity to modulate mRNA expression at the synapse. Functional inhibition of miR-137 at the synapse resulted in upregulated target mRNA expression of NCOA1, KLF4 and HNRNPU. One limitation in our study is that we did not investigate whether miR-137 is also able to regulate the protein synthesis of its target mRNAs. However, it is well known that synaptic plasticity depends upon local protein synthesis and we show that miR-137 modulates mRNA expression at the synapse; therefore it is tempting to assume that miR-137 might be involved in regulating mGluR-LTD mediated local protein synthesis. It is likely that the mGluR-LTD related protein synthesis needs to be downregulated after a short time interval. Since miRNAs are negative regulators of mRNA translation, we suggest that the upregulation of miR-137 after mGluR-LTD induction might be part of a mechanism that prevents excessive protein synthesis by inhibiting mRNA translation. Precise control of translation following synaptic plasticity is important, because dysregulated protein synthesis can cause deficits in learning and memory (Huber, Gallagher, Warren & Bear, 2002).

Consistent with this idea, a correlation could be made between the importance of controlled protein synthesis and ID phenotypes. Multiple lines of evidence show that the ID phenotype FXS is characterized through the loss of the translational repressor protein FMRP. Absence of this RNA binding protein leads to excessive mGluR5 signalling causing increased LTD protein synthesis (Bear et al., 2004; Dolen et al., 2007). Previous studies linked miRNAs to neurodevelopmental disorders by reporting that FMRP associates with the miRNA pathway (Cheever & Ceman, 2009; Kocerha, Kauppinen & Wahlestedt, 2009). It is thought that miRNAs control neuronal gene circuitries important for local changes at the synapse and that their disruption leads to defects in post-transcriptional regulation which may lead to abnormalities in synaptic plasticity as seen in ID phenotypes (Kuhn et al. 2010; Jin et al., 2004). Further evidence for the hypothesis that miR-137 might be involved in a process preventing excessive protein synthesis is in line with previous work. A recent study suggested a possible correlation between miR-137 and ID phenotypes. ID patients had significantly reduced expression levels of miR-137, at the same time the patients displayed increased expression of miR-137 downstream targets (Willemsen et al., 2011). Moreover, it is also known that miRNAs might be involved in modulating synaptic plasticity (Lambert, Storm & Sullivan, 2010).

To conclude, the substantial upregulation of miR-137 by mGluR-LTD suggests a potential role of this miRNA in controlling mGluR-LTD related protein synthesis. Therefore it is tempting to speculate that miR-137 is involved in the machinery that downregulates the protein synthesis upon neuronal activity. We show that mGluR-LTD influences miR-137 expression within 15 minutes and the period before miR-137 is upregulated may allow sufficient time for protein synthesis to support mGluR-LTD.

Our results further reveal that the mechanism by which group I mGluRs upregulates miR-137 involves the activation of mGluR5. This is of particular interest as mGluR5 inhibition has been shown to have therapeutic effects in FXS. In the absence of FMRP, it is believed that mGluR5 signalling is exaggerated (Bear et al., 2004), this hypothesis is supported by the findings of pharmacological studies that showed that application of mGluR5 inhibitors are able to correct the abnormal protein synthesis in LTD (Dolen et al., 2007; Michalon et al., 2012).

We further speculate that mGluR5 regulates mature miR-137 expression following mGluR-LTD via calcium dependent intracellular increase. Group I mGluRs are G-Protein coupled receptors and act via second messenger cascades to stimulate intracellular calcium release (Fagni, Chavis, Ango & Bockaert, 2000).

Interestingly, it was reported that mGluR5 stimulation lead to sustained calcium responses, that were blocked by specific mGluR5 antagonists (Kumar, Fahey, Jong, Ramanan & O'Malley, 2012). Also only calcium oscillations were found in hippocampal cells that expressed mGluR5 but not mGluR1 (Flint, Dammerman & Kriegstein, 1999). Additionally, individual activation of mGluR1 did not induce calcium increase, only a single transient calcium release was detected (Uchino et al., 2004; Kim, Braud, Isaac & Roche, 2005). This indicates that mGluR1 and mGluR5 could differently contribute to the neuronal response following DHPG application and that blocking mGluR5 might subsequently alter intracellular calcium levels important for pre-miR-137 processing. Our speculation fits into the model suggested by Smalheiser and Lugli (2009), that Dicer is activated in a calcium dependent manner upon synaptic plasticity. Calcium increase beyond a threshold activates the protease calpain, which in turn activates the RNAse III activity of Dicer (Lugli, Larson, Martone, Jones & Smalheiser,

2005; Smalheiser & Lugli, 2009). This mechanism is a probable explanation for the effect we saw during blockade of mGluR5 following DHPG application. Mature miR-137 expression might have been inhibited due to decreased intracellular calcium release, causing Dicer to remain in the inactive form.

With regard to hippocampal long-term potentiation (LTP), mGluRs group I play also an important role in LTP. A common mechanism of mGluRs in their function in synaptic plasticity involves the intracellular calcium signal generated by activation of those receptors (Mukherjee & Manahan-Vaughan 2012). Previous work suggests that the intracellular calcium signal determines whether LTD or LTP is generated, with LTD requiring a modest rise in calcium (Cummings, Mulkey, Nicoll & Malenka, 1996) and LTP requiring a large rise beyond a critical threshold value (Malenka 1991). It is also well known that mGluR5 also triggers dendritic protein synthesis in LTP, therefore miR-137 might be also involved in regulating LTP mediated protein synthesis.

Together, our data suggests that miR-137 expression is linked to mGluR5 stimulation and that this miRNA might be a critical regulator for activity-dependent mRNA translation upon mGluR-LTD induction.

4.2 Mib1 protein is upregulated following LTD induction

The identification of the proteins that are synthesized following the induction of LTD remains challenging. Based on the finding that the ubiqutin ligase Mib1 is targeted by miR-137 and that FMRP also targets Mib1 (Smrt et al., 2010; Darnell et al., 2011), we investigated Mib1 expression following mGluR-LTD induction. Our data indicates an upregulation of the miR-137 target Mib1 upon mGluR-LTD induction. On the surface, this result might seem contradictory for miRNA mediated translation repression upon mGluR-LTD. However, recent work provides evidence for a temporal shift in downregulating target mRNAs by miRNAs. Lee et al. (2012) report that following neuronal activity miR-188 was upregulated and that its target mRNA NRP2 was 1 hour later downregulated. Therefore we speculate that miR-137 inhibits Mib1 translation at a later time point. To uncover the interaction between Mib1 and miR-137, future work should address a temporal expression pattern of Mib1 protein upon mGluR- LTD induction.

4.3 Assessing miR-137 role in synaptogenesis

The potential functions of miRNAs at dendritic spines are still under active investigation. Various miRNAs have been detected to be enriched and functionally active in the synaptic compartment. Our study investigated the function of the synaptically enriched miR-137 in neuronal maturation. The results show that overexpression of miR-137 in cultured hippocampal neurons did not affect synaptogenesis. However, previous studies report that miRNAs are able to influence neuronal development. For example, gain of function studies of miR-138 and miR-132 identified a specific function for those miRNAs during neuronal development. Overexpression of miR-138 reduced spine size in hippocampal neurons, whereas miR-132 increased spine volume (Siegel et al., 2009; Edbauer et al., 2010). This indicates that individual miRNAs might have specialized tasks in dendritic and synaptic development. Our study only investigated the effects of miR-137 for synaptogenesis, therefore it is possible that miR-137 is not specialized in influencing the gene circuitry important for synaptogenesis, but rather of other stages during neuronal development.

Despite the fact that our study implies no involvement of miR-137 in synaptogenesis, previous studies have shown that miR-137 overexpression reduced spine density in vivo (Smrt et al., 2010). This contrast in effects could be explained by differences in methodology. First, we conducted the experiments in vitro and the development of cultured hippocampal neurons might differ from developing neurons in vivo. Second, we waited for twelve or thirteen days before we live-labelled the transfected neurons for the synaptic protein GluA1, during this period the miR-137 mimic could be inactivated or degraded which might explain that no effect is seen. Consequently, it is possible that miR-137 might affect synaptogenesis, therefore further investigation should shift the approach to in vivo studies or utilize lentiviral transfection to prevent possible inactivation of miR-137 overexpression and to increase transfection efficiency. Additionally, transfected neurons should be imaged within 48-hours post-transfection.

5. Conclusion

The present study provides mechanistic insight into the role of miRNAs and synaptic plasticity. We

provide further motivation to investigate whether miRNA mediated regulation of mRNA translation represents a general mechanism to regulate local protein synthesis following neuronal activity.

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7. References

Anggono, V. & Huganir R.L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. *Current Opinion Neurobiololgy, 22*(3), 461-469.

Bassell, G. J. & Warren, S. T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron, 60*(2), 201-214.

Bear, M. F., Huber K. M., & Warren, S.T. (2004). The mGluR theory of fragile X mental retardation. *Trends in Neuroscience, 27*(7), 370-377.

Beaumont, V., Zhong N., Fletcher R., Froemke, R. C. & Zucker, R. S. (2001). Phosphorylation and local presynaptic protein synthesis in calcium- and calcineurin-dependent induction of crayfish long-term facilitation. *Neuron, 32*(3), 489-501.

Cheever, A. & Ceman, S. (2009). Phosphorylation of FMRP inhibits association with Dicer. *RNA, 15*(3), 362-366.

Choe, E. A., Liao, L., Zhou, J. Y., Cheng, D., Duong, D. M., Jin, P., … & Peng, J. (2007). Neuronal morphogenesis is regulated by the interplay between cyclin-dependent kinase 5 and the ubiquitin ligase mind bomb 1. *Journal of Neuroscience, 27*(35), 9503-9512.

Citri, A. & Malenka, R. C. (2008). Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology, 33* (1), 18-41.

Collingridge, G. L., Peineau, S., Howland, J. G., & Wang, Y. T. (2010). Long-term depression in the CNS. *Nature Reviews Neuroscience, 11*(7), 459-473.

Costa-Mattiolo, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*(1), 10-26.

Cummings, J. A., Mulkey, R. M., Nicoll, R. A. & Malenka, R. C. (1996). Ca2+ signaling requirements for long-term depression in the hippocampus. *Neuron, 16*(4), 825-833.

Darnell, J. C., van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., … & Darnell, R. B., (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell, 146*(2), 247-261.

Dolen, G., Osterweil, E., Rao. B. S., Smith, B., Auerbach, B. D., Chattarji, S., & Bear, M. F. (2007). Correction of fragile X syndrome in mice. *Neuron, 56*(6), 955-962.

Edbauer, D., Neilson, J. R., Foster, K. A., Wang, C. F., Seeburg, D. P., Batterton, M. N., … & Sheng, M. (2010). Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron, 65*(3), 373-384.

Fagni, L., Chavis, P., Ango, F., & Bockaert, J. (2000). Complex interactions between mGluRs, intracellular Ca2+ stores and ion channels in neurons. *Trends in Neuroscience, 23*(2), 80-88.

Feng, W. & Feng, Y. (2011). MicroRNAs in neural cell development and brain diseases. Science *China Life Sciences, 54*(12), 1103-1112.

Flint, A. C., Dammerman, R. S., & Kriegstein, A. R. (1999). Endogenous activation of metabotropic glutamate receptors in neocortical development causes neuronal calcium oscillations. *Proceedings of the National Academy of sciences of the United States of America, 96*(21), 12144-12149.

Gray, E. G. & Whittaker, V. P. (1962). The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. *Journal of Anatomy, 96*, 79-88.

Gunaratne, P. H., Creighton, C. J., Watson, M., & Tennakoon, J. B. (2010). Large-scale integration of MicroRNA and gene expression data for identification of enriched microRNA-mRNA associations in biological systems. *Methods Molecular Biology 667*, 297-315.

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., Mcgaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *Journal of Neuroscience, 20*(11), 3993-4001.

Huber, K. M., Gallagher, S. M., Warren, S. T., Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings Of the National Academy of sciences of the United States of America, 99*(11), 7746-7750.

Huber, K. M., Kayser, M. S., & Bear, M. F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science, 288*(5469): 1254-1257.

Hunsberger, J. G., Austin, D. R., Chen, G., & Manji, H. K. (2009). MicroRNAs in mental health: from biological underpinnings to potential therapies. *Neuromolecular Medicine, 11*(3), 173-182.

Hunt, D. L. & Castillo, P. E. (2012). Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Current Opinions Neurobiology, 22*(3), 496-508.

Izumi, Y. & Zorumski, C. F. (2012). NMDA receptors,

mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. *Neuropsychopharmacology, 37*(3), 609-617.

Jin, P., Zarnescu, D. C., Ceman, S., Nakamoto, M., Mowrey, J., Jongens, T. A., ... & Warren, S. T. (2004). Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nature Neuroscience, 7*(2), 113-117.

Kim, C. H., Braud, S., Isaac, J. T., & Roche, K. W. (2005). Protein kinase C phosphorylation of the metabotropic glutamate receptor mGluR5 on Serine 839 regulates Ca2+ oscillations. *Journal of Biological Chemistry, 280*(27), 25409-25415.

Kim, V. N. (2005). MicroRNA biogenesis: coordinated cropping and dicing. *Nature Reviews Molecular Cell Biology, 6*(5), 376-385.

Kindler, S., & Kreienkamp, H. J. (2012). Dendritic mRNA targeting and translation. *Advances in Experimental Medicicine and Biology, 970*, 285-305.

Kocerha, J., Kauppinen, S., & Wahlestedt, C. (2009). microRNAs in CNS disorders. *Neuromolecular Medicine 11*(3), 162-172.

Kuhn, D. E., Nuovo, G. J., Terry, A. V. Jr., Martin, M. M., Malana, G. E., Sansom, S. E., … & Elton, T. S. (2010). Chromosome 21-derived microRNAs provide an etiological basis for aberrant protein expression in human Down syndrome brains. *Journal of Biological Chemistry, 285*(2), 1529-1543.

Kumar, V., Fahey, P. G., Jong, J., Ramanan, N., & O'Malley, K. L. (2012). Activation of intracellular metabotropic glutamate receptor 5 in striatal neurons leads to up-regulation of genes associated with sustained synaptic transmission including Arc/Arg3.1 protein. *Journal of Biological Chemistry, 287*(8), 5412-5425.

Lambert, T. J., Storm, D. R., & Sullivan, J. M. (2010). MicroRNA132 modulates short-term synaptic plasticity but not basal release probability in hippocampal neurons. *PLoS One, 5*(12), e15182.

Lee, K., Kim, J. H., Kwon, O. B., An, K., Ryu, J., Cho, Y. H., … & Kim, H.S. (2012). An activity-regulated microRNA, miR-188, controls dendritic plasticity and synaptic transmission by downregulating neuropilin-2. *Journal of Neuroscience, 32*(16), 5678-5687.

Lugli, G., Larson, J., Martone, M. E., Jones, Y., & Smalheiser, N. R. (2005). Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. *Journal of Neurochemistry, 94*(4), 896-905.

Malenka, R. C. (1991). Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron, 6*(1), 53-60.

Manakov, S. A., Grant, S.G., & Enright, A. J. (2009). Reciprocal regulation of microRNA and mRNA profiles in neuronal development and synapse formation. *BMC Genomics, 10*, 419.

Mathonnet, G., Fabian, M. R., Svitkin, Y. V., Parsyan, A., Huck, L., Murata, T., ... & Sonenberg, N. (2007). MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. *Science, 317*(5845), 1764-1767.

Michalon, A., Sidorov, M., Ballard, T. M., Ozmen, L., Spooren, W., Wettstein, J. G., … & Lindeman, L. (2012). Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron, 74*(1), 49-56.

Moult, P. R., Gladding, C. M., Sanderson, T. M., Fitzjohn, S. M., Bashir, Z. I., Molnar, E. & Collinridge, G. L. (2006). Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. *Journal of Neuroscience, 26*(9), 2544-2554.

Muddashetty, R. S., Nalavadi, V. C., Gross, C., Yao, X., Xing, L., Laur, O., … & Bassel, G. J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Molecular Cell, 42*(5), 673-688.

Mukherjee, S., & Manahan-Vaughan, D. (2012). Role of metabotropic glutamate receptors in persistent forms of hippocampal plasticity and learning. *Neuropharmacology*.

Nomura, T., Kimura, M., Horii, T., Morita, S., Soejima, H., Kudo, S., & Hatada, I. (2008). MeCP2-dependent repression of an imprinted miR-184 released by depolarization. *Human Molecular Genetics, 17*(8), 1192-1199.

Olde Loohuis, N. F., Kos, A., Martens, G.J., van Bokhoven, H., Nadif Kasri, N., & Aschrafi, A. (2012). MicroRNA networks direct neuronal development and plasticity. *Cell Molecular Life Sciences, 69*(1), 89-102.

Petersen, C. P., Bordeleau, M. E., Pelletier, J., & Sharp, P. A. (2006). Short RNAs repress translation after initiation in mammalian cells. *Molecular Cell, 21*(4), 533-542.

Poon, M. M., Choi, S.-H., Jamieson, C. A. M., Geschwind, D. H., & Martin, K. C. (2006). Identification of process-localized mRNAs from cultured rodent hippocampal neurons. *Journal of Neuroscience, 26*, 13390-13399.

Ruberti, F., Barbato, C., & Cogoni, C. (2012). Targeting microRNAs in neurons: Tools and perspectives. *Experimental Neurology, 235*(2), 419-26.

Schmittgen, T. D. & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocol, 3*(6), 1101-1108.

Schratt, G. (2009). microRNAs at the synapse. *Nature Reviews Neuroscience, 10*(12), 842-849.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., & Greenberg, M. E. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature, 439*(7074), 283-289.

Siegel, G., Obernosterer, G., Fiore, R., Oehmen, M., Bicker, S., Christensen, M., … & Schratt, G. M. (2009). A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. *Nature Cell Biology, 11*(6), 705-716.

Siegel, G., Saba, R., & Schratt, G. (2011). microRNAs in neurons: manifold regulatory roles at the synapse. *Current Opinions Genetics & Development, 21*(4), 491-497.

Smalheiser, N. R., & G. Lugli (2009). microRNA regulation of synaptic plasticity. *Neuromolecular Medicine, 11*(3), 133-140.

Smrt, R. D., Szulwach, K. E., Pfeiffer, R. L., Li, X., Guo, W., Pathania, M., … & Zhao, X. (2010). MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem Cells, 28*(6), 1060-1070.

Uchino, M., Sakai, N., Kashiwagi, K., Shirai, Y., Shinohara, Y., Hirose,K., … & Saito, N. (2004). Isoform-specific phosphorylation of metabotropic glutamate receptor 5 by protein kinase C (PKC) blocks Ca2+ oscillation and oscillatory translocation of Ca2+-dependent PKC. *Journal of Biological Chemistry, 279*(3), 2254-2261.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., van Roy, N., de Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology, 3*(7), RESEARCH0034.

Waites, C. L., Craig, A. M., & Garner, C. C. (2005). Mechanisms of vertebrate synaptogenesis. *Annual Review Neuroscience, 28*, 251-274.

Waung, M. W., & Huber, K. M. (2009). Protein translation in synaptic plasticity: mGluR LTD, Fragile X. *Current Opinion Neurobiology, 19*(3), 319-326

Wienholds, E., Kloosterman W. P., Miska, E., Alvarez-Saavedra, E., Berezikov, E., De Bruijn, E., … & Plasterk, R.H. (2005). MicroRNA expression in zebrafish embryonic development. *Science, 309*(5732), 310-311.

Willemsen, M. H., Vallès, A., Kirkels, L.A., Mastebroek, M., Olde Loohuis, N., Kos., A., … & Kleefstra, T. (2011). Chromosome 1p21.3 microdeletions comprising DPYD and MIR137 are associated with intellectual disability. *Journal of Medical Genetics, 48*(12), 810-818.

Abstracts

Proceedings of the Master's Programme 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

Increased Basal Ganglia White Matter Integrity Associated with Impaired Updating During Working Memory

Carsten Bundt, Martine van Schouwenburg, Roshan Cools

Working memory (WM), the ability to maintain and update information in a goal-directed way is crucial for successful behavior. Whereas attentional maintenance is commonly associated with the prefrontal cortex (PFC), recent studies emphasize the crucial role of the basal ganglia (BG) in attentional information updating. Interestingly, primate and patient studies suggest that attentional maintenance and attentional updating during working memory are reciprocal in its nature. However, the evidence for this reciprocity in healthy individuals is lacking. To assess the potentially inverse relationship of these processes and to pinpoint it's neural basis, healthy subjects were scanned using diffusion tensor imaging and were subsequently tested on a delayed match to sample task. Behaviorally, results do indicate an inverse association between attentional maintenance and attentional updating in individuals during WM. Moreover, this reciprocity was correlated with the white matter integrity in/around the basal ganglia extending into prefrontal, thalamic, and midbrain regions. These results highlight the interplay between PFC-BG regions in moderating attentional maintenance and attentional updating during working memory.

Doors and Space: Examining the Association Between Object, Location, and Context

Daniel Fängström, Alejandro Vicente-Grabovetsky, Christian Doeller

The relation between memory and space is frequently noticeable, although at times difficult to accurately determine. Certain objects are bound to certain locations, and particular places bring to mind particular objects. Previous behavioural experiments have suggested an effect on object memory simply by walking through a doorway. In this study, we set out to replicate the behavioural effect by using a similar experimental design as previous studies. The results we obtained differed from previous literature and further studies of the object-location relation were performed using a greatly different layout. The association of objects, location, and overall context was examined in a virtual reality environment that was altered from previous studies. Participants were trained to remember associations between these three factors and performed a reactivation task in a magnetic resonance imaging (MRI)-scanner. The experiment provided results of activation of parahippocampal areas in object-location memory, although examining the data using multivariate representational similarity analysis gave less clear results.

Entrainment of Slow Brain Activity Preceding Leg Movements

Simone Heideman, Peter Praamstra, Erik te Woerd, Floris de Lange

The time course of the contingent negative variation (CNV), as well as power in the alpha and beta range have been shown to entrain to regular task rhythms, revealing implicitly evoked anticipatory timing. Thus far these effects have been established for manual responses only. In the current experiment the same approach was used to investigate entrainment preceding leg movements. In this experiment, people were standing and responded to series of rhythmically presented arrow stimuli by making brisk leg movements. The standard stimulus onset asynchrony differed between series and was either 1500 or 2000 ms, sometimes perturbed by a deviant interval. Entrainment was visible in the CNV time course, with the maximum amplitude being reached just before the next stimulus was presented. Entrainment was also expressed in the pattern of alpha and beta (de)synchronization that similarly adjusted to the rhythm of the task. CNV scalp topographies were different from a previous experiment using manual responses, suggesting effector dependency of CNV activity. Four of sixteen participants were analyzed separately because they showed a deviant CNV morphology, while showing similar oscillatory patterns. The results are relevant with regard to Parkinson's Disease, where problems are found in rhythm processing and temporal preparation. Investigation of the neural correlates of leg movement entrainment is important for linking entrainment to cueing of gait and relevant behavioral outcomes in this group.

Improving Memory by Taking Brain States into Account

Maarten van den Heuvel, Til Ole Bergmann, Ole Jensen

Memory is a long studied topic in neuroscience, but so far, surprisingly little attention has been paid to practical application of our present knowledge. In this light, we ask the question: can we put our theoretical knowledge to practical use by improving memory by taking brain states into account? Subsequent memory effects (SMEs) - brain signals during learning of an item that predict later memory performance for that item - have been observed in various brain regions in both functional magnetic resonance imaging (fMRI) and magnetoencephalographic (MEG)/electroencephalographic (EEG) studies. We attempt to test the feasibility of using knowledge about SMEs to construct a semi-online MEG Brain-Computer-Interface (BCI) setup where trials in a memory task are selectively re-introduced on the basis of single-trial alpha (7-13Hz) power during learning. This setup is based on the notion that re-introducing/repeating items that would otherwise be forgotten - as predicted by alpha power - should improve memory performance compared to random repeats on a subsequent recognition memory task. Based on the notion of alpha as active inhibition, we expect higher alpha in task-irrelevant areas to predict a higher chance of successful memory encoding, while higher alpha in task-relevant areas is expected to predict forgetting. The feasibility of this setup is assessed in three different memory tasks: words, complex objects and photographs. All tasks are separated into a stimulus interval (visual presentation) and a rehearsal interval (blank screen). In the word task, we expect higher alpha - inhibition - in task-irrelevant right hemispheric areas and lower alpha - disinhibition - in task-relevant left cortical areas to be predictive of good memory performance. In the visual (object and photograph) tasks we mainly expect lower alpha in task-relevant right hemispheric or bilateral areas to be beneficial, as well as higher left hemispheric alpha. The data mostly confirm the expected sites of the SME, and show that a performance increase can be obtained by repeating items on the basis of alpha power in these sites. However, the predicted performance increase - although significant - is rather low, in part because most subjects do not benefit strongly from re-introducing items that would otherwise be forgotten and partly because initial high performance leaves little room for improvement by repeating. Therefore, before taking the proposed BCI online, we may need to rethink the setup in order to gain maximal benefit from the SMEs that have been found.

Neural Mechanisms of Second Language Vocabulary Learning in Adults Under Incidental and Intentional Learning Conditions

Toru Hitomi, Annika Hultén, Hubert Fonteijn, Roel Willems

Learning a new language in adulthood is mundane occurrence in an ever more globalized world. Effects of intention during learning new words on behavioral and neural consequences are however not well understood. In this functional magnetic resonance imaging (fMRI) study we aimed at revealing neural mechanisms underlying second language (L2) vocabulary learning and effects of intentionality during encoding form-meaning associations. Dutch native speakers were presented of a Japanese word-picture pairs of common objects under two learning conditions that differed in terms of the level of intention towards learning. In the Intentional condition, participants were instructed to intentionally learn the Japanese word, whereas in the other Incidental learning condition, they were told that these words did not need to be learned as they would not be tested. On the next day, their learning was assessed with a picture naming task, which required participants to speak aloud a name of a presented picture. The blood-oxygen dependent level signals during both learning (encoding) and testing (retrieval) phases were measured with fMRI. Behaviorally higher rate of successful learning of the intentionally learned words, yet also found successful learning of incidentally trained words. In the brain, intentional learning activated the regions in the left inferior frontal gyrus (BA 45), the left superior frontal gyrus (BA 6) and the left middle frontal gyrus more compared to incidentally trained items, indicating a higher load on the phonological processing. Conversely, incidental training showed a higher activation in the right supramarginal gyrus, reflecting less efficient phonological processing. A region of interest analysis demonstrated that the signal level of the left hippocampus was positively correlated with behavioral performance in both learning conditions, suggesting the left hippocampus plays important roles at the initial encoding phase of word learning regardless of intentionality. The left inferior parietal lobe also showed a positive correlation only during the intentional condition, indicating intentionality during encoding modulated the neural responses during retrieval. In conclusion, the intentionality during encoding modulated the neural mechanisms of L2 word learning.

Dynamics of the Extracellular Potential

Charl Linssen, Paul Tiesinga

Electrodes inserted in the brain measure the extracellular potential (ECP), from which through high pass filtering the multi unit activity is obtained, representing spiking activity close to the electrode, and from which through low pass filtering the local field potential is extracted, which represents pop- ulation activity. The ECP is generated by currents through the neuronal cell membrane due to action potentials and synaptic inputs. In the standard approach it is theoretically determined as the solution of the Poisson equation with these currents as sources. This implies that the ECP directly follows the dynamics of the source without any frequency dependent attenuation, which is inconsistent with experimental measurements of a frequency dependent dielectric constant. Our primary objective was to determine the time scales relevant for ECP dynamics. We determined that the propagation effects in Maxwell's equations were too fast to affect the dynamics and indeed found the quasistatic formulation to be adequate. We further evaluated the role of the geometry of extracellular space, Ohmic transport, diffusion and polarization within the context of the Poisson-Nernst-Planck equation in numerical simulations using the Finite Element Method. Previous studies by Bédard et al. (2004; 2006) have suggested that charge dynamics in extracellular space is dominated by the Maxwell-Wagner timescale, which is in the order of nanoseconds for neural tissue. Hence, in order to account for the frequency dependence of the dielectric constant for low frequencies, the conductivity near the cell membrane needs to be cho- sen much smaller than is supported by experimental measurements. We find that polarisation effects introduce a timescale proportional to the neuron size and the Debye length, and inversely proportional to the diffusion constant. This timescale is in much closer agreement with experimental data and suggests that geometry is a crucial determinant of charcteristic timescales in the extracellular potential and frequency filtering properties of extracellular space.

Simulation Types During Natural Story Comprehension: A Functional Magnetic Resonance Imaging Study

Annabel Nijhof, Roel Willems, Peter Hagoort

Despite the great amount of neuroscientific language research being done, little is known about the comprehension of language in its natural form. In the current study functional magnetic resonance imaging (fMRI) was used to investigate if and how people engage in (different kinds of) mental simulation when listening to story fragments. Brain activity was found in different regions for different kinds of story content, among which regions that are normally associated with perception and action, but also several regions of the Theory-of-Mind network. Besides, there were strong indications of individual differences in how much participants engaged in (different kinds of) mental simulation. The results are in line with existing theories about higher-order language comprehension from both literary science and cognitive science.

Pain Perception and the Relation with EEG Changes in a Rat Model of Endometriosis

Maria Panagiotou, Saskia Mulder, Ard Peeters, Clementina van Rijn

Chronic pain is a widespread disabling health problem, pathophysiology and effective treatments of which are elusive. Endometriosis is a chronic pain condition, defined as the presence of endometrial tissue outside the uterus, causing pelvic pain. Endometriosis in estrous animals is induced by transplanting endometrial tissue to ectopic sites. Our study is investigating the effect of ectopic endometrial lesions on pain behavior and on the electroencephalography (EEG) changes in WAG/Rij rats. By conducting behavioral experiments, using the Skinner box apparatus, we demonstrate that the endometriosis animals cross the separator less often compared to controls and shams due to abdominal pain; results have not been correlated to the estrous cycle. In addition, since all animals of this inbred strain show Spike Wave Discharges (SWDs) in the electroencephalogram, a correlation has been found between SWDs and the estrous day of the cycle. No significant differences are reported regarding the theta and delta peak and the mean delta thalamus and cortex activity between groups; only a trend of increased mean theta activity in thalamus of the endometriosis animals can be reported. Nevertheless, interesting differences can be seen between dark and light conditions of both groups in the electroencephalogram.

The Role of Emotional Discourse on Metaphorical Language Comprehension

Dalya Samur, Vicky Tzuyin Lai, Peter Hagoort, Roel Willems

We often talk about our emotions metaphorically and sometimes with the use of embodied expressions; when we are happy we hold on to our joy and when we are sad we push the sorrow away. It is shown that these metaphorical expressions rely on sensory-motor areas similar to the literal ones. Even though the role of emotion on metaphor use has been prominent in metaphor research, how our brain relates to this association regarding the sensory-motor mapping has not been addressed yet; thus we examined the interaction between emotional discourse and metaphorical language processing through sensory-motor activations. We created 147 stories with ambiguous target sentences (e.g., he got it) that the action/motion verb can be metaphorical (he understood the idea) or literal (he caught the ball) depending on their preceding discourse. In addition, we varied how emotional the preceding context is: High-emotional and low-emotional. We hypothesized that context can turn these target sentences metaphorical or literal, and when the context is more emotionally valenced, the metaphorical target sentence map on sensory-motor areas in a different degree than the literal one. The interaction effect is found on Visual Motion (MT+) areas; when the context is emotional then the mapping gets stronger for metaphorical condition and remains same for the literal condition. The primary motor cortex gets more activated for high emotional context in metaphorical condition and not in literal one. Thus, this study provides evidence for the fact that the emotional context can change how much sensory motor areas are involved in language processing, but such modulation is only important for metaphors, not for the literal. In addition to that, sensory motor activation in language comprehension is context-dependent.

Behavioural Facilitation Through Hippocampal Sequence Learning

Peter Smulders, Branka Milivojevic, Floris de Lange, Christian Doeller

The goal of the study was to investigate how sequences of events are encoded in the hippocampus and how this hippocampal sequence learning facilitates behaviour by predicting upcoming events and encoding uncertainty. Functional magnetic resonance imaging (fMRI) was used to record brain activity while participants learned both predictable and uninformative sequences consisting of three items (triplets). For the predictable sequences, the final item and response could be predicted based on the first two items in the triplet, while for the uninformative sequences, the final item and response could not be predicted from the preceding two items. Using a combination of behavioural analyses, univariate fMRI analyses and multi-voxel pattern analyses, evidence was found for sequential encoding of future events in the hippocampus. In addition, we observed a link between this hippocampal sequential encoding and response facilitation to predictable items via a hippocampal-striatal mechanism, and observed evidence for a link between hippocampal sequential encoding and the expectation of uncertainty via a hippocampal-striatial-Anterior Cingulate Cortex mechanism. These effects occurred before the critical third item, suggesting that behavioural facilitation occurs as soon as sufficient evidence is available to predict which events will occur in the future.

Functionally Defined Amygdalar Subdivisions as Imaging Biomarkers for Predicting Behaviour in Autism

Wei Zhang, Wouter Groen, Maarten Mennes, Christian Beckmann, Jan Buitelaar

Deficits in social interaction are among the core characteristics of autism spectrum disorders. Since the amygdala, a subcortical structure in the brain, is involved in social perception and behaviour, its role in the etiology of autism has received much scientific attention. Yet, many previous neuroimaging studies have investigated the amygdala as a single entity, while it is composed of multiple nuclei with different functions including social perception, affiliation and aversion. Here we used resting-state functional magnetic resonance imaging in order to functionally segment the amygdala into three subdivisions (subserving social perception, affiliation and aversion) in 12- to 21-year old participants with autism and matched controls. We measured the volume of each segmented amygdalar subdivision and found that participants with autism had larger left dorsal amygdala volume (subserving the aversion network; $p < 0.05$) and larger right ventrolateral amygdala volume (subserving the social perception network; $p < 0.01$). Furthermore, we also found the volume of the left dorsal amygdala correlated with social behavior in the autism group ($p < 0.05$), but not in the control group. These results indicate that the functionally defined subdivisions of the amygdala are abnormal in autism. The volume-behavior correlation provides a novel biomarker and directly links functionally defined amygdala subdivision with real life social skills in autism.

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