Table of Contents

From the Editors-in-Chief of the CNS Journal

Dear Reader,

We are pleased to present the second issue of the sixth volume of the Proceedings of the Master's Programme Cognitive Neuroscience of the Radboud University. The increase in high quality research being conducted in the Master's programme in the 2010/2011 academic year, has resulted in the publication of a second issue. We are very proud to have contributed to this new milestone and to present for the first time in the history of the CNS journal a second issue published in a single academic year. This journal is unique not only because it is exclusively run by students but also because it strives to follow the publishing procedures of highly-ranked, professional scientific journals.

This issue continues a tradition that was introduced six years ago by students in the Research Master's Cognitive Neuroscience at Radboud University. The journal is an important outlet for the research conducted by students in the Master's programme and gives them the opportunity to develop writing, editing and reviewing skills. This experience with academic publishing is an important asset for young researchers entering the scientific community and complements the first-rate training in cognitive neuroscience and valuable hands-on research experience offered by the interdisciplinary Master's programme at Radboud University.

In this issue you will find articles that cover a variety of scientific topics and methodologies: genetics, immunohistochemistry, brain-computer interface and pharmacology. This selection of theses showcases the diversity of neuroscientific research carried out at Radboud University. In addition, in this issue you will find the abstracts of all articles submitted this year. A complete version of these articles can be found on our website: www.ru.nl/master/cns/journal.

This issue is the culmination of the dedication and hard work of the authors, reviewers, and all members of the journal committee. We would like to thank them cordially for their contribution to the realization of the journal you are now holding in your hands. We hope that you will share our excitement and pride after reading it. We wish you a pleasant journey through the pages of this journal and through the world of cognitive neuroscience!

On behalf of the CNS Journal board we thank you for your interest in the CNS Journal.

Klodiana-Daphne Tona & Flora Vanlangendonck
Editors-in-Chief


Photo: Luis Victor

From the Director of the Donders Centre for Neuroscience

Dear Reader,

You are holding the newest issue of the Proceedings of the Master's Programme Cognitive Neuroscience in your hands. This serious scientific journal, which already pre-published numerous influential articles in high-profile journals over its six years of existence, is initiated and edited by students only. The educational aim is to understand scientific publishing as a comprehensive process. It entails not only the side that an ordinary scientist sees when writing, submitting, and revising a manuscript, it also includes the side of the editor covering reviewer selection, assessing response letters, selecting the articles, and handling the entire production process. Beside this crucial educational emphasis, each and every issue of this journal is an achievement that all authors and editors are rightly proud of. There is a nice tradition that editorial board members hand over a copy or two of the Proceedings to guests of the Donders Institute. The responses are overwhelmingly positive. For instance, John Gabrieli (MIT) told me later that he was truly impressed by the professionalism of the journal and the quality of the research published therein.

And now the second issue of the sixth volume is published. It is again a pleasure to see how it continues to strive for scientific quality while showing the diversity of neuroscientific research at the Donders Institute. The research presented pulls research fields into the focus of cognitive neuroscience that where recently still at its periphery at best. This issue contains papers reporting neurocognitive research on brain-computer interfaces, pharmacology, genetics, and mitochochondrial biology . Neuroscience is by its nature multi- and interdisciplinary, but this breadth of approaches with a stringent focus on our four research themes is a unique feature of the Donders Institute and its Graduate School perfectly presented in this issue of the Proceedings. However, it is important to note that scientific publishing is a selection process and many manuscripts that were rejected from this journal describe also high-quality research that will be visible in other scientific journals soon.

Finally, I would like to congratulate and thank every author, referee and editor. You are making an important contribution to the distinguished esteem that the Donders Institute and its Graduate School has achieved.

Enjoy reading this "preview" - coming soon to a top journal near you!
All the best,

Guillén Fernández
Director of the Donders Centre for Neuroscience

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

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Brain-Computer Interfaces with User Chosen Features - A Free BCI

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In brain-computer interface (BCI) research, two approaches can be distinguished. Either the experimenter explicitly instructs the user to perform a certain mental task that evokes a known brain activity, or the user has to implicitly learn how to control a feedback signal that represents aspects of his brain activity. Both these approaches suffer from inter-subject and inter-session variability. A particular case of inter-subject variability is BCI illiteracy, the problem that some subjects cannot produce the brain activity needed to control conventional BCI paradigms. We will show an approach that aims to improve current BCI approaches in these regards. In a single session experiment, users are presented rich feedback representing a manifold feature space. Users can freely find controllable aspects in this complex feedback environment without explicit task instruction. This is statistically verified with a binary regularized kernel logistic regression and yields classification rates significantly different from chance for five out of eight users ($p<.01$). However, we also show that subjectively important aspects of the feedback do not match objectively important aspects in the feature space, leading to the necessity of mutual adaptation for future studies. Freeing users from rigid task instructions, and hence allowing dynamic and mutual interaction within the system, might be a way to overcome BCI illiteracy.

Keywords: free bci, user centered, user-customized, rich feature bci, brain-computer interface, self-regulation, neurofeedback, user adaptation, operant conditioning

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1. Introduction

Learning to control a brain-computer interface (BCI) means learning to voluntarily produce detectable patterns of brain activity without using the peripheral nervous system (cf. Wolpaw et al., 2000). Brain activity is recorded invasively or non-invasively using techniques like electrocorticography (ECoG) or electroencephalography (EEG), respectively. While invasive methods have a much better signal-to-noise ratio, thus a better signal to work with, there is a risk of tissue damage, and hence impairing brain functioning (see Haselager, Vlek, Hill & Nijboer, 2009). This paper and all cited papers are on non-invasive BCIs using EEG unless stated otherwise.

In both approaches, the recorded brain activation patterns are sent to a computer that processes and interprets this data to produce some control signal. Various external output devices can be connected to that computer, like a robot arm (Bell, Shenoy, Chalodhorn & Raod, 2008), a computer screen visualizing the control signal for example as done in a P300 speller (Farwel & Donchin, 1988) or in form of a mouse cursor (McFarland, Krusienski, Sarnacki & Wolpaw, 2002). Patients who have lost or will lose control of their muscular system, as for example in amyotrophic lateral sclerosis, may benefit most from brain-computer interfacing as a new communication channel.

In contrast to learning other, more natural skills like body movements, there is no inner sense for producing detectable brain patterns. Therefore, perceptual senses such as vision or audition have to be exploited here: Through extrinsic feedback provided by the experiment the user can understand when his brain patterns are detected. This way, users can successfully learn to control a BCI and adapt their mental strategy according to the extrinsically provided feedback.

Learning and adaptation can both be explained using a reinforcement model, that is, by computing an error signal that compares the result of an action with the goal to be achieved. The human body utilizes feedback control systems that control for the status of the current behavior and the goal in an online manner, that is, correcting for errors during performance (see Desmurget & Grafton, 2000). For example, in neuromuscular control feedback is provided by internal muscle senses (proprioceptive feedback and kinesthesis) as well as visual feedback. In contrast to visual feedback, proprioceptive feedback and kinesthesis exclusively use pathways within the central nervous system and are therefore considerably faster than pure visual feedback, leading to improved learning and faster adaptation. For a more detailed description of the neuromuscular system see Scott (2003).

From early life on, people learn and adapt to their environment and its behavior by making use of feedback signals. One popular example is how babies learn to speak. Infants learn to produce speech sounds by imitating perceived sounds – a process that is called babbling (Oller & Eilers, 1988). A very similar mechanism applies when infants learn to control body movement through body babbling (Meltzoff & Moore, 1997). Here, a key claim is that the infant performs imitation with the help of an active feedback loop. This feedback serves as a matching-to-target process by an error signal, that is encourages the infant to imitate as good as possible.

In brain-computer interfacing, extrinsically provided feedback is the only source of information for the users about their performance. In learning to control a device with brain activity, two approaches can be distinguished: Explicit and implicit paradigms. The implicit paradigm is also called neurofeedback. In the neurofeedback approach, a feedback signal is based on some single feature of the recorded signal, for example, a horizontal bar that extends or contracts according to the ratio of power in frontal theta-band (4-8 Hz) and frontal beta-band (12-20 Hz) (Leins et al., 2007). The subject has no explicit task except trying to control the presented feedback. This means that the user implicitly learns to create a pattern of brain activity that evokes a suitable response in the presented feedback. In this approach, subjects learn to regulate their electrical brain activity in as few as five to seven sessions (Walker, 2010). A session usually lasts between one hour and half a day. After learning, subjects can voluntarily and reliably control the feedback signal. Such implicit learning of control over an output signal related to own (mental) actions resembles a very intuitive way of learning – similar to babies learning through (implicit) babbling.

In contrast to the neurofeedback approach, the experimenter can also specify a mental task explicitly. Such mental tasks are known to elicit reliable patterns of brain activity, though with individual difference. For example, an imagined arm movement usually elicits a two-folded pattern in the beta-band. On the hemisphere contralateral to the movement side, an event-related desynchronization (ERD) occurs, that is a decrease in power in this frequency band. In contrast, on the ipsilateral hemisphere, there is an event-related synchronization (ERS), that is an increase in power. Both the ERD and the ERS

are located around the subject's motor cortex (Pfurtscheller & Lopes da Silva, 1999). Most users can learn to control an 'explicit' BCI in a single session due to explicit task instructions and machine learning algorithms that adapt to the user specific brain activity pattern.

Few approaches combining the explicit with the implicit approach to BCI have been attempted so far. In Wolpaw and McFarland's study (2004), users had to implicitly learn to control a mouse cursor on a computer screen. The cursor position was controlled by explicit experimenter-defined features of the user's brain activity. However, this control signal was based on features usually obtained from imagined movement. Accordingly, subjects reported that they exploited imagined movements in the beginning of the experiment, but could control the cursor implicitly during the course of the experiment, without giving a clear report on how they were doing this. This study consisted of two to four half hour sessions a week, in total 22 to 68 sessions. Congedo, Lubar and Joffe (2004) presented a feedback signal based on a complex function of the raw power measurements of the alpha and beta bands. These two features can be modulated by explicit tasks, i.e. relaxation and motor related behavior. Users were trained with an implicit learning paradigm and were able to enhance beta and slightly suppress alpha activity near the anterior cingulate cortex in as few as six sessions.

While extracranial EEG BCI experiments focus on learning a single feature in isolation or a combination of two features, more complex feature spaces were investigated using invasive approaches. Miller et al. (2007) and Lachaux et al. (2007) found direct correlates of behavioral paradigms and acoustic perception on real time estimates of the frequency spectrum of brain activity recorded by ECoG and intracranial EEG, respectively. Thus, time-frequency estimations of brain oscillations can be used as real-time feedback signals in BCI as they represent correlates of (mental) actions– although they might be very complex. By feeding back comprehensible aspects of such time-frequency correlates, BCI users can learn about the influence of their (mental) actions on the feedback.

Despite huge advances in recent years, research in brain-computer interfacing is still confronted by two problems: inter-subject variability and inter-session variability. Inter-subject variability describes the issue that brain activity patterns vary significantly across subjects for a specific mental task. This also leads to a significant performance difference across subjects, even after more than twenty sessions (e.g. Wolpaw et al., 2004). Furthermore, in all known BCI approaches, a substantial group of users cannot produce reliable patterns of brain activity – a problem generally termed BCI illiteracy, which is a particular case of inter-subject variability. Recently, Vidaurre and Blankertz (2009) claimed that BCI illiteracy could be solved by applying adaptive machine learning algorithms to reduce inter-subject variability. However, the reliability of current paradigms is further diminished by inter-session variability. This variability describes the fact that nearly all subjects are unable to reliably produce stable brain patterns across sessions. They may perform well one day while being unable to produce detectable patterns another day. Both these problems show that the relation between brain activity patterns and mental activity is not fully understood in current BCI approaches.

The free BCI presented here tackles the problem to either make the (explicit) tasks or (experimenter-chosen) features more suitable and robust. A feature can be any mathematically transformed electrophysiological phenomenon; feedback represents a set of features or a feature space. In both the explicit and the implicit approaches, the user is not involved in selecting the features to control the BCI. In a free BCI, users are free in selecting a personal subset of the presented feature space or personal (mental) tasks. They can adapt their strategy and decide to focus on a different feature or task if some other strategy does not work. This way, users are confronted with their own variability by the perceived feedback – a problem similar to those naturally faced in life.

To give users such a high degree of freedom, the feedback must be intuitively understandable and cover a high dimensional feature space. We present the user with such a rich feedback signal in the form of a color surface-shape (Figure 1). The color surface-shape can reflect a multitude of features, varying from induced or evoked responses to higher order features like phase coherence. Colors represent feature strength. There has been extensive research on the validity and influence of color on task performance (Maier, Barchfeld, Elliot & Pekrunn 2009; Mehta & Zhu, 2009) and quality attainment (Elliot, Maier, Moller, Friedman & Meinhardt, 2007). The study presented here uses induced power in the time-frequency domain over specific frequency bands as features. Without any specific instruction the user learns to control aspects of this intuitive but complex feedback by changing the shape or color of areas. He can learn implicitly in a neurofeedback manner - but personally select controllable features

- or learn explicitly what influence his mental task has on the feature space. The user has to recognize a stable and regular pattern in the feedback. In later stages of the free BCI project, adaptive machine learning algorithms could be applied to adapt the feature space to the detected pattern of brain activity of the user in order to increase quality and usability of the feedback. Note that making the feature space more appropriate to the individual user has recently been tackled in the BCI2000 project (Schalk, Brunner, Gerhard, Bischof & Wolpaw, 2007). In that study, Gaussian mixture models were used to model the frequency distribution of the signal in order to obtain user specific features for classification. In the free BCI experiment presented here, we leave out the machine learning side. We apply a fixed set of features and a spatial filter with a fixed topology. The here presented study investigates solely how the user adapts to a rich feedback space and whether the system converges to a stable - and hence usable - state.

In this paper, we show results testing the feasibility of the free BCI project. We will test the following four hypotheses by a single session experiment:

1. We expect all users to gain control over the feedback in two distinct ways (two classes) by thoughts alone i.e. that users are able to evoke two distinct feedback color patterns. We will measure this statistically and by subjective user rating.

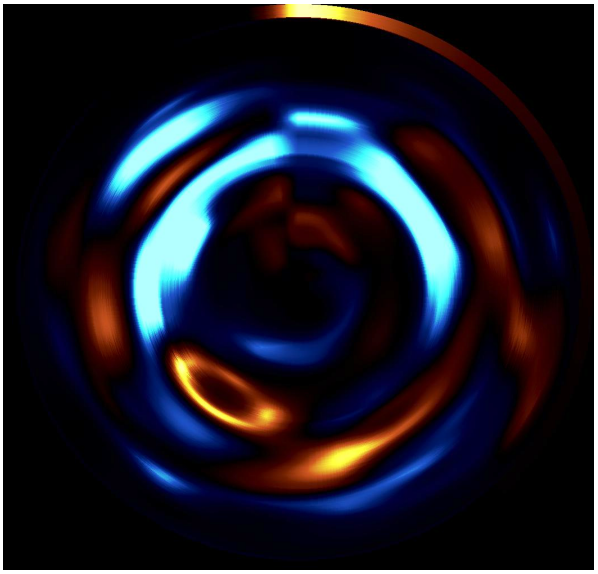

Fig.1 Feedback presented to the free BCI user. Presented features are induced power in the time-frequency domain over the last eight trials. The outer ring shows amplitude in the time domain of the last trial. Blue means high activity, red means low activity. A near-zero or highly variable feature value is shown as black color. The feedback is updated every second - at the onset of a new trial - synchronously to an acoustic stimulus. Time runs clockwise starting at 12 o'clock. Low frequencies are on the inside of the surface shape. Frequencies increase with farther distance from the centre. For detailed information see Methodology.

2. However, the features that the users utilize for getting informed about their performance will match only partially with statistically important aspects of the feedback. This hypothesis is based on Bayliss' finding (2003) that users' subjective rating of quality does not necessarily meet objective performance criteria.

3. In the motor control domain, the guidance hypothesis (Maslovat, Brunke, Chua & Franks, 2009) states that feedback is necessary to maintain a high level of performance when feedback was provided during the training period. Thus, when users are not provided with feedback in the testing phase, we expect both the subjective performance rating and the statistical performance to drop.

4. The predefined feature space reflects user-specific class information only suboptimally. We assume that the statistical difference between the two classes will significantly increase when using a larger set of features. Thereby we will show that users may benefit from having applied machine-learning adaption online, which might help the user in making the two distinct feedback representations maximally different.

In a formal experiment, users, from now on also referred to as subjects, start with freely exploring how to influence the feedback by mental activity. When the subject reports control over the feedback in two distinct ways, the experiment continues with an instructed part. Here, the subject has to reproduce one or the other mental activity and so the feedback representation. Subjects report what aspects of the feedback they think they are able to control, which is statistically verified in an offline analysis.

2. Methodology

2.1 Apparatus and equipment

All experiments took place in an electrically and acoustically shielded room to minimize environmental noise. We used a Biosemi (BioSemi, Amsterdam, The Netherlands, http://www.biosemi.com) ActiveTwoAD-box amplifier to record 64 Ag/AgCl active electrodes according to the international 10-20 system (Klem, Luders, Jasper & Elger, 1999) at a sampling frequency of 2048 Hz. Electrode offsets were kept below 25mV at the beginning of the experiment. In addition we recorded vertical electrooculographic (EOG) activity from

below and above the left eye and horizontal EOG activity at the outer canthi of the left and right eye. Electromyographic (EMG) activity was recorded with a bipolar pair at the glottis, similar to the setup by Jorgensen, Lee & Agabone, 2003. Additionally, an accelerometer for detecting head movements was attached between the central channels labeled 'Cz', 'CPz', 'CP1' and 'C1' according to the international 10-20 system.

As recording software ActiView (BioSemi, Amsterdam, The Netherlands) was used. All other analyses were carried out in Matlab, with toolboxes BrainStream (http://www.brainstream.nu) for experiment management, Psychtoolbox (Brainard, 1997; Pelli, 1997) for stimulus presentation and FieldTrip (Oostenveld, 2010) for data analysis. BrainStream and FieldTrip are open source toolboxes for Matlab developed at the Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands.

2.2 Subjects

We recruited eight subjects, five male and three female between 23 and 54 years. All subjects were right-handed and had normal or corrected to normal vision. All but one subject were free of neurological disorders and medication. Subject 8 had an attention deficit hyperactivity disorder and was medicated by methylphenidate. He had no previous BCI experience, while all other subjects had BCI research experience of a few months up to several years.

2.3 Subject instructions

Before the experiment started, all subjects asked to fill out a form with general details about age, handedness, possible disorders, medication and BCI experience. Next, they were comfortably seated in the experiment room on a chair placed at a distanced of approximately 50 cm from a 17 inch computer screen. During the cap fitting procedure, subjects viewed a tutorial that explained the setup and goal of the experiment. They were instructed to create patterns in the feedback color representation that were as distinct as possible. The goal was to gain control over the presented feedback in two different ways (classes) using thoughts alone.

2.4 Experimental procedure

The experiment consisted of one session, subdivided into five blocks; one free exploration block, the length of which was decided by the subject, and four verification blocks lasting approximately 7.5 minutes (see Figure 2). A trial lasted one second and its beginning was indicated by an acoustic stimulus (metronome click). For the first five subjects, a baseline recording of 32 trials preceded every block. For all other subjects, there

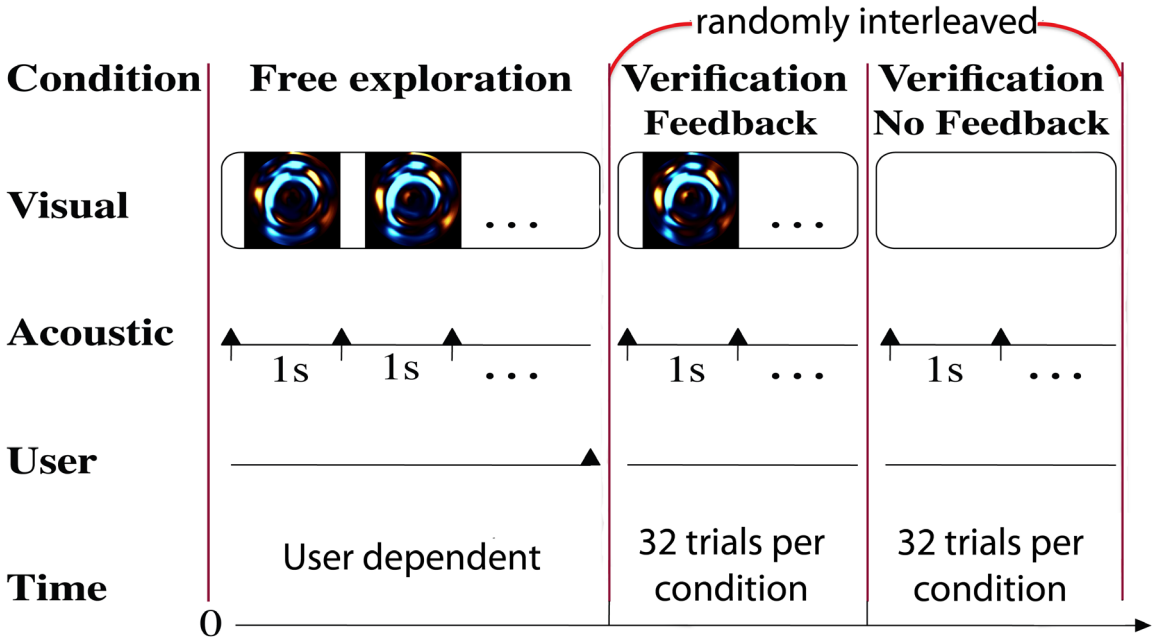

Fig. 2 Experimental setup. Subjects began a free exploration phase and indicated when they were able to control some of the features, i.e. a new class was found. Then, we instructed the subject to reproduce two found classes in a specific randomized order ('sequence'). This allowed verifying that they could indeed intentionally control the newly discovered mental task. After each sequence, the participant had to report his subjective sense of control.

was only one baseline recording preceding the free exploration block. In the free exploration block, the subject got acquainted with the feedback and then had to find two methods that made the feedback as distinct as possible. When the subject found a way (class) to control the feedback, he or she indicated this by pressing a button. When the subject ended the block, we presented screenshots of the representations of the found classes in which the subject had to indicate the major areas of difference in the two chosen classes.

The verification blocks consisted of randomly instructed sequences of either one of the individual classes. A sequence lasted 32 trials. As an additional factor, the feedback was turned off in half of the sequences, resulting in a 2x2 factorial design (mental task x feedback on/off). Each of these four conditions was tested three times per block. Thus per block 384 trials were collected, that is 96 trials per condition. In total, 1536 trials were collected, 384 per condition. Due to technical problems, the data for Subject 1 consists of only 352 trials per condition, so one sequence less.

After every block, subjects had to fill out a questionnaire, in which they reported their level of arousal, excitement and motivation. Additionally, they had to indicate which areas of the feedback they believed they were controlling and reported their feeling of control on a scale from 1 to 5, where 1 meant total randomness and 5 meant total control over some aspects of the feedback

2.5 Timing

Accurate timing was guaranteed by using Psychtoolbox, optimized for precise timing in psychophysical experiments using OpenGL (www.opengl.org) for graphic representation and OpenAL (http://connect.creativelabs.com/openal) for audio playback. Corresponding to the trial length of one second, the feedback was fully updated once every second. The beginning of a trial was indicated by an auditory cue (a metronome click), synchronized with the visual update. The onset of a new trial, and so of the metronome click, corresponded to the 12 o'clock position in the feedback representation.

2.6 Signal preprocessing

The signal preprocessing steps leading to the visual feedback presentation (Figure 3) were based on the work of Vlek and colleagues (Vlek, Schaefer, Gielen, Farquhar and Desain, 2011). Bad channels were identified from the raw EEG signal for each trial with an algorithm sensitive to four properties. Initially, any channel with a DC offset exceeding 30 mV was marked as 'bad', as well as channels with a power exceeding 3500 μV2 in the 50 Hz band (45 to 55 Hz) or a maximum derivative larger than 200 μV/sample. Horizontal and vertical EOG channels were band-pass filtered between 0.2 and 15 Hz and decorrelated from the EEG (Schlögl et al., 2007), thus removing eye drifts or blinks if present. The raw EEG signal, originally sampled at 2048 Hz, was temporally downsampled to a sampling frequency of 256 Hz. Additionally, as a fourth property for identification of bad channels, within-trial variance was computed and channels exceeding a variance of 2000 μV2 were marked 'bad'. Bad channels were reconstructed by interpolation from the remaining good channels with a spherical spline interpolation algorithm (Perrin, Pernier, Bertrand & Echallier 1989). The interpolation step was motivated by the intention to provide continuous feedback at all times with a stable number of channels. Data was re-referenced to a common average reference and linearly de-trended. The same preprocessing was used for all subsequent analyses. Finally, we constructed two virtual channels by applying a Laplacian spatial filter, which reduces the signal's sensitivity to noise. The location of the filter was motivated by successful piloting and a previous imagined movement paradigm. One filter with positive weights was located posterior to the channel labeled 'C3' in the international 10-20 and one with negative weights was located around the channel 'C4'. These will be referred to as virtual channels (Figure 4). The width of the spatial filter was defined as two average electrode distances.

2.7 Feature space

The feature space consisted of two components: induced power in the time-frequency representation (TFR) and signal amplitude in the time domain. The first component is computed as the difference between the two virtual channels in the Fourier transformed time-frequency data. The transformation used 32 evenly spaced frequency bins between 4 Hz and 32 Hz in 8 time windows (Hanning window). The size of a time window depended on the corresponding frequency bin and was chosen such that four full cycles of that frequency fit into one window. The feature space consisted of induced power in the TFR over the last eight trials. The second component was the difference in the event-related potential (ERP)

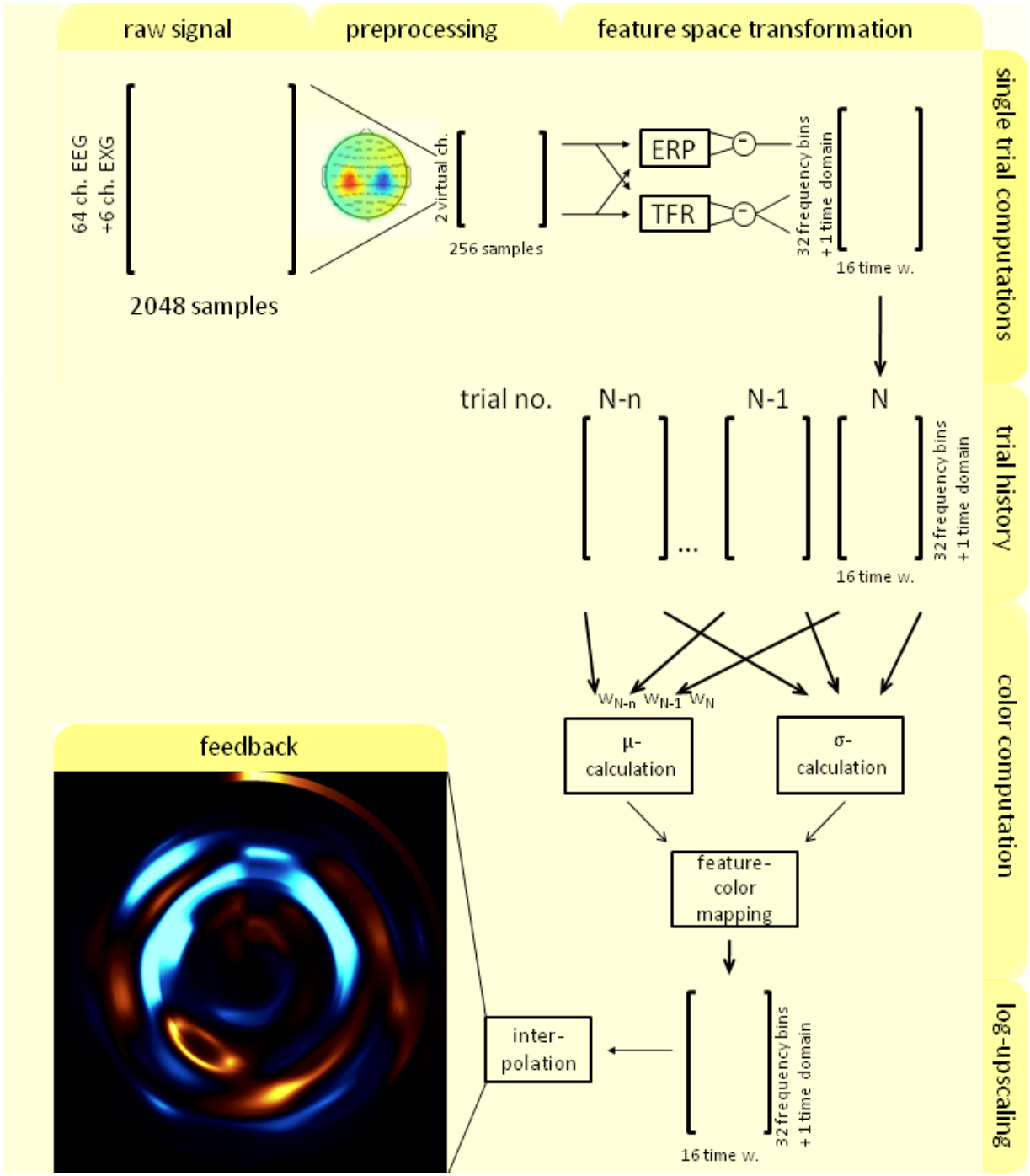

Fig. 3 Data processing. The raw signal with a trial length one second is first preprocessed and spatially filtered (see Section 2.6). Spatial filtering results in two virtual channels that are then used for computing the difference event-related potential (ERP) and the difference time-frequency representation (TFR, see Section 2.7). For estimating the induced power in the time-frequency domain, a trial history of size n is used (here n = 8). From the trial history a pseudo mean and pseudo standard deviation is computed (Section 2.8), with which a single feature space for this trial (trial N) is built. The feature space consists of induced power in the time-frequency domain over the last n trials and the event-related

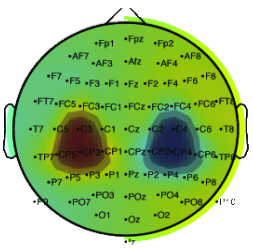

Fig. 4 The location of the applied spatial filter around channel C3 with positive weighting (red color) and around C4 with negative weighting (blue color). The location of the spatial filter was based on an imagined movement paradigm. Channel labels conform to the international 10-20 naming convention.

between the virtual channels, i.e. the difference in magnitude per recorded sample in the time domain filtered between 0.5 Hz and 20 Hz. This feature was a single trial measurement.

2.8 Visual feedback

Any set of features (or feature space) can be visualized in form of a spectrogram, with feature represented on the y-axis, time on the x-axis, and feature power as color. Here, we presented visual feedback as a polar transformation of such a feature space spectrogram (Figure 5). The resulting circle represents time running clockwise starting at the 12 o'clock position that serves as trial start and endpoint. Analogous to a regular spectrogram, individual features were binned that were represented as ring segments on the circle. Originally in a polar representation, ring segments near the centroid have a small radius, which increases with increasing distance to the centre. To make information distribution more similar independent of segment location, the frequency axis was scaled in a logarithmic fashion, such that the outer quarter of rings represents only half of the feature space. Frequencies were sorted such that low frequencies were on the inside and frequency increases towards the outside. The ERP feature was visualized as an additional ring segment with doubled width to make it more salient.

Values in the feature space were mapped to the interval of the color scale. A color scaling in 256 hues served to indicate feature power. The color scaling ranged from light red for low feature power (RGB value: 1/1/0.7 on a scale from 0 to 1) to black for zero-power (0/0/0) to light blue for high feature power (0.7/1/1). While the mapping of the ERP was linear and accordingly straight forward, the mapping of the TFR was more complex. When showing TFR power averaged over the last trials, an outlier (a single high power value that makes the TFR estimation unreliable) can drastically influence the feature value. Therefore, we applied a feature value transformation that penalizes a high variance over the last trials, thereby reinforcing smooth transitions and stability in brain activity.

The TFR feature matrix consists of all frequency bins f in all time windows t. The final TFR feature value is calculated by the inverse of an exponential function. This exponential function (Equation 3) depends on two parameters: The pseudo mean and pseudo standard deviation of the current trial N and the frequency bin f and time window t. This function is symmetrical along the x-axis for positive and negative pseudo means and converges to half the range of the color scale, i.e. a black color, for high pseudo standard deviations (Figure 6). The pseudo mean (Equation 1) is obtained by a normalized linearly weighted average over the last eight trials of the feature power contrasted with the average baseline power of that feature. As normalization factor the sum of the average baseline power and its standard deviation over all frequency bins and time windows in the baseline is used. The pseudo standard deviation (Equation 2) is obtained in a similar way, except we did not weigh individual trials and the normalization factor was three times the baseline standard deviation over all features. The exact mathematical description is found below.

Equation 1 $$\hat{m}_N^f = \frac{1}{m^b + s^b}\left(\sum_{k=N-n}^{N} w_k p_k^f - m_b^f\right)$$

Equation 2 $$\hat{s}_N^f = \frac{1}{3s^b}\left(s_N^f - s_b^f\right)$$

Equation 3 $$f_N^f = \frac{1}{2} + \left(\frac{1}{2}\right) \Big/ \left(1 + e^{4.8\,\tilde{s}_N^f}\right)$$

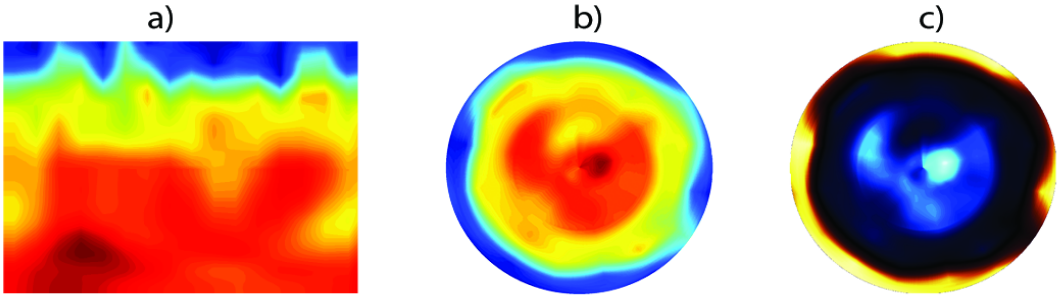

Fig.5 **(A)** A regular spectrogram with time on the x-axis, features (e.g. frequency bands) on the y-axis and feature power as colors. Red colors indicate high feature power, blue colors low feature power, green represents zero power. **(B)** Polar visualization of this feature space. The inner half of the polar representation contains three quarter of all the features (roughly the red part). **(C)** The free task visualization of this feature space. Note how near-zero power values become nearly black, high power is blue and low power is red.

Equation 1 describes the calculation of the normalized weighted mean of the feature power for time window t, frequency bin f in trial N. The weights were linearly distributed so that the weight of the current trial N is weighted n-th time the weight of trial N-n. As already stated, we used the last eight trials for all our calculations, i.e. n=8. The weighted mean was contrasted with the average baseline of that tf-feature, where b denotes the baseline. The result was then normalized by the sum of the mean of the baseline over all features and its standard deviation over all features.

Equation 2 explains the calculation of the normalized standard deviation of trial N by the standard deviation of the feature and the standard deviation of the baseline . Only the latest 8 trials were used for calculating the feature space and thus also for the standard deviation for trial N. We normalized by three times the standard deviation of the baseline .

After this step, parameter values below 0 and above 1 were cut to 0 and 1, respectively, denoted as pseudo mean and pseudo standard deviation . These two parameters served as input for Equation 3. The formula penalizes a high variance by converging exponentially to one half that is black color (Figure 6). A low variance had little or no influence on the final feature value. The resulting feature value decided the color hue of that feature in time window t, frequency bin f for trial N.

2.9 Offline analysis

Offline analysis was based on Vlek et al. (2011). Matlab and the Fieldtrip toolbox (Oostenveld, 2010) served as tools for offline analysis. For EOG and EMG channels (referred to as EXG) as well as for the EEG channels nearly the same preprocessing steps as in the online case were used. If – according to the four properties mentioned on page 8 – more than 20 % of the channels in a trial were bad, the trial was excluded from further offline analysis. Here, we used a 50 Hz cutoff value for marking trials as bad of 5000 µV instead of 3500 µV. This was because the data from Subject 4 suffered from 50Hz noise, leading to dismissing most of the trials due to this noise. With a higher cutoff value the data from this participant could be used, while other data remained mainly unaffected. The signal from the head movement sensor was only linearly de-trended.

For EEG channels, the same transformation to the time-frequency domain as in the online experiment was used, which will be referred to as predefined features. In addition to the TFR settings used for the online experiment, we also calculated power in the time-frequency domain from 4 Hz to 64 Hz in 2 Hz steps in 51 Hanning windows of size 200 ms each (referred to as all features). EOG and EMG channels and the head movement signal were analyzed in the time domain after being bandpass filtered between 4 Hz and 64 Hz.

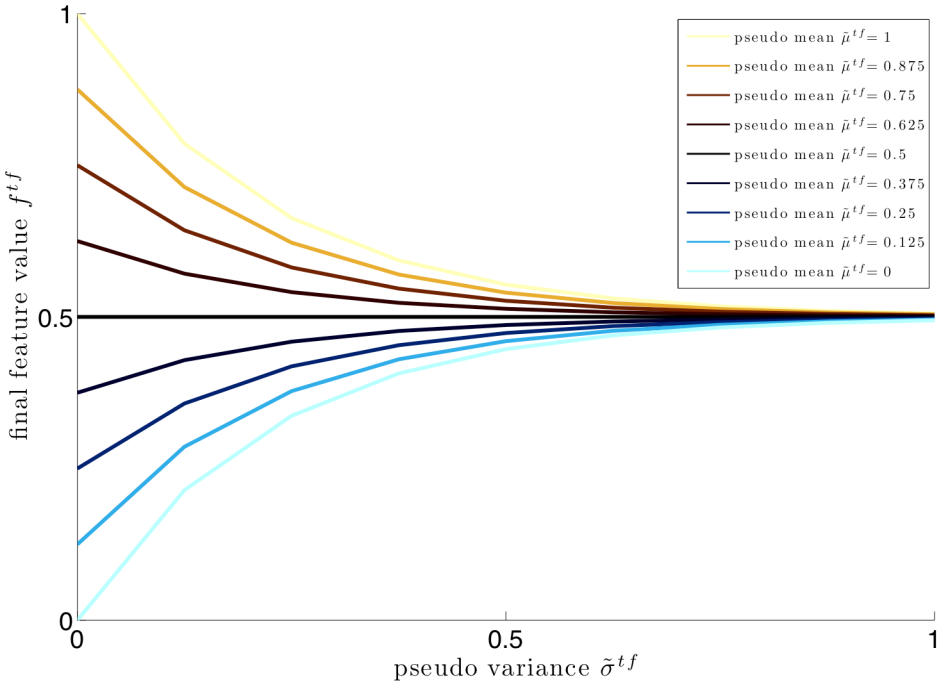

Fig. 6 The influence of variance of a feature on the feature value. The final value (color) of a feature was determined by its weighted mean feature power and the feature variance. High variance penalized the final value towards 128, i.e. a black color. Colors of the lines indicate what color the mean feature power would have represented.

Logistic regression (denoted as KLR) was used for classification (Tomioko, Aihara & Müller. 2007), which was further L2-regularized to prevent overfitting (Farquhar, 2009). Due to the experimental block design, we used a blockwise leave-one-out validation method. The reported classification rates and standard errors correspond to the mean classification rate over all folds and the standard error over all folds for the regularization parameter with the highest performance. Statistical analyses were performed with 95 % and 99 % confidence intervals and a two-sided t-test.

After having applied the classifier, the obtained weight matrix was compared with the subject's report about which aspects of the feedback they believed they were controlling. We assumed the weight matrix of the classifier to be an objective measure of class information. Features that are weighted with extreme values are statistically more important than features weighted around zero. For this analysis only weights with a magnitude of at least a third of the maximal weight in absolute terms were used. Subjectively important features were obtained at the end of each experimental block. This analysis was carried out by eye without statistical analysis.

In order to assess whether class information from one type of sensor (EOG/EMG, EEG, head movement sensor) leaked into another type, we correlated the classifier output (or decision values) of one sensor type with the decision values from another type using Pearson's correlation coefficient and a 95 % confidence bound. We speak of leakage of class relevant information if there is a significant correlation between the decision values.

3. Results

3.1 Behavioral Results

The free BCI project allows users many degrees of freedom in interacting with the feedback system. After a period of approximately ten to fifteen minutes, subjects got acquainted with the feedback and then searched for reliable ways to control aspects of the presented feedback. Subjects found two classes and thus ended training phase after approximately 48 minutes on average. The length of the training phase could not be related to performance measures that have been computed. Table 1 provides an overview of subject specific mental tasks. Note that although the naming of some tasks might be similar, subjects performed similar mental tasks in a different manner.

Although subjects reported that the feedback was reliable in the majority of trials (average rating 2.8/5), feedback quality dropped for four of the first five subjects towards the end of the experiment (Subject 1, 2, 3 and 5, average rating at the end of the experiment 1.9/5). In this respect, it was fairly apparent that the feedback representation changed drastically with every new baseline recording. An example of such a change and hence of subjectively important features is given in Figure 7. This example is representative for Subject 1, 3 and 5. For Subject 6, 7 and 8, we decided not to record additional baseline periods but contrasted the features with

Subject	Task 1	Task 2
S1	*Imagined Counting* Imagining counting from 1 onwards to the metronome	*Imagined Right Hand* Movement Imagining playing drums time-locked to the metronome
S2	*Verbal Imagination* Saying 'Bonjour' time-locked to the metronome	*Acoustic Imagination* Hearing a siren sound syncopated to the metronome
S3	*Mental Arithmetic* Adding 7 to some starting number, time-locked to the metronome	*Imagined Tongue Movement* Time-locked with the metronome
S4	*Mental Arithmetic* Counting back from 100 in steps of 7	*Imagined Dancing* Exercising ballet
S5	*Imagined Hand Movement* Boxing against a boxing ball, time-locked to metronome	*Imagined Feelings* Thinking about happy feelings and events in the past
S6	*Imagined Finger Movement* Playing a piece of Vivaldi on the flute	*Imagined Foot Movement* Imagining playing a fast rhythm on a bass drum with the right foot
S7	*Implicit Learning* Letting a yellow blob appear, later associated to gentle and relaxed feelings and positive coaching	*Implicit Learning* Letting a blue blob appear, later related with restlessness and distraction and subdividing the metronome
S8	*Visual Imagination* Imagining a candle burning, learnt in meditation class	*Imagined Visual Rotation* Imagining the same candle rotate downwards/sagitally

Table 1. Overview of the individual mental tasks and task descriptions per subject. Subjects used a variety of different mental tasks to gain control over the feedback.

Fig. 7 Example of the effect of introducing a new baseline to subjectively important features. All shown drawings are from the same individual class of Subject 5 but from different blocks. (A) After the free exploration phase, Subject 5 was relatively sure to be able to reliably control the feedback (3/5). (B)-(E) Although the feedback representation, and by such subjectively important features, changed continuously in every verification block, Subject 5 reported no major loss of reliability (rating 3/5 for the first two blocks, rating 2/5 for the last two blocks). The feedback still responded reliable, but the effect on the feedback changed with every block.

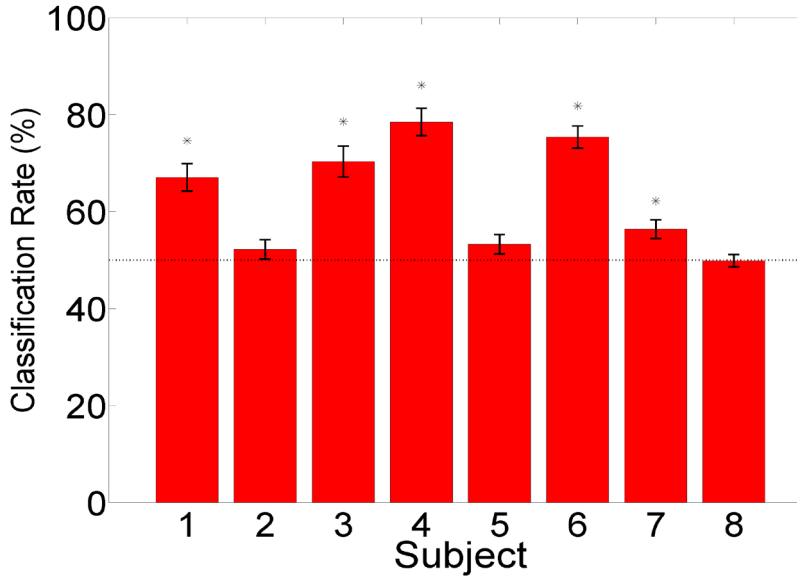

Fig. 8 Classification rates on single trial basis of the individually found classes per subject using the predefined feature in the feedback condition. Error bars indicate standard error. For five out of eight subjects, classification rates were significantly different from chance ($p < .01$).

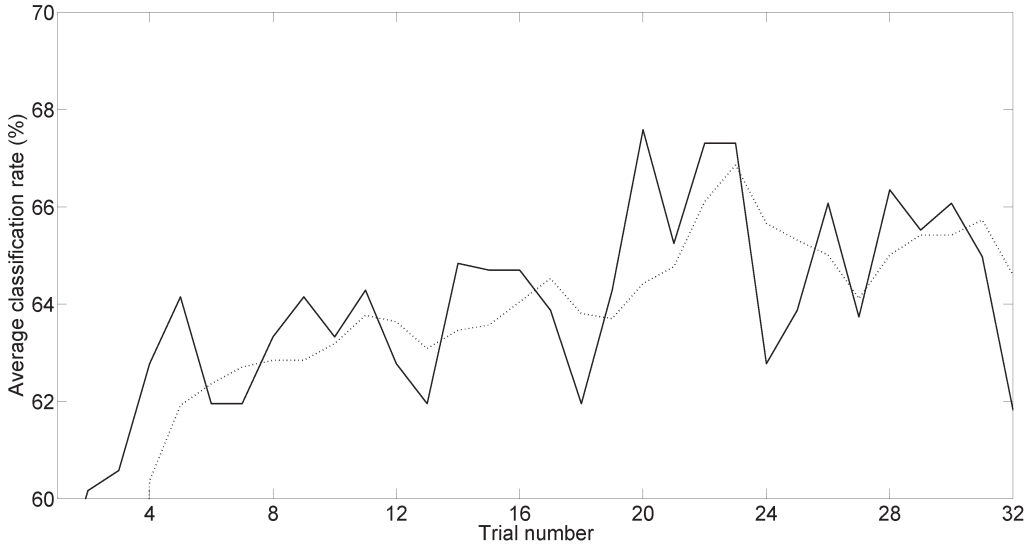

Fig. 9 Average classification rates of trials within a sequence across subjects (pooled for both sets of EEG features). The classification rates are obtained using the predefined features in the feedback condition. The trial number is on the x-axis, the average classification rate on the y-axis. The dotted line indicates a moving average over the last four trials. There is a significant difference in classification performance between the first four trials and the last four trials of a sequence across subjects ($t(727) = -3.08$, $p < .01$).

the baseline recorded at the beginning of the experiment. These subjects reported a very stable feedback representation (rating 3.3/5), also at the end of the experiment (rating 3.1/5).

Subject 3 complained about problems with her back during the experiment and Subject 7 reported a loss of concentration at the end of the experiment. There were no other behavioral results worth mentioning.

3.2 Controllability of the feedback

Per subject, we trained a binary classifier on the two individually found classes. The classifier input consisted of only the predefined time-frequency features, which were fed back to the subject. The classification rate was significantly different from chance for five out of eight subjects ($p<.01$; Figure 8). However, trials in the beginning of a sequence yielded lower classification rates than trials near the end of a sequence, see Figure 9. So, some sequences started with a transient period until classification performance reached a stable level. This was verified by a significant difference across subjects between the classification rate of the first four trials of a sequence and the last four trials ($t(727)=-3.08$, $p<.01$).

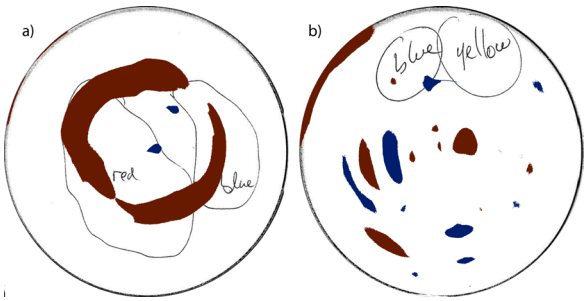

Fig. 10 Overlap between subjectively important features and objectively important features obtained from classifying on the predefined features in the feedback condition. The polar representation corresponds to the free BCI visualization. Blue color blobs indicate a high feature weight of at least a third the highest weight in absolute terms; red blobs indicate a low feature weight of maximally a third of the additive inverse of the highest weight in absolute terms. Line drawings and written text is scanned in from the behavioral questionnaire. (A) Example of a subject with a high classification rate of above 70% (Subject 6). The left area labeled 'red' represents subjectively important features for class 1. The area labeled 'blue' indicates subjectively important features for class 2. There is a partial overlap for area 'red', but the objectively important areas extend up to the 'blue' area. (B) Example of a subject with a low classification rate around 50% (Subject 7). The area on the top labeled 'blue' represents subjectively important features for class 1. The area labeled 'yellow' indicates subjectively important features for the other class. Neither of the two areas overlaps grossly with objectively important areas.

Figure 12 shows the difference TFR (right panel) and difference in the frequency domain (left panel) between the two individual classes per subject. Whilst the TFR of Subject 1, 2, 4, 6 and to a lesser extent also of Subject 3 and 5 mainly reveals activity in the alpha and low beta band around 10 Hz (Figure 12 a, b, d, f and c and e, respectively), the main activity in Subject 7 and 8 is less clear (Figure 12 g and h, respectively). These two subjects achieved a modulation of the high beta- and low gamma-band. Furthermore, there is little structure in the time domain in higher frequencies for Subject 3, 5, 7 and 8 (Figure 12 c, e, g and h, respectively).

3.3 Reliability of subjective reports

Previous BCI studies have shown that subjective ratings of performance do not always match objective performance criteria (Bayliss, 2003). This part of the analysis compares statistically important features with subjectively important features. We overlaid a representation of individual subjective patterns with objective classifier weights. In order to do so, the weight matrix was transformed into the free BCI polar representation. The analysis was complicated by the fact that the online feedback representation was dependent on the baseline recording. We recorded a new baseline for the first five subjects at the beginning of every block, which changed the respective feedback drastically for most subjects (see also Section 3.1). In the further analysis, we excluded Subject 1, 3 and 5 due to their changing report on which aspects of the feedback were subjectively important.

The remaining five subjects reported a stable feedback representation and thus similar patterns

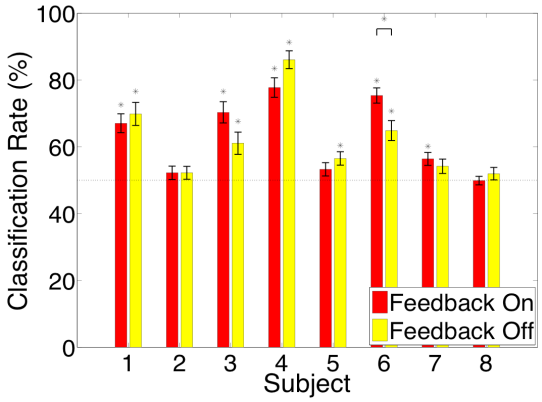

Fig. 11 Classification rates for the feedback and the no feedback condition. Error bars indicate standard error. There is no significant difference ($p > .05$) between the two conditions for all but one subject (slight significance, $p < .05$). For Subject 6, receiving no feedback decreases class discriminability.

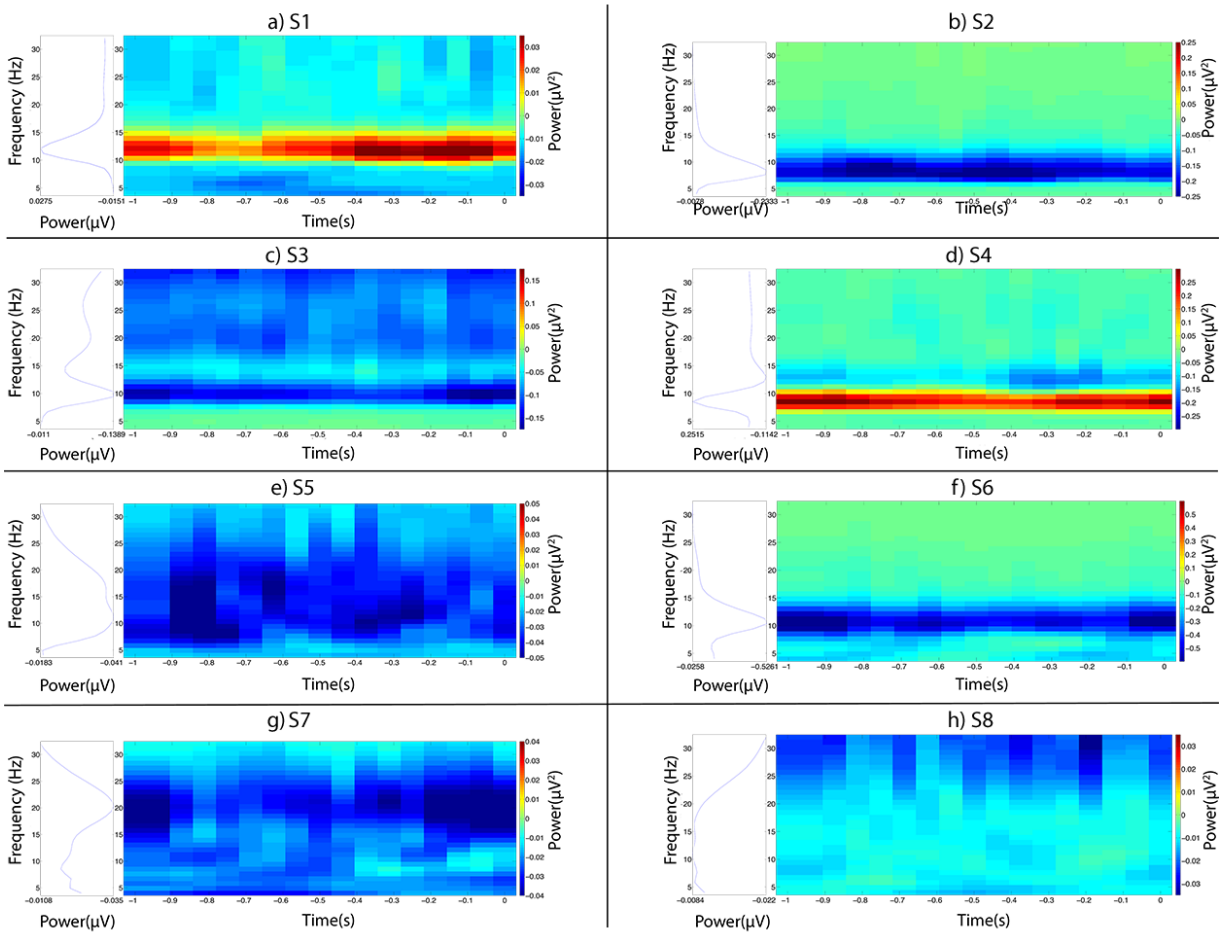

Fig. 12 Time-frequency representation of the difference between the subject-specific mental classes. Left panel per subfigure shows the power in the pure frequency domain, the right panel consists of a spectrogram showing induced power in the time-frequency domain. Subfigures (A) to (H) show data from Subject 1 to 8, respectively.

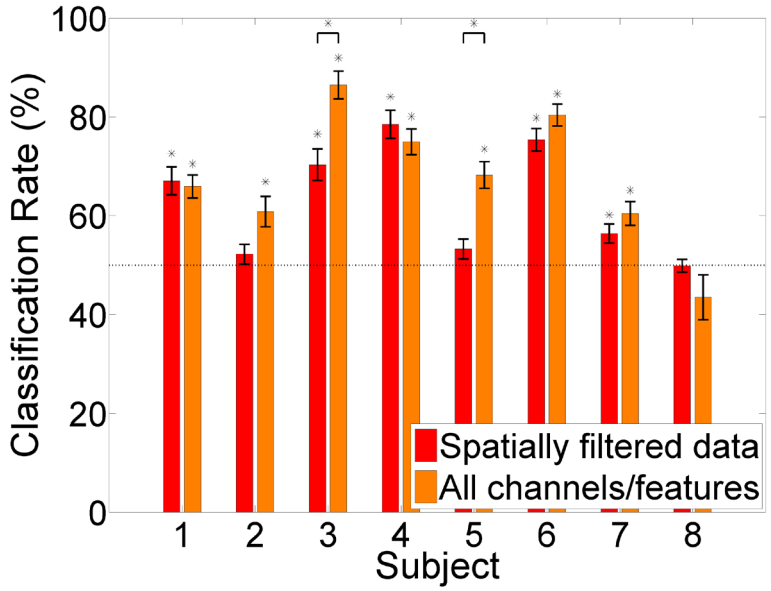

Fig. 13 The effect of feature selection on classification performance. Error bars show the standard error. When all features are used, classification rates for two subjects increase significantly ($p < .01$). The classification rate for Subject 2 has a tendency towards significance ($p < .1$). All other comparisons show no significance ($p > .05$).

for their individual classes across all experimental blocks. Here, two cases can be distinguished. On the one hand, Subject 4 and 6, who performed very well in terms of classification rate, created a brain activity pattern that was suited very well for the used features, because the activity overlaps with the location of the applied spatial filter. But, their subjectively important features only marginally overlapped with statistical weighting (See for example Figure 10a). Still, these subjects were very sure about the reliability of the feedback (average rating 3.5 on a scale from 1 to 5). On the other hand, subjects with low performance showed an even stronger mismatch. This was the case for Subject 2, 7 and 8. Here, subjective reports on feature importance had almost no correspondence with objective weights (see Figure 10b for an illustration). These subjects also reported the feedback to respond reliably to their mental tasks in most cases (average rating 3.2/5), with small doubts near the end of the experiment (average rating 2.8/5).

3.4 The effect of feedback

The setup of the experiment also aimed to test whether receiving feedback facilitates task performance (guidance hypothesis). We compared the feedback with the no-feedback condition (Figure 11). There was a significant difference between the two conditions for only Subject 6 ($p<.05$), but not for any of the other subjects ($p>.05$). After having found the individual classes using the feedback, seven out of eight subjects were able to reproduce the classes equally well, whether or not they received feedback. This speaks against the guidance hypothesis with our experimental setup.

3.5 Class information in the feedback feature space

We hypothesized that the predefined set of features reflects class information only suboptimally. Therefore, we compared the classifier performance using only the predefined features with the performance of a classifier using all TFR features from all EEG channels, see Figure 13. The latter features included higher frequency bins up to 64 Hz as well as a more precise time windowing (see Section 2.9). For two out of eight subjects, classification rates were significantly different from each other

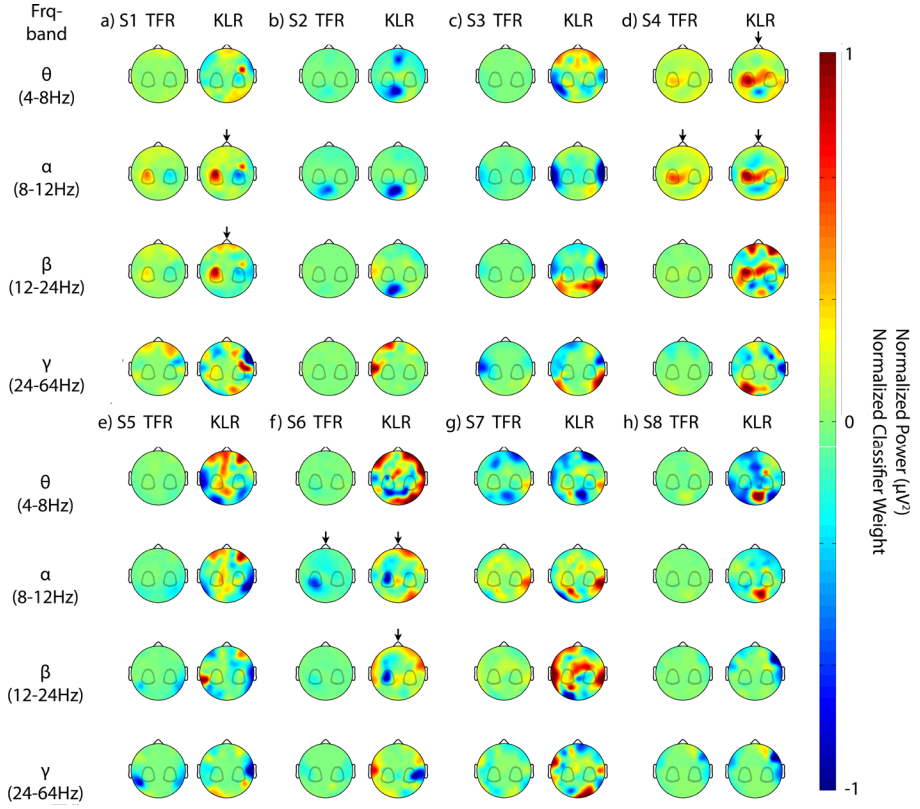

Fig. 14 Topographic distributions for all subjects (S1 to S8) of power in the time-frequency domain (left part per subfigure) and weights of the KLR classifier (right part per subfigure). Different frequency bands (theta, alpha, beta, and gamma) run down the y-axis. Grey shadings indicate the main foci of the predefined spatial filter around C3 and C4. Arrows highlight topologies, in which main activity coincides with the spatial filter. For most other subjects, it matches only partially the main activity or classifier weights, though there only is a significant difference in terms of classification performance between the predefined and all EEG features for Subject 3 and Subject 5.

($p<.01$). For Subject 2, the difference was close to significance ($p<.1$).

Figure 14 shows the subject-specific topographic distributions of the four major frequency bands per subject. Subject 1, 4 and 6 could achieve a brain activity pattern that is matched very well by the predefined spatial filter. Contrary, Subject 2 shows occipital activity and Subject 3, 5, 7 and 8 show very lateralized activity, which is matched only marginally by the spatial filter.

The topographic distributions of the classifier weights differ from those of the TFR power especially near the most posterior and anterior as well as lateral sides. The classifier utilizes activity there that was not picked up by the predefined spatial filter. However, keep in mind that comparing the classification on the predefined features with all channels and features yielded only a significant difference for Subject 3 (Figure 14c) and Subject 5 (Figure 14d).

3.6 Confounding factors analysis

In the analysis of the EOG and EMG signals (together referred to as EXG signals), classification rates for the individual classes were significantly different from chance for seven out eight subjects. This especially constitutes a worry for Subject 7, because he obtained a classification rate around 70 % for classifying on the EXG channels, which was significantly better than when classifying on the EEG channels ($p<.01$). A correlation analysis was set up to test whether class relevant EXG information leaked into the EEG signal.

In the following, we argue that EXG movement signals are no confounding factor for the reported EEG classification results. There was no significant correlation between the EXG features and the predefined EEG features ($p>.05$), but between the EXG features and all EEG features for Subject 7 and 8 ($p<.01$). So, information leaked into some EEG channels, but class relevant information from the EXG channels has not improved classification rates on all EEG features, because there is no significant difference between the classification rates of the predefined and the one of all EEG features for these subjects ($p>.05$). Therefore, we consider the possible leakage of class relevant information not to be a confounding factor of our analysis. A similar analysis of the classification results obtained from the head movement sensor led to no significant correlations ($p>.05$).

4. Discussion

4.1 Behavioral Results

Within one block, the first five subjects reported the feedback to be stable and reliable. The feedback changed drastically once a new baseline recording was used for calculating feature power as done at the beginning of every block. So, per subject, a new baseline period resulted in different features. Although we found no explanation coming from recorded behavioral factors (level of arousal, excitement and motivation), the change in baseline may be caused by many other factors, for example task engagement. Since the influence of the baseline was so high, we decided to change the experimental protocol to using the same individual baseline period for all experimental blocks for Subject 6, 7 and 8. As expected, the feedback representation was much more stable and reliable when using a fixed baseline. Subjects benefitted from using only one baseline recording as shown by their subjective rating: The feedback appeared more stable during the whole experiment.

In future experiments, we propose to keep using the same baseline period for the whole experimental session. An extension of the length of this baseline period might lead to further improvements. This way, random fluctuations will be more likely to be averaged out, and hence the TFR estimation will be even more reliable. In other words, subjects will get a better estimate of their mental activity, which is especially useful in the free exploration phase when subjects need to rely on the feedback.

4.2 Controllability of the feedback

Five out of eight subjects were able to achieve classification rates significantly different from chance. This result confirms that BCI users can adapt to an extrinsically provided and very rich feedback signal, even if they are not informed about precisely what the feedback represents. Users can reliably control personal aspects of the feedback and reproduce stable patterns by relating their thoughts to a task known from everyday life or by implicit actions. Note however, that although classification rates are significantly different from chance, the achievable bit rates are hardly usable for an everyday BCI.

A BCI for daily use needs an instantaneously high performance. Our experimental setup used a block design, which is not suited for such usage. We

found that - on average - the first trials of a sequence yielded a significantly lower classification rate than trials near the end of the sequence, thus after various repetitions of the mental task. This effect is not constant for all subjects, but it is possible that some mental tasks might need a longer preparation time - for example emotion modulation - than other mental tasks such as imagined movement. Nevertheless, the range of mental tasks that could possibly be used to control a BCI is much wider than the currently used tasks. A free BCI can also be used for rapid prototyping to find and test novel mental tasks. However this comes with the risk of testing mental tasks that are not suitable for controlling a BCI, as happened to some subjects here.

There were three subjects who could not achieve a performance different from chance, nevertheless these subjects reported controllability of the feedback using their individual mental strategies. A reason for this may be that these subjects detected patterns that were not always stable, but showed some reoccurrence. Especially when the feedback pattern did not resemble the expected pattern, it might have been that they changed their mental strategy and by so created a different brain activation pattern. However, if subjects detected patterns that were not reliable and stable, one would assume that their subjective rating would drop constantly throughout the experiment. This was not the case, as we will discuss in the following.

4.3 Reliability of subjective reports

Bayliss (2003) found that subjective performance criteria do not always match objective performance criteria. Our comparison of subjective and objective measures goes one step further by showing that subjectively important features do not match objectively important features. So, subjects believed that they had achieved control over some aspects of the feedback, which in fact they had, statistically measured, no control over. As already stated, recording a new baseline constituted a major problem for the reliability of the feedback representation. But even when all relevant factors for the computation of the feedback signal, including the baseline, were kept constant, subjects were not able to give a reliable report on what aspects of the feedback they could control. Especially surprising is that subjects performing at or near chance level also reported that they could control the feedback.

There are several possible reasons for such an observation. A psychological account might be that subjects wanted to see an influence of their performance on the feedback – a placebo effect. This could have happened because of impatience, low level of concentration and especially the expectation to see controllable, reoccurring patterns during the experiment. A different account could be that this difference comes from humans having different capabilities to recognize patterns than statistical methods have. From this, a critical question emerges: Did subjects see patterns that did not exist, or did the statistical analysis not reveal all important patterns? Making the subjects rely on statistical importance could reduce a possible placebo effect and as such shed light on the two mentioned hypothesis. As machine-learning algorithms detect statistically important features, we will propose how an adaptive algorithm could hint the user to features that are of class relevance in two possible ways.

First, improving visualization of class importance in the feedback. Subjects reported mostly features in the inner or middle part of the circle to be of importance. This was maybe because higher frequencies have lower amplitude than low frequencies have. We applied baseline contrasting to account for the 1-over-f power law. Still, changes in high frequencies might not have become apparent. By using a frequency dependent normalization method, subjects might have detected patterns in high frequencies. Future experiments should include a normalization method that normalizes individual frequency bins separately, for example by sphering and centering the data. Centered data is zero-average and the covariance of sphered data is equal to 1. This will hence eliminate power differences across frequency bins. Another possible explanation for subjects reporting mainly low frequency features to be of importance might be that the field of view focused on the inner circle. In the inside of the feedback, information is more condensed in less space. Therefore, rearranging how the feedback displays the feature space might help subjects to find controllable aspects in the feature space, for example by allowing important features to take more space and shrinking space for less important features. Additionally, features could be rearranged by moving statistically important features to the centre of the feedback. In future experiments of the free BCI project, more important features should be in the inside of the circle and occupy more space while less important feature should be moved to the outer part of the circle and occupy less space.

Second, adapting the feature space. Applying a statistical weighting to adapt the feature space can help visualize class relevant features, similar to what

has recently been done in the BCI2000 project (Schalk et al., 2007). This way, a new spatio-spectral feature space will evolve, making the features more suitable for the subject-specific mental tasks. However, the explicit mapping of several statistically important features to a class label might be complicated for subjective interpretation, in particular for multiclass problems. Such an approach also raises the question of whether a mutual adaptive system, consisting of man and machine will converge to a stable state. In order to study this approach thoroughly, a step-by-step procedure is recommended. Future experiments should first incorporate discrete steps of machine adaptation, so that machine and man adaptation do not occur simultaneously but turn after turn. This may improve objective and subjective performance criteria, because they can rely more on the feedback that reflects objective performance criteria directly.

4.4 The effect of feedback

The guidance hypothesis, originally coming from the realm of motor control, states that a task learnt through feedback can not be reproduced as good in the absence of feedback.. In other words, feedback is necessary to maintain the same level of performance. We tested this hypothesis in the free BCI setup but could not verify it. We found that visual input does not deteriorate class performance, but also does not improve it as the guidance hypothesis states. Only for one subject we found a decrease in performance in the no-feedback condition compared to the feedback condition. Since we found no effect in the opposite direction, feedback does also not have a distracting effect. Two different explanations might account for this disagreement with the guidance hypothesis:

1. As subjects received feedback in a later sequence about the same mental task, this discrete feedback was sufficient to be informed how to reproduce the expected feedback pattern.
2. The free exploration phase does not reflect a learning phase but only a search for appropriate ways to produce a reliable pattern using well-known mental tasks.

So, on the one hand, our experimental setup might not be valid for testing the guidance hypothesis – a concern raised by explanation 1. In case of doubt, subjects could verify their performance by waiting for a later sequence that showed feedback again. On the other hand, it might be that the individual cognitive tasks were not complex enough for having the need to rely on feedback, and thus do not need to be learnt – a concern raised by explanation 2. To test whether subjects rely on the feedback in the verification phase (as proposed by hypothesis 1), longer sequences without feedback are needed. If a degradation of performance over time occurs in no-feedback blocks but not in feedback blocks, the guidance hypothesis can be claimed valid. The other hypothesis (hypothesis 2) can only be tested by a multi-session experiment. If there is a learning effect, thus performance is increasing in later sessions, then the cognitive tasks can be learnt and hence the experimental setup is valid to test for the guidance hypothesis. Else, the guidance hypothesis does not apply in this free BCI setup.

4.5 Class information in the feedback feature space

We found that classification performance using the predefined features did not differ from using all channels and features for six out of eight subjects. Three subjects, Subject 1, 4 and 6, could find ways to strongly modulate their brain activity nearly exclusively around the location of the spatial filter (see Figure 14a, d and f). This can be explained by the location around the motor cortex and the parietal lobe. These areas are highly engaged in motor planning and imagined execution of motor movements. For all these subjects, at least one if not both strategies to control the feedback was related to planning of coordinated movements. It is rather common to use a motor-related task to control a BCI. Nevertheless, an explicit experimenter-defined task execution might not have resulted in such strong brain activation patterns. The subjects tried BCI tasks that are closer to conventional BCI tasks as well, but did not succeed in finding reliable feedback. Subject 4 explicitly reported that imagined arm movement did not result in a stable and reliable pattern. This might be related to the fact that the spatial filtered was located more posterior than an ideal motor imagery spatial filter would be. However, Subject 4 managed to find a very stable and reliable pattern using a different mental motor task – this is also confirmed by the high classification rate around 80 %. Note that we did not optimize our processing pipeline for imagined movement classification.

Subject 2 used auditory imagery and language production imagery and was able to induce alpha-band desynchronization at a centro-occipital location that was marginally picked up by the spatial

filter. Such activity has most often been related to memory related processes (Jensen, Gelfand, Kounios & Lisman, 2002). There is evidence that music imagery exposes brain activity in fronto-central areas (Vlek et al., 2011), but rarely in the occipital lobe. The predefined spatial filter did not operate on this centro-occipital locus of activity, but classifying on all channels and features showed no significant improvement than classifying on the predefined features. Still it yielded a result significantly different from chance, which was not the case for classification on the predefined features, i.e. a small but important improvement. Applying advanced machine learning techniques that focus on this centro-occipital difference might improve class performance even more.

For Subject 3 and 5, the predefined feature space was not reflecting the difference in class information optimally. Subject 3 used mental calculation and imagined tongue movement to control the feedback. Subject 5 used imagined hand movement and happy feelings. For these subjects, classification performance could be significantly improved when using the full range of available EEG channels and features. Both these subjects showed lateral gamma band activity that was not matched by the spatial filter. Although it might be tempting to conclude a considerable improvement in classification rate would be possible when using more suitable features, it is important to rule out confounding factors beforehand.

4.6 Confounding factors analysis

Confounding factors in BCI experiments can arise from two sources: environmental noise or subject movement. Our setup already aimed at reducing environmental noise, but the bigger source of artifacts is the subject himself. Especially muscle movements can disturb the signal. Although explicitly instructed to not use any muscle to control the feedback, subconscious movements might have occurred and leaked into the EEG. Therefore we analyze eye movements and glottis muscle movements by EOG and EMG (EXG) and head movements by an accelerometer separately. Our correlation analysis serves as evidence that class relevant information from these did not improve EEG classification results. It is noteworthy nonetheless that Subject 7 achieved a much higher classification rate on the time domain data of the EXG than on the EEG features.

Movement measurements using additional sensors are limited to experimenter-defined locations. In the last years, there has been increasing interest in identifying EEG signal characteristics caused by movements (e.g. Gonchorova, McFarland, Vaughan & Wolpaw, 2003). It has been proposed that high frequencies near the gamma range on frontal and temporal electrodes may be corrupted by movement artifacts. In our analysis, we could identify such characteristics in signals from three subjects:

Subject 1 showed high gamma activity around the frontal electrodes (Figure 14a). As Yuval-Greenberg et al. (2008) pointed out, microsaccades could lead to activity in the gamma range around this area. In this subject, class difference in the gamma-band was fairly stable in the grand average over all trials. However, there was no increase in classification performance when using all features compared to when using the predefined feature space and therefore this frontal activity had no influence on our results. Still, future work should also deal with investigating further improvements of algorithms that dismiss non-brain activity caused by eye movements from the EEG signal.The EOG decorrelation used here works only on frequencies below those affected by microsaccades.

Subject 3 showed high gamma activity at the outer temporal electrodes T7 and T8; see Figure 14c. Also, the 1-over-f power law did not apply here (Figure 12c). As Goncharova et al. (2003) pointed out, contraction of facial muscles can lead to activity in the gamma range near the temporal lobe. It should also be noted that Subject 3 complained about back problems and did not sit comfortable during the course of the experiment. This may have caused her to be unsettled and disturbed, and thereby producing movement artifacts. But on the other hand, studies using magnetoencephalography (MEG) (e.g. Kissler, Müller, Fehr, Rockstroh & Elbert, 2000) found left-lateralized gamma band activity around the frontal lobe for mental arithmetic tasks – a task that Subject 3 used. Functional-magnetic resonance imaging (fMRI) studies also find left and right lateralized activity in mental arithmetic (e.g. Rivera, Reiss, Eckert & Menon, 2005). Thus, the cause of the gamma-band activity in Subject 3 can be debated, but no safe conclusion about its origin can be made at this point.

A similar reasoning can be applied to the discussion about lateralized activity from Subject 5. Again, there was strong lateral gamma band activity. Subject 5 modulated her emotions to control the feedback. The effect of emotion modulation in the gamma-band in the temporal lobe has been shown by MEG and fMRI studies (e.g. Luo et al., 2009),

and is also described in EEG studies (e.g. Aftanas, Reva, Varlamov, Pavlov & Makhnev, 2004). Facial muscle movements might also have caused this lateral activity.

In Summary, data from the latter two subjects show EEG classification rates that may be influenced by movement artifacts. For Subject 3 and 5, the classifier picked up the possibly contaminated temporal channels as class relevant. By assuming that this gamma-band activity comes from facial muscle movements as explained by Goncharova et al. (2003), the subjects controlled a hybrid brain-computer interface, which means that the control signal was a mixture of unconscious muscle movements and pure brain responses. Note that this gamma-band susceptibility is not specific to our approach, but in fact to any EEG recording. Mathematical methods such as beamforming or independent component analysis (Onton & Makeig, 2009) can help to identify and filter out activity coming from sources outside of the brain. Apart from a mathematical solution to this problem, there are the following three choices how to deal with such noise:

1. Exclude the most temporal and frontal channels from the analysis. Frontal and temporal channels are most susceptible to saccadic or facial muscle artifacts that cannot be filtered out. Channel exclusion constitutes the most conservative way to deal with artifacts.
2. Exclude frequency bands in the high-beta and gamma range, because these frequency bands are most susceptible to muscle artifacts due to their low power in EEG recordings.
3. Allow for such unconscious muscle use that cannot be decorrelated or filtered out, but admit that the patterns in the feedback might have occurred due to use of the peripheral nervous system, similar to a hybrid BCI.

All three options have their downsides. Removing the most temporal and frontal channels is not necessarily enough due to volume conduction. Specific electrical activity is not picked by one channel alone – it spreads over several electrodes. Although this way, the danger that muscle artifacts will leak into the analysis will be reduced, it is unlikely that all artifacts are removed. In fact, by relying on this method the danger to conclude unjustified insights from data is higher as one could assume that muscle artifacts were no matter of concern anymore. Excluding high frequencies from the analysis will reduce the amount of ways to control the feedback, because many high and low-level processes in the human brain correlate with activity in these bands. Especially gamma-band activity has been shown to be a crucial mechanism for neuronal communication (Fries, 2005).

For the free BCI we propose to allow the usage of a hybrid BCI. This, however, is subject to two conditions: First, in the data analysis possible muscle artifacts have to be identified and reported. It is of great importance to be aware of such muscular contributions – also in order to research the influence of (unconscious) muscle movements on the EEG signal. Thereby, advanced detection and filtering algorithms can be developed that might detect or remove muscle artifacts by using additional sensors near facial muscles. Second, muscle contribution to the EEG signal must occur subconsciously. Subjects must still have the impression of controlling the feedback patterns without using the peripheral nervous system. Otherwise, they might exploit tasks that elicit muscle movement on purpose, making the experiment unrelated to brain-computer interfacing.

5. General discussion and future work

The results of the present study show that subjects are able to find personal strategies to control aspects of a rich feedback space. Subjects used a learning-by-doing approach – an approach that could be called neurobabbling. When they found reoccurring patterns, they tried to reproduce them until they were sure that they were reliably controlling certain aspects of the feedback. The subject's cognitive system was able to find controllable regularities in its own response, therefore showing that a free BCI can be successful.

This finding is, however, impaired by the fact that subjectively important features are not of statistical importance – machine adaptation is therefore crucial. Subjects and statistical methods need to optimize similar aspects of the feature space to achieve an optimal result. When statistically important aspects become more salient in the feedback space, subjects might be tempted to try to control these aspects. Moreover, we used a fixed set of features that did not represent subject specific brain activity patterns optimally. If subjects are presented with more appropriate features for their personal mental strategies, a better representation of class performance by the feedback can be expected. In such a mutual adaptation setting the subjectively perceived, as well as the objectively measured performance might increase. However, whether such

a system converges to a stable state is still a subject of great concern and needs thorough investigation.

Implementing online machine adaptation might lead to a great benefit for subjects to find reliable ways to control the feedback and to create patterns, but the susceptibility to high-frequency artifacts might steer the machine-based adaptation in the wrong direction. These artifacts can be filtered away only to a certain extent. Caution has to be taken when there is high-frequency activity in EEG channels that cannot be guaranteed to arise from muscle artifacts as it has also been shown that pure brain responses induce gamma-band activity in a number of tasks (Fries, 2005, Kissler el al., 2000, Onton & Makeig, 2009). In future experiments, we therefore suggest to decorrelate or filter those components out that can be identified as artifacts, but leave in the components that might be either muscle artifact or brain activity. In such a system, similar to a hybrid BCI, muscle movements will not be made conscious. It should also be kept in mind that signal classification rates might then be lower for patients who cannot exploit unconscious muscle artifacts, but also note that we could only identify a few subjects which might have benefitted from such muscle movement.

When it has been shown that a mutually adaptive system converges to a usable state, future studies can focus on multiple session experiments, testing the reliability of mental tasks over time. Until now, subjects used the free BCI only in a single session, yielding stable results for most subjects. The free BCI project also aims to deal with inter-session variability so that subjects can control similar aspects of the feedback by the same mental strategies, independent of non-stationarity of the signal across sessions. This can only be tested in a multi-session experiment. Likewise, an adaptive system makes it possible that subjects achieve similar classification rates across sessions – at least a classification rate above chance. If this is shown, subjects that were unable to control BCIs using conventional tasks, so called BCI illiterates, can be tested. We hypothesize that they can find personal mental tasks to gain control over aspects of the feedback independent of their performance in other BCI setups. Freeing users from rigid task instructions, and hence allowing dynamic and mutual interaction within the system, might be a way out to overcome BCI illiteracy and improve future work on brain-computer interfacing.

Acknowledgment

I would like to thank Professor Peter Desain and Rutger Vlek for their great supervision, inspiring discussions and great support. Furthermore, I would like to thank the whole CAI group for welcoming me and letting me become part of the group, Eric Pankrath for proofreading, Kevin H. Stecyk for teaching me a lesson in how to write (I owe you a lot!), Peter, Marijn, Goedie and Cecilia for a great time during my Master's and my girlfriend Anne, who always supports me. Thanks for staying by my side!

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Mitochondrial dysfunctioning in chronic stress and depression

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Stress adaptation is a plastic process that requires considerable energy. Mitochondria provide the energy required through oxidative phosphorylation. Inhibition of the activities of the respiratory chain complexes has been implicated in the pathogenesis of stress-related brain diseases, such as major depression. Furthermore, mitochondria undergo continuous cycles of fission and fusion. This determines mitochondrial morphology, which is tightly linked to function, including electron transport. We hypothesize that chronic stress will influence mitochondrial morphology and functioning in various stress-sensitive brain areas. Immunohistochemistry was used to determine the effect of chronic stress on mitochondrial morphology, i.e. fission and fusion, and functioning in various stress-sensitive brain areas. Also, a genetic meta-analysis was conducted to determine the association between genes coding for OXPHOS complexes and depression. And, the prevalence of depressive symptoms in pediatric patients with a mitochondrial disease was evaluated using questionnaires. The data show a clear association between genes and depression and there was an increased incidence of depressive symptoms in children with mitochondrial dysfunction compared to the norm population. Furthermore, OPA1 was significantly down-regulated after chronic stress. We argue that this could result in a loss of/damaged mtDNA, which eventually could lead to a significant dysfunctioning of the OXPHOS complexes. When this reaches a certain threshold, this will lead to an insufficient energy production and as such impairs stress adaptation. Together these findings support a mechanism whereby stress-induced alterations in mitochondrial functioning might contribute to stress-related psychopathology.

Keywords: stress adaptation, depression, mitochondria, oxidative phosphorylation, fission, fusion

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1. Introduction

Major depressive disorder (MDD) is a stress-related brain disorder. There is a clear association between stressful life events and depression. Successful adaptation to these stressful events, coordinated by the CRF-corticosteroid system, is a plastic process (De Kloet et al., 2005; Bale & Vale, 2004; Kozicz, 2007). When a situation is perceived as stressful, the brain activates various limbic-forebrain and brainstem neuronal networks, regulating mood, that modify their structures and communication to adapt to the demand. Van Wijk et al. (unpublished data), for instance, showed an activation of the non-preganglionic Edinger-Westphal nucleus (npEW; e.g. upregulation of UCN1 and mRNA production) in addition to signs of plasticity (e.g. increased secretion and number of excitatory synapses) in rats after chronic stress. Besides the npEW other neuronal networks include the hippocampus, prefrontal cortex (PFC), central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), hypothalamic paraventricular nucleus (PVN), and the dorsal raphe nucleus. When this neuronal circuitry fails to successfully adapt (maladaptation), i.e. there is impaired plasticity, this increases the risk for stress-induced brain disorders like MDD (de Kloet et al., 2005; Reul & Holsboer, 2002; Kozicz et al., 2008).

Several theories exist as to the cause of such impaired plasticity, including reduced levels of neurotrophic factors and glutamate toxicity (Nestler et al., 2002; Carlson et al., 2006). Another possibility could be insufficient energy. The mitochondria are the main energy providers of cells and their dysfunctioning could result, among others, in insufficient production/availability of adenosine triphosphate (ATP). Neurons, being high-energy requiring cells, are especially dependent on these organelles for normal functioning and survival. This is evidenced by the fact that mitochondrial diseases mainly involve neuronal tissue. Moreover, mitochondrial dysfunctioning has been implicated in aging and neurodegenerative diseases like Alzheimer's disease (Calabrese et al., 2001). Specifically, mitochondria are critically important in synaptic development and plasticity; many of the proteins and processes that are involved in plasticity require ATP (Mattson et al., 2008; Mattson, 2007). Interestingly, mitochondria also appear to mediate some of the effects of glutamate and BDNF on synaptic plasticity (Bindokas et al., 1998; Burkhalter et al, 2003).

Mitochondria produce ATP mainly through oxidative phosphorylation (OXPHOS). This process involves the action of five multi-subunit complexes (complex I - V) embedded in the inner mitochondrial membrane. These complexes form the electron transport chain. Electrons are fed into this chain and transferred across the different complexes. This transference releases energy which is used for the translocation of protons from the mitochondrial matrix to the intermembrane space. The generated proton gradient is subsequently used by the ATP synthase (complex V) to produce ATP through the phosphorylation of adenosine diphosphate (ADP).

Mitochondria are dynamic organelles that undergo continuous cycles of fission and fusion, mediated by specific proteins (Chen & Chan, 2005). Fusion is mediated by mitofusins 1 and 2 (Mfn1, Mfn2) and optic atrophy protein 1 (OPA1). Fis1 and dynamin-related protein 1 (Drp1) regulate fission. Mitochondrial morphology, which constitutes a tubular network, is determined by the balance between these events. Disruption of fusion causes mitochondrial fragmentation, whereas disruption of fission causes excessively elongated and interconnected mitochondria. Both morphologies have been implicated with impaired energy production (Benard et al., 2007; Amati-Bonneau et al., 2005), indicating that mitochondrial morphology plays a role in OXPHOS. Fission and fusion also appear to be involved in mitochondrial movement, which is essential for synaptic plasticity (Li et al., 2004; Verstreken et al., 2005).

There is increasing evidence for the involvement of mitochondrial dysfunctioning in MDD. For instance, Madrigal et al. (2001) showed an inhibition of the mitochondrial respiratory chain in rat brain after chronic stress, an animal model for depression. POLG-mutant mice, an animal model for mitochondrial DNA depletion, have been shown to display depressive-like behaviors (Kasahara, 2006). Koene et al. (2009) found an increased incidence of MDD in children with a genetic defect of OXPHOS. Furthermore, recent studies found an increased frequency of certain mitochondrial DNA mutations and biochemical signs of insufficient energy production in patients with MDD (Kato, 2007; Shao et al., 2008).

The present study aims to further explore the involvement of mitochondria in depression. It is hypothesized that psychological stressors and/or genetic factors affect the body's energy expenditure, which could eventually result in exhaustion of energy stores. This will ultimately lead to disturbances in the functioning of the stress system. Such dysregulation of the stress adaptation response will consequently

increase vulnerability to depressive-like phenotype.

In order to test this hypothesis, an animal model for depression will be used to evaluate the effects of chronic stress on mitochondrial functioning and morphology. Specifically, the expression of a protein (COX4I1) involved in complex IV activity will be explored as well as the expression of several fission and fusion proteins. This will be done in various stress-sensitive brain areas, i.e. the npEW, PVN, amygdala and BNST. Furthermore, the prevalence of depressive symptoms in children with a mitochondrial disease will be assessed using several questionnaires and the occurrence of mutations in genes coding for the OXPHOS complexes in depression will be evaluated through a genetic meta-analysis.

2. Methods

2.1 Animal study

2.1.1 Animals and experimentation

Twelve adult (3-months old) male Wistar-R Amsterdam rats were used. They were housed under standard vivarium conditions (22°C; lights on at 6 am, lights out at 6 pm) and food and water was available ad libitum.

The rats were divided into two groups, i.e. control rats (n=6) and chronically stressed rats (n=6). The chronically stressed rats were exposed daily to a series of unpredictable variable mild stressors (CVMS; Marin et al., 2007) for 14 days (table 1). Control rats were handled in exactly the same way as stressed animals but were not exposed to stress. All rats were sacrificed after the last exposure to a stressor, between 10 and 11 am.

All animal procedures were conducted at the Anatomy Department of Pécs University in accordance to the Declaration of Helsinki and the animal use guidelines from the Medical Faculty Committee for Animal Resources of Pécs University, Pécs, Hungary. All efforts were made to minimize the number and suffering of rats.

2.1.2 Fixation

Rats were deeply anesthetized with Nembutal (sodium-pentobarbital; Sanofi, Budapest, Hungary; 100 mg/kg body weight), their chest cavity opened, and a small incision was made in the left ventricle. A blood sample of 1 ml was collected in an EDTA-containing tube, centrifuged at 3,000 rpm for 10 min, and stored as 50 µl serum aliquots at -20°C until further use. Meanwhile, rats were transcardially perfused with 50 ml of 0.1 M sodium phosphate-buffered saline (PBS; pH 7.4) for 2 min, followed by perfusion with 250 ml of ice-cold 4% paraformaldehyde in 0.2 M Millonig sodium phosphate buffer (pH 7.4) for 20 min. All blood samples were taken and perfusion was initiated within 5 to 10 min after the first touching of the animals' cage. After perfusion, rats were rapidly decapitated, their brains quickly removed and post fixed in the same fixative solution. The brains were then transferred to 30% sucrose in 0,1 M PBS. When they were completely submerged, 25-µm coronal sections were cut through the brain using a HM 440 E freezing microtome (Microm, Walldorf, Germany). The sections were subsequently saved in sterile antifreeze solution (0.05 M PBS, 30% ethylene glycol, 20% glycerol), at -20 °C, until histological processing.

Table 1. Chronic variable mild stress (CVMS) paradigm

Day	Stress
1	swim stress, 2 min (4°C); humid sawdust, 3 h
2	food/water deprivation, permanent
3	lights on, overnight; humid sawdust, permanent
4	lights off, 180 min; swim stress, 2 min (4°C)
5	food/water deprivation, overnight; isolation,
6	cold isolation (4°C), 15 min; lights off, 120 min
7	swim stress, 4 min (12°C); food/water deprivation, overnight
8	inverted light/dark cycle; humid sawdust, over-
9	constant light, overnight; food/water deprivation, overnight
10	lights off, 180 min; humid sawdust, permanent
11	isolation, overnight; food/water deprivation,
12	restraint stress, 60 min; lights on, overnight
13	inverted light/dark cycle; food/water deprivation, overnight
14	restraint stress, 60 min

2.1.3 Physiological measurements

Body weights of control and CVMS rats were determined at day 0 and day 14. The adrenal glands and thymus, including capsule, were removed after decapitation and placed into a physiological saline. They were subsequently dried on the outside with paper and fat tissue was removed. The organs were weighed on a Sartorius L2200P scale and normalized to total body weight.

Corticosterone radioimmunoassay was performed with 5 µl of a blood serum sample, as described previously (Gaszner et al., 2004), using

3H-corticosterone (12,000 cpm; 90-120 Ci/mmol, NET-399; Perkin-Elmer, Boston, MA) and CS-RCS-57 antiserum (Jozsa et al., 2005). The inter- and intra-assay co-efficient of variation was 9.2% and 6.4%, respectively.

2.1.4 Double Immunohistochemistry

Only sections containing the BNST, PVN, CeA or npEW were selected. After 6 x 10 min rinses in 0.1 M PBS, the Heat-Induced Epitope Retrieval (HIER) method was used in order to improve the reactivity of the antigens. The sections were transferred to 0.01 M citrate buffer (pH 6.0) and heated to 90°C by means of a microwave (10 min) or a water bath (15 min). Afterwards, they were slowly cooled down in the buffer for 30 min. Following a 15 min rinse in 0.1 M PBS, the sections were treated with 0.5% Triton X-100 (Sigma Chemicals, St. Louis, CA, USA) in 0.1 M PBS for 30 min in order to increase cell permeability. Then they were rinsed 3 x 10 min and incubated in 2% normal donkey serum (NDS; Jackson Immunoresearch Laboratories) in 0.1 M PBS with 0.5% TSA blocking reagent (PSB-B TSA; NEN Life Science Products, Boston, MA, USA) for 60 min, followed by a 3 night incubation in (a mixture of) primary antisera in 2% NDS in PBS-B TSA at 4°C. The following sera were used: polyclonal (rabbit) anti-CRF (1:700; gift from Dr. W. W. Vale), polyclonal (goat) anti-UCN1 (1:200; R-20; sc-1825; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), or polyclonal (rabbit) anti-UCN1 (1:2000; gift from Dr. W. W. Vale) with polyclonal (rabbit) anti-FIS1 (1:100; IMG-5113A; Imgenex, San Diego, CA, USA), polyclonal (chicken) anti-MFN1 (1:75; NB110-58853; Novus Biologicals, Littleton, CO, USA), monoclonal (mouse) anti-OPA1 (1:25; 612607; BD Biosciences), monoclonal (mouse) anti-DRP1 (1:100; 611112; BD Biosciences), or (rabbit) anti-COX4I1 (1:100; HPA002485; Sigma, St. Louis, MO, USA). After 3 x 10 min rinses in 0.1 M PBS, the section were incubated in (a mixture of) secondary antisera in PBS-B TSA for 2 hours. The following sera were used: Cy3-conjugated donkey-anti-rabbit IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA), donkey-anti-rabbit biotinylated IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA), donkey-anti-mouse biotinylated IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA), or donkey-anti-chicken biotinylated IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA). A third incubation in a mixture of Cy3 –conjugated streptavidin (Jackson Immunoresearch Laboratories, West Grove, PA, USA) and secondary antisera in 2% NDS in PBS-BTSA for 2 hours followed after 3 x 10 min rinses, with exception of the anti-FIS1 incubation; these sections were mounted. The following sera were used: Cy2-conjugated donkey-anti-rabbit IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA), or Cy2-conjugated donkey-anti-goat IgG (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA). Finally, after 3 x 10 min rinses in 0.1 M PBS, the sections were mounted on gelatin coated glass slides.

2.1.5 Image analysis

Fluorescence images were taken with a confocal laser scanning microscope (Leica microsystems). When necessary, brightness and contrast of the images was adjusted using Adobe Photoshop 11.0.1. For quantification of the different proteins in the npEW, cell bodies that showed a co-localization with UCN1 were counted. For quantification in the BNST, PVN and CeA, all immunoreactive cells were counted. In order to determine the amount of the different proteins per cell, the specific signal density (SSD) was measured for 10 random cells. Both cell count and SSD was determined using the ImageJ software (NIH, Bethesda, MA, USA). Number of cells and SSD was determined in 3-5 sections per animal, and the outcomes averaged. Furthermore, percentage increase/decrease between groups was determined.

2.1.6 Statistical analysis

The data were analyzed in Excel 2003, using a Student's t-test for independent samples (α =5%), and are expressed as mean ± standard error of the mean (SEM).

2.2 Clinical study

2.2.1 Patients

Twenty-three pediatric patients with a mitochondrial disease, 13 males and 10 females aged 2-17 years (mean ± SEM: 7.4 ± 0.9), were evaluated for depressive symptoms. The control group consisted out of 14 pediatric patients with a non-mitochondrial metabolic disease, e.g. GSD IX, MELAS, hypoglycemia. This group consisted out of 7 males and 7 females aged 3-18 years (8.7 ± 1.2). Besides patient data, norm data was also used. The questionnaires were filled in separately by both parents of the patients. The parents/patients

were approached for participation within a period of 9 months during their regular standard outpatient clinics follow-up at the UMC St Radboud and by way of telephone. In total 37 patients were evaluated. For 5 patients only one of the two questionnaires was scored as they did not meet the age requirement for the other questionnaire (see below). From 9 patients only one set of questionnaires was obtained, filled in by the mother.

2.2.2 Questionnaires

In order to evaluate the prevalence of depressive symptoms two questionnaires were used, i.e. the Dutch version of the child behavior checklist (CBCL) and the sociaal-emotionele vragenlijst (SEV). The CBCL (Achenback, 2000) is a screening tool used to evaluate emotional and behavioral problems in children. There are two versions, i.e. one for children aged 1.5-5 years and one version for children aged 6-18 years. The CBCL contains several subscales, e.g. competence scales, internalizing, externalizing. Only the syndrome scales and the DSM-oriented scales are of interest for the current study. Within these, the T-scores for anxious/depressed, withdrawn (/ depressed) and affective problems were used for statistical analysis. The norm data for this test is a T-score of 54 for all the scales evaluated.

The SEV (Scholte & van der Ploeg, 2005) is also a screening tool that evaluates problems in social-emotional development in children (aged 4-18 years). Subscales include ADHD and autism. The scores on the anxious/depressed scale were used for statistical analysis. Unfortunately, no norm data was available.

2.2.3 Statistical analysis

Data was analyzed using SPSS for windows 15.0.1 software (SPSS Inc., Chicago, IL, USA). An independent-samples t-test was used in order to determine the difference between the two patient groups. A one-sample ANOVA was used to compare the two groups with the norm data.

2.3 Genetic meta-analysis

In order to assess the occurrence of genetic mutations of the OXPHOS complexes in depression, the SLEP database was used (https://slep.unc.edu/evidence/). This database only contains information for nuclear-encoded genes and thus mitochondrial DNA was not included in the search. All genes coding for one of the 5 mitochondrial complexes were evaluated. Both primary studies and meta analyses were included in the search. Mutations were evaluated with respect to bipolar and major depression. Furthermore, the following settings were used: GWL 1 mb, GWA 100 kb, MA 5 kb, CNV 100 kb, SignPost 50 kb. GWL studies yielding a linkage score below 3, or below 2 when a meta analysis, were disregarded. Furthermore, the gene had to be within the linkage interval. In case of GWA studies, only p-values $\leq$ 10-4 were of interest and the finding had to be within 100 kb of the gene. For

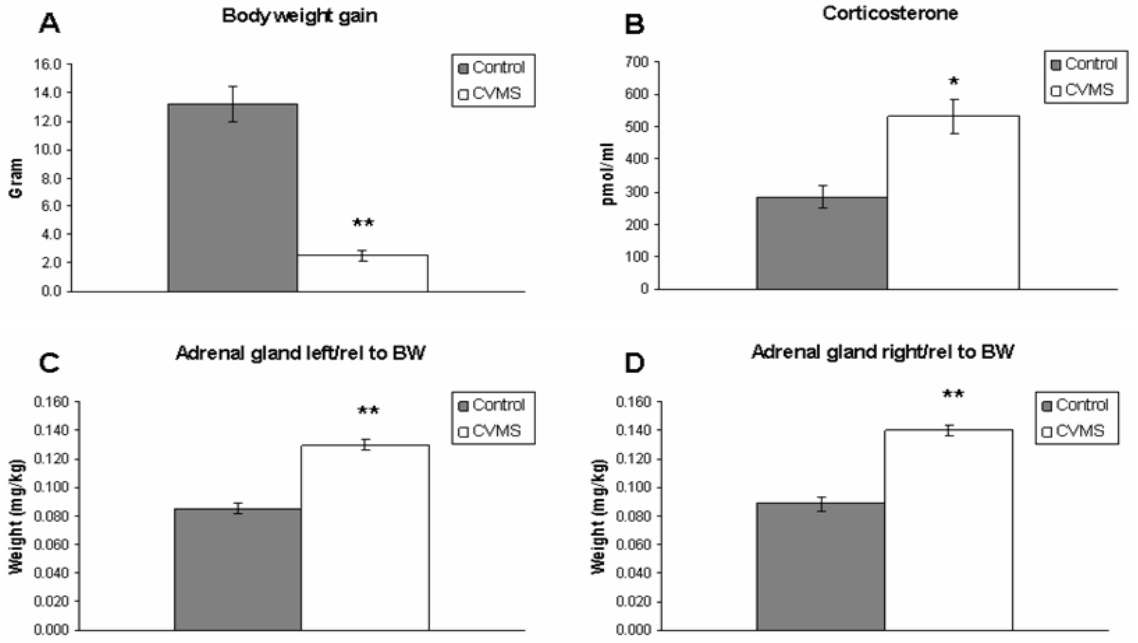

Fig 1. HPA-axis activation. A. Measurements of body weight gain. B. Corticosterone levels. C. Left adrenal weight relative to body weight. D. Right adrenal weight relative to body weight. Mean values ± SEM are shown. *p < 0.01; **p < 10-6.

CNV studies, the gene had to be present in the CNV. All microarray studies were included, SignPosts were disregarded.

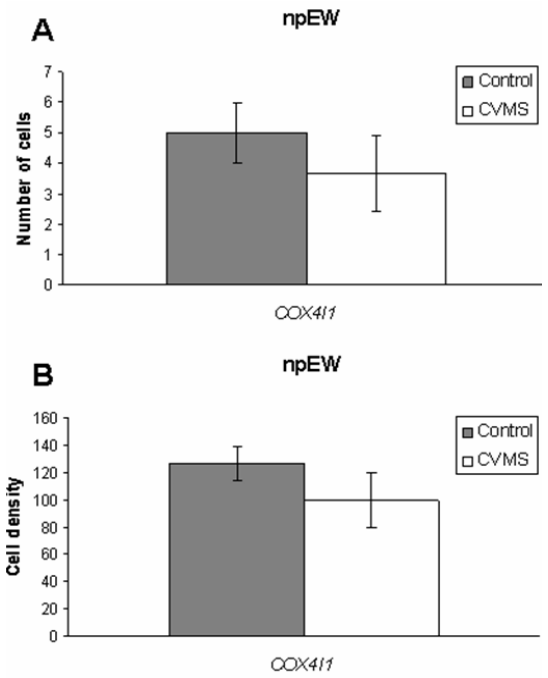

Fig 2. Expression of COX4I1 in the npEW. A. Number of cells expressing COX4I1. B. The average amount of COX4I1 per expressing cell. Mean values ± SEM are shown.

3. Results

3.1 Chronic variable mild stress paradigm

3.1.1 Physiological measurements

In order to establish the occurrence of chronic stress, several physiological measurements were taken (figure 1). There was a significant difference ($p < 0.01$) between control (531.7 ± 54.8) and CVMS (282 ± 1.2) rats with respect to corticosterone values at 120 min after the initiation of the last stressor. Furthermore, chronic stress resulted in a significant difference ($p < 0.01$) in weight gain (control: 13.2 ± 1.2 vs. CVMS: 2.5 ± 0.4). In addition, the total weight of the adrenals showed an approximately 50% increase in CVMS rats compared to control rats.

3.1.2. Non-preganglioninc Edinger-Westphal nucleus

The expression of COX4I1 was not significantly different ($p > 0.05$) between the control and the CVMS rats (figure 2A+B). Both number of cells (control: 5 ± 1.0 vs. CVMS: 3.7 ± 1.2) and SSD (control: 126.9 ± 12.4 vs. CVMS: 99.2 ± 20.1) showed no change.

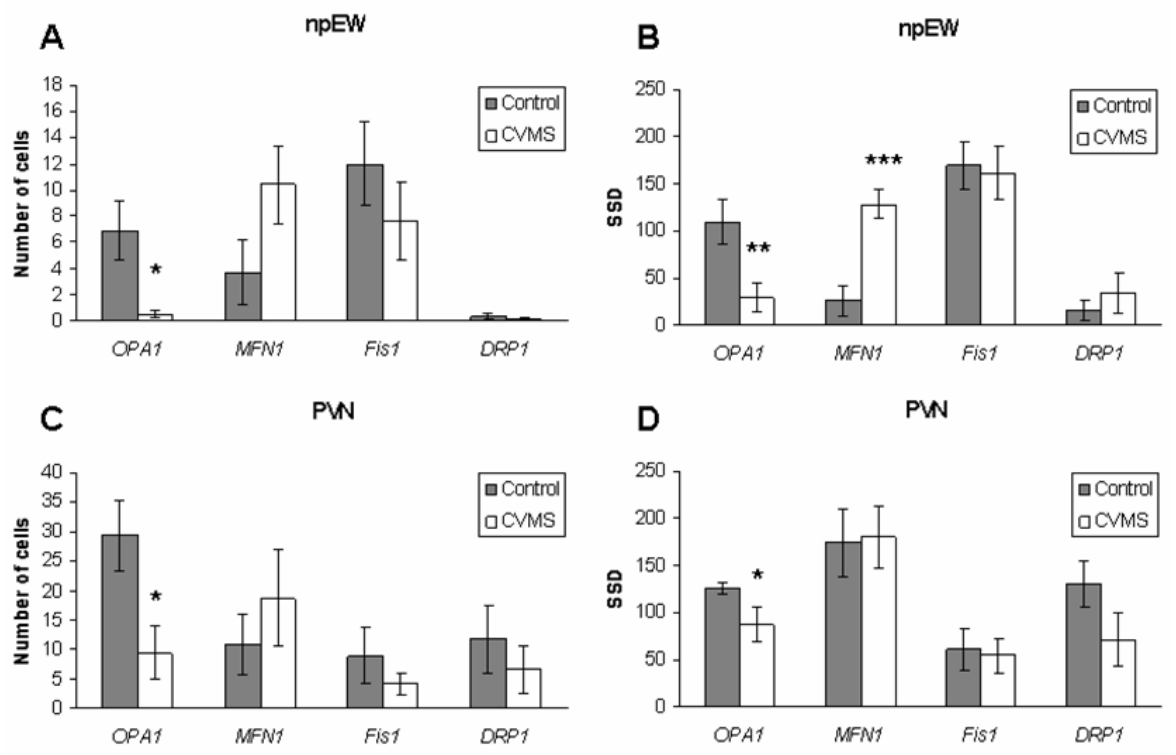

Fig 3. Expression of fission and fusion proteins. A. Number of cells expressing the different fission and fusion proteins in the npEW. B. The average amount of the different fission and fusion proteins per expressing cell in the npEW. C. Number of cells expressing the different fission and fusion proteins in the PVN. D. The average amount of the different fission and fusion proteins per expressing cell in the PVN. Mean values ± SEM are shown. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

As shown by figure 3A+B, the levels of the fusion proteins OPA1 and Mfn1 were significantly different between the two groups. With respect to OPA1, there were 93% fewer neurons expressing this protein in the CVMS rats (0.5 ± 0.3) than in the control rats (6.9 ± 2.3; $p < 0.05$). Furthermore, the SSD decreased with 98% in the chronically stressed group (29.0 ± 15.0) compared to the control group (110.4 ± 24.2; $p < 0.01$). For Mfn1 the number of cells did not change (control: 3.7 ± 2.4 vs. CVMS: 10.4 ± 2.9; $p > 0.05$). However, there was an increase of approximately 395% in the SSD in the CVMS rats (127.9 ± 15.0) compared to the control rats (25.8 ± 16.3; $p < 0.001$).

Figure 3A+B also shows the results for the fission proteins Fis1 and Drp1. Neither protein displayed a significant change with respect to their expression. There was no significant difference with respect to the number of cells expressing Fis1 (control: 12.0 ± 3.2 vs. CVMS: 7.6 ± 3.0; $p > 0.05$) or Drp1 (control: 0.4 ± 0.3 vs. CVMS: 0.1 ± 0.1; $p > 0.05$), nor was there for the SSD of Fis1 (control: 169.5 ± 25.3 vs. CVMS: 161.5 ± 28.2; $p > 0.05$) or Drp1 (control: 15.9 ± 10.3 vs. CVMS: 34.4 ± 20.7; $p > 0.05$)

3.1.3 Hypothalamic paraventricular nucleus

With respect to the fusion proteins (figure 3C+D), only OPA1 expression was significantly different after chronic stress. The SSD was decreased with 30% in the CVMS rats (87.5 ± 18.6) compared to the control rats (125.0 ± 6.2: $p < 0.05$). Also, there were a 68% lower number of cells expressing OPA1 in the CVMS rats (29.3 ± 6.0) compared to the controls (9.4 ± 4.5; $p < 0.05$). The number of cells expressing Mfn1 in the CVMS rats (18.7 ± 8.1) was not significantly different from the control rats (10.9 ± 5.2; $P > 0.05$). There was also no significant difference between controls (17.3 ± 35.3) and chronically stressed rats (180.2 ± 33.0; $P > 0.05$) with respect to Mfn1 SSD.

The levels of the fission proteins were also not significantly different in the PVN (figure 3C+D). The number of cells expressing Fis1 or Drp1 did not increase/decrease in the CVMS rats (Fis1: 4.1 ± 1.9; DRP1: 6.6 ± 4.1) compared to the control rats (Fis1: 8.9 ± 4.8; $p > 0.05$; DRP1: 11.8 ± 5.8; $p > 0.05$). The SSD was also the same between the groups for both Fis1 (control: 60.6 ± 22.6 vs. CVMS: 54.3 ± 19.0; $p > 0.05$) and Drp1 (control: 130.4 ± 24.2 vs. CVMS: 71.4 ± 28.3; $p > 0.05$).

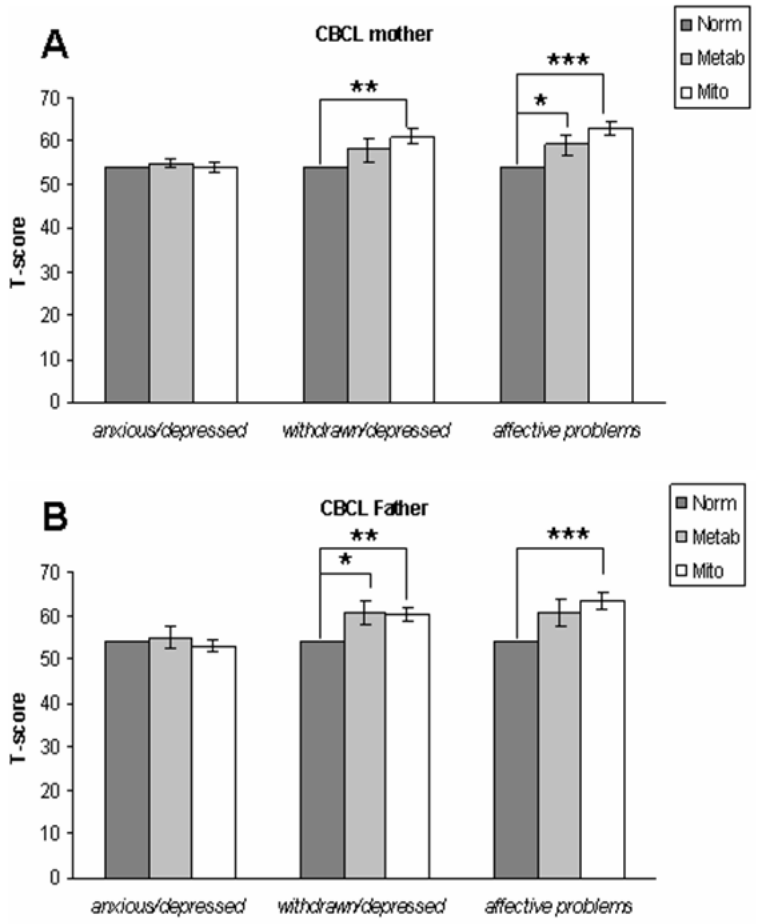

Fig 4. CBCL scores. A. Scores from the mother. B. Scores from the father. Mean values ± SEM are shown. *$p < 0.05$; **$p < 0.01$;

Table 2. Mean and standard error of the mean (SEM) for the CBCL and SEV

	Mean		SEM	
	Mother	Father	Mother	Father
Anxious/ depressed (SEV)	2.1	0.38	1.1	0.3
Anxious/ depressed	55.5	54.8	2.7	2.4
With-drawn/ depressed	58.0	60.6	2.3	2.8
Affective problems	59.3	60.7	2.3	3.4

3.1.4. Amygdala and bed nucleus of the stria terminalis

The immunofluorescent staining in these regions was such (diffuse) that no reliable quantification could be made.

3.2 Questionnaires

Figures 4A+5A show the results from the mother. The patients with a mitochondrial disorder scored significantly higher on the withdrawn/depressed ($t = 3.8$, $p < 0.01$) and affective problems scales ($t = 5.4$, $p < 0.01$) of the CBCL than the norm population. The metabolic group only showed a significant higher score on the affective problems scale ($t = 2.4$, $p < 0.05$). There was no difference with respect to the withdrawn/depressed scale between the metabolic group and the norm population, nor was there for the anxious/depressed scale of both the SEV and CBCL for both groups. Furthermore, there was no significant difference between the metabolic group and the mitochondrial group on any of the scales. Table 2 shows the mean and SEM for the two groups across the different scales.

The results from the father were similar to that of the mother (figure 4B+5B). Both the patients with a mitochondrial disorder as the metabolic group scored significantly higher on the withdrawn/depressed scale (mitochondrial: $t = 3.7$, $p < 0.01$; metabolic: $t = 2.3$, $p < 0.05$) compared to the norm population. However, only the mitochondrial group scored higher on the affective problems scale ($t = 5.2$, $p < 0.01$). There was no significant difference between the metabolic group and the norm population on this scale. Also, both groups scored the same as the norm population on the anxious/depressed scale of both the CBCL and SEV. Moreover, no differences were found between the mitochondrial and metabolic group on the different scales. Table 2 shows the mean and SEM for the two groups across the different scales.

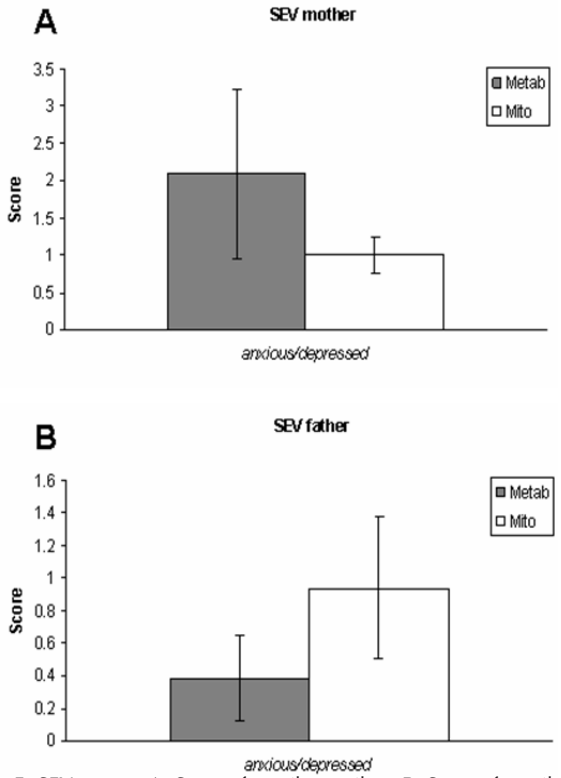

Fig 5. SEV scores. A. Scores from the mother. B. Scores from the father. Mean values ± SEM are shown.

3.3 Genetic review study

The search in the SLEP database yielded a number of interesting associations between polymorphisms in various mitochondrial complex genes and depression. Table 3 shows which genetic polymorphisms met the criteria. With respect to MDD, three polymorphisms were identified. Two were in genes coding for complex V, i.e. an assembly factor and a subunit, and one polymorphism in a gene coding for a subunit of complex I.

4. Discussion

The present study was aimed at exploring the possible role of mitochondria in the development of MDD. It was hypothesized that chronic stress results in insufficient energy, leading to a dysfunction of the stress system and subsequently increasing the risk for a depression. Figure 6 shows a model of this hypothesis. This model was adapted from the LEARn model for disease progression (Lahiri et al., 2009). Figure 6A shows the natural decline in mitochondrial function that occurs with age in every healthy individual. However, if someone were to experience a significant stressful event (hit),

Table 3. Polymorphisms in mitochondrial complex I, IV and V genes associated with depression. Chr = chromosoom, PMID = PubMed ID, MDD = major depressive disorder, BIP = bipolar disorder, GWA = genome-wide association study, GWL = genome-wide linkage study, MA = microarray.

Gene	Function	Chr	Gene Region	Location	Disorder	Study Type	Study Method	Score Stat	P-value	PMID
NDUFB3	Subunit Complex I	2	201,644,706-201,658,718	201,677,185-201,763,557	MDD	Primary	GWA	-	0.00002405	19065144
ATPAF2	Assembly factor Complex V	17	17,862,058-17,883,205	17,205,258-36,247,990	MDD	Primary	GWL	2.1	-	18615541
ATP6V1G2	Subunit Complex V	6	31,620,218-31,622,606	31,628,405-31,650,461	MDD	Primary	GWA	-	0.0006673	19065144

this could damage the mitochondria, resulting in a decline in functioning (figure 6B). At a certain point this will go below the normal range, meaning there is an insufficient energy production. This will subsequently increase the risk for depression.

In order to test this hypothesis, rats were chronically stressed using the CVMS paradigm. This model was successful in generating stress given the fact that all physiological parameters point to a chronic activation of the HPA-axis. This model also leads to depressive behavior as evidenced by the forced swim test (Van Wijk et al., unpublished data).

The chronic stress led to a change in the expression of the fusion proteins in both the npEW and the PVN. OPA1 was reduced in both areas, whereas Mfn1 was increased in the npEW. There was no change with respect to the expression of the fission proteins.

Another aspect investigated was the link between mitochondria related genes and depression. A variety of genetic studies were analyzed and it was shown that various polymorphisms in genes coding for the OXPHOS complexes were associated with mood disorders. Furthermore, a clinical study with pediatric patients was conducted. This confirmed an increase in depressive symptoms in children with a mitochondrial dysfunction compared to a healthy population.

4.1 Increased incidence of depressive symptoms in mitochondrial dysfunction

Although there was an increase in depressive symptoms in children with a mitochondrial dysfunction, there was no difference between these children and the pediatric patients with a different type of metabolic disorder. This could indicate that the increase in depressive symptoms is primarily caused by the fact that these children are chronically ill. However, this does not support our hypothesis, or the literature. A study by Morava et al. (In Press), for instance, showed that there was a difference between children with a mitochondrial disorder and children with Sotos syndrome, which is also a chronic disease. It is believed that, although being chronically sick will increase the prevalence of depressive symptoms, a mitochondrial dysfunction would add to this increase due to its proposed role in stress adaptation.

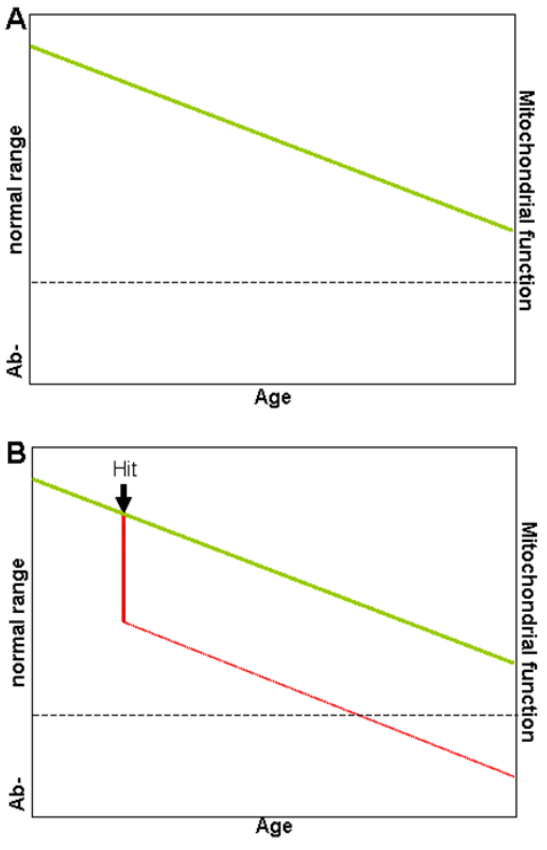

Fig 6. Model for progression of mitochondrial function. A. Normal situation with age associated decline in mitochondrial function. B. Decrease in mitochondrial function (red line) after a hit.

Looking at the data, the problem probably lies within the number of patients used for this study, i.e. in total and per group. For the mitochondrial group there were 24 patients, whereas for the metabolic group there were only 13 patients. This means that there is data from only 37 patients. A power analysis actually revealed that at least 50 patients per group were needed in order to obtain a statistically meaningful result. Thus, more questionnaires need to be collected before any meaningful conclusions can be drawn from these questionnaires.

4.2 Genetic complex alterations in depression

As said, a number of interesting polymorphisms within genes coding for the mitochondrial complexes were found to be associated with mood disorders. This is indicative of an involvement of mitochondria in stress-induced brain disorders. The majority of the polymorphisms were associated with a bipolar disorder. Although this is very interesting, this disease does not represent a pure depression in the sense that it also includes mania. Looking at MDD, only three polymorphisms were associated. However these associations are quite weak. It is believed that the current methods, used in the studies analyzed, are successful in identifying common genes that have a relatively large effect (e.g. Saxena et al., 2007). However, they often fail to identify the less common genes and genes that have small effects for the more complex traits (Maher, 2008). A better strategy would be to collectively test genes involved in specific biological pathways, such that the combined effects of genetic variants with a small effect can be examined (Torkamani et al., 2008; Ruano et al., 2010).

In the current study only the nuclear-encoded genes that code for the five complexes were evaluated. Given the results from the animal study (see below), it would be interesting to also analyze the genes coding for the different fission and fusion proteins, as well as the mtDNA.

4.3 Effects of chronic stress on mitochondrial fission and fusion

The chronic stress clearly had an effect on the morphology of mitochondria in various stress-sensitive brain areas, by way of changing the balance between fission and fusion. As stated previously, the balance between these events has been found to play a crucial role in the functioning and distribution of mitochondria.

Fusion plays a crucial role in maintaining the health of the mitochondrial population, in that it allows for the mixing and exchange of small molecules, proteins and mtDNA (Chen & Chan, 2004). Disruption of this process has been associated with a loss of mitochondrial membrane potential and OXPHOS dysfunction, caused probably by the loss of mtDNA or metabolic substrates (Chen & Chan, 2005; Amati-Bonnea et al., 2005; Chen et al., 2007; Chen et al., 2003). Furthermore, fusion has also been associated with a disruption of mitochondrial distribution, which is essential for synaptic plasticity. Mfn2 deficient cells, for instance, display a clustering of mitochondria in the cell body together with a reduction in number of spines (Chen et al., 2007).

Fission is required for the elimination of dysfunctional mitochondria and it appears to be involved in apoptosis (Twig et al., 2008; Suen et al., 2008) and the redistribution of mitochondria in response to local changes in the demand for ATP (Skulachev, 2001). Disruption of the fission process has similar effects to disturbed fusion. First of all, given its role in distribution, it has also been implicated in mitochondrial movement. Mutation in Drp1 has been shown to negatively influence distribution and thus, as such, the morphology and plasticity of spines and synapses (Li et al., 2004; Verstreken et al., 2005). In addition, Benard et al. (2007) demonstrated that disruption of fission also leads to an impaired energy production. Parone et al. (2008) showed similar results. Furthermore, they describe an inhibition of cell proliferation, an increase in ROS levels, autophagy and loss of mtDNA. Whether the increased ROS levels or the loss of mtDNA is the causal factor here, is still unclear.

The current study showed an increase in Mfn1 in the npEW, which would indicate an increase in fusion. Various studies have shown that this represents an adaptive response to changed cellular energy demands (Hackenbrock, 1966; Hackenbrock, 1968; Hackenbrock et al., 1971; Rossignol et al., 2004; Jakobs et al., 2003). Upon activation of the OXPHOS system for ATP production, there appears to be a transformation from the so-called "orthodox" to a "condensed" configuration, i.e. they become elongated, strongly branched and interconnected. Furthermore, Tondera et al., (2009) found that exposure to apoptotic stimuli resulted in the same reconfiguration. They state that this might represent an adaptive pro-survival response to stress. Although there is an important difference between the stimuli used, the same might be occurring here,

as several studies have shown that stress is also an apoptotic stimulus (Lee et al., 2006; Lowy et al., 1995). On an important note, fusion cannot occur without Mfn2 (Chen et al., 2003). Unfortunately there is no data available for this protein. Future studies should include this mitofusin in order to obtain a complete picture.

Based on the Mfn1 data it would seem that the mitochondria within the npEW display an adaptive response to the chronic stress. However, in the PVN such a response is not seen. Furthermore, in both regions the expression of OPA1 is decreased. Both OPA1 and mitofusins mediate fusion. However, what this decrease in OPA1 means for this process is unclear. Based on the literature there are three possibilities, i.e. no fusion, incomplete fusion or transient fusion.

There is conflicting data as to whether fusion can occur without OPA1. Some studies show that a disruption of OPA1 blocks fusion and results in a fragmented network (Chen et al., 2005; Olichon et al., 2003). However, several other studies have shown that the mitofusins and OPA1 regulate sequential steps in the fusion process (Song et al., 2009; Malka et al., 2005). Mfn1 and Mfn2 regulate fusion of the outer membrane and OPA1 that of the inner membrane. Fusion of the outer membrane does not require the presence of OPA1. Thus, mitochondria depleted of OPA1 are still capable of fusion, albeit incomplete fusion. Such an incomplete fusion would prevent the exchange of mtDNA, and leads in many instances to mitochondria with multiple matrix compartments (Song et al., 2009).

As said a third possibility is that of transient fusion. Liu et al., (2009) have recently shown that there are two distinct fusion events, i.e. transient and complete fusion. Transient fusions are of extremely short duration and maintain the original shape of the participating mitochondria. Importantly, transient fusion seems to display a distinct dependency on OPA1. Whereas complete fusion decreases and disappears with either low or extremely high levels respectively, transient fusion increases. Furthermore, transient fusion promotes mitochondrial motility and appears to be sufficient to support mitochondrial metabolism. However, due to its fast nature, it does not allow for the exchange of slow moving proteins and mtDNA.

In order to determine which scenario is occurring here, morphometric data on the (ultra)structure of these organelles is required. Furthermore, as said, the expression of Mfn2 needs to be established to determine whether transient or incomplete fusion is a possibility for this paradigm.

Whichever type of fusion occurred, all are associated with a lack of protein and mtDNA exchange, resulting in depletion and/or damage. Chen et al. (2007) propose that under normal circumstances depletion also occurs, but it is rare due to the transient nature resulting from mitochondrial dynamics. However, during long-term periods of fusion-deficiency mitochondria lacking mtDNA, and thus respiratory activity, accumulate. (Chen et al., 2007). Furthermore, mtDNA mutations and deletions will no longer be 'repaired', resulting in a rapid accumulation of mutations and deletions in the mitochondrial genome (Chen et al., 2010). When these reach a certain threshold this can affect OXPHOS (DiMauro & Schon, 2003; Taylor & Turnbull, 2005).

Although the current study did not find a change in complex IV activity within the npEW, this does not say anything about the overall functioning of the OXPHOS system as it is only one of five complexes. An animal study by Madrigal et al. (2001), using immobilization stress, also found that complex IV activity in the cortex was not affected by chronic stress. The activity of complexes I, II and III was, however, significantly reduced. The finding that complex II activity was reduced is interesting, in that it is the only complex fully encoded by the nuclear genome and therefore a clear indication that other factors besides mtDNA alterations are involved. Rezin et al. (2008), using the chronic stress model, found similar results, i.e. reduced complex I and III activity. However, they also showed a reduction of complex IV activity, but not of complex II activity. Furthermore, they only obtained these results within the cortex and cerebellum, not within the hippocampus. Rodenburg et al. (unpublished data), however, did find changed complex activity within the hippocampus. Using the CVMS paradigm to induce chronic stress, he looked at complex I and IV activity within the hippocampus, PVN, BNST and CeA. He found a significant reduction of complex 1 activity within the hippocampus and CeA, and decreased complex IV activity within the BNST. No changes with respect to PVN were found, but activity of complex II, III and V was not examined. Unfortunately no data is available for the npEW. These studies show a clear effect on the OXPHOS system after chronic stress.

Interestingly, Madrigal et al. (2001) did not find a reduction in the ATP production. This is surprising given the reduced complex activity, and the increased mtDNA mutations and reduced ATP production found in patients with depression (Videbech, 2000; Gardner et al., 2003; Shao et al., 2008). However, it

appears that energy production is not affected until a certain threshold of reduced complex activity has been reached (Davey & Clark, 1996; Madrigal et al., 2001).

To conclude, the present study strengthens our hypothesis, i.e. there are clear indications that mitochondria play a role in stress-induced brain disorders. The data fit nicely with the model for the progression of mitochondrial function (figure 6). The chronic stress represents the hit, which causes a defiency in the fusion process. This results in a loss of and/or mutations and deletions in mtDNA and, when this reaches a certain threshold, subsequent dysfunctioning of the OXPHOS system (red line). When this again reaches a specific threshold (dotted line), this will lead to insufficient energy production (abnormal range), a dysfunction of the stress system and subsequently an increased risk for depression. An interesting question that warrants further study is whether this damage is reversible or not.

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Dopaminergic medication effects in Parkinson's disease on probabilistic selection reflect value-based choice rather than learning

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Dopamine has long been implicated in reward-based learning and the expression of learning on choice. Although effects on learning and choice enjoy ample theoretical and empirical support, teasing them apart has proven challenging. We have adapted a classic test of value-based learning, the probabilistic selection task, to investigate effects of dopamine on value-based choice rather than on learning per se. Dopaminergic medication in patients with mild Parkinson's disease potentiated reward-based approach but not punishment-based avoidance, both in terms of accuracy and response vigor. These data demonstrate that effects of dopamine on probabilistic choice are at least partly mediated by effects on the expression of learning rather than on learning per se, and help refine current models of dopamine's role in reward.

Keywords: Parkinson's disease, motivation, dopamine, reinforcement learning, decision-making, value-based choice

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1. Introduction

Midbrain dopamine (DA) is involved in a wide range of cognitive functions including working memory (Brozoski, Brown, Rosvold, & Goldman, 1979), task-switching (Cools, Barker, Sahakian, & Robbins, 2001), motivation (Berridge & Robinson, 1998), and reinforcement learning (Hollerman & Schultz, 1998). Current models of dopamine's role highlight its contribution to driving behavior by reward, through effects on learning, motivation, overcoming effort, or more generally, behavioral activation (Berridge & Robinson, 1998; Robbins & Everitt, 2007; Salamone, Correa, Mingote, & Weber, 2005). The predominant view in computational and systems neuroscience holds that DA serves to promote reinforcement learning (RL), that is trial-and-error instrumental learning to choose rewarding actions (Houk & Wise, 1995; Montague, Dayan, & Sejnowski, 1996; Paton, Belova, Morrison, & Salzman, 2006; Samejima, Ueda, Doya, & Kimura, 2005; Schultz, Dayan, & Montague, 1997). This idea was derived from electrophysiological recordings from neurons in the midbrain dopaminergic nuclei of primates performing simple tasks for reward (Hollerman & Schultz, 1998; Ljungberg, Apicella, & Schultz, 1992; Waelti, Dickinson, & Schultz, 2001), together with the insight that the phasic firing of these neurons quantitatively resembles a 'reward prediction error' signal used in computational algorithms for RL to improve action choice so as to obtain more rewards (Bayer & Glimcher, 2005; Frank, 2005; Montague, et al., 1996; Montague, Hyman, & Cohen, 2004; Sutton & Barto, 1998). Consistent are recordings in both nonhuman primates (Hollerman & Schultz, 1998; Schultz, 1998) and humans (Zaghloul et al., 2009), which show that the phasic response of DA neurons in the midbrain is proportional to the difference between expected and actual outcome.

Supporting evidence for a role of dopamine in human RL comes from controlled medication withdrawal studies in Parkinson's disease. Parkinson's disease (PD) is associated with dopaminergic cell loss in the substantia nigra pars compacta (Hassler, 1938), which projects to the basal ganglia through mesolimbic and nigrostriatal projections. PD is commonly treated by alleviating DA depletion through DA enhancing drugs (levodopa and/or DA receptor agonists) and the role of DA in human cognition can be analysed by examining the effects of withdrawing dopaminergic medication in PD.

Studies employing this approach have revealed effects of DA on reinforcement learning by showing effects on value-based choice (e.g. Frank, Seeberger, and O'Reilly (2004) and Rutledge et al. (2009). For example, Frank and colleagues (2007; 2004) have shown that dopaminergic medication in PD alters the relative tendency to learn from appetitive versus aversive feedback. However, one major problem with this approach is that performance on the tasks used to assess RL depends not only on the gradual learning of associations between stimuli and responses based on reinforcement, but also on the expression of such learning on choice. Accordingly, effects on learning may partly reflect effects on the expression of learning, i.e. value-based choice rather than on learning per se. In the present study, we aim to disentangle effects of DA on value-based choice from those on reinforcement learning.

Dissociating dopamine's role in choice from learning is pertinent, because dopamine is increasingly recognized to be involved less in acquisition and more in the performance of motivated behavior. Indeed, the most pronounced effects of causal DA manipulations tend to be on performance rather than learning, with DA promoting behavioral vigor or activation more generally (Berridge, 2007; Ikemoto & Panksepp, 1999; Robbins & Everitt, 2007; Salamone, Correa, Farrar, & Mingote, 2007). Two current interpretations characterize these effects as arising via dopaminergic modulation of incentive motivation (Berridge, 2007) or cost/benefit tradeoffs (Salamone, et al., 2007). Other authors writing from a similar tradition have provided a more general activational account (Robbins & Everitt, 1982, 1992, 2007), stressing both a performance-based energetic component to DA as well as reinforcement-related functions more akin to those posited in the computational RL models, e.g. conditioned reinforcement and stamping-in of stimulus-response habits (Wise, 2004). Indeed, early experimental work by Gallistel et al. (1974) argued for both reinforcing and activational effects of (putatively dopaminergic) brain stimulation reward, distinguished as progressive and immediate effects of contingent versus non-contingent self-stimulation.

To disentangle effects of DA on learning from those on choice, we designed an adapted version of the probabilistic selection task by Frank et al. (2004). Like the original version, the adapted version also consisted of a learning phase and a test phase. However, unlike the original learning phase, our learning phase required subjects to learn a series of associations between affectively neutral cues and affectively neutral outcomes, rather than

Table 1. Demographic variables and neuropsychological test scores. **(A)** Demographic and disease characteristics of participants. UPDRS: Unified Parkinson's Disease Rating Scale. **(B)** Background neuropsychological tests. Asterisks indicate significance level of $p < 0.05$ in two-tailed control versus patient student t-tests, uncorrected for multiple comparisons. DART: Dutch Adult Reading Test; BDI: Beck Depression Inventory; FAB: Frontal Assessment Battery; MMSE: Mini-Mental State Examination; BIS: Barratt Impulsivity Scale. For relevant references on the tests, see methods. Values in A and B represent Mean(SEM).

A

	n	sex ratio m:f	age (years)	education (years)	UPDRS ON	UPDRS OFF	L-dopa equivalent dose (mg)	disease duration (years)
patients	18	12:6	55.4 (2.2)	5.2 (1.2)	20.8 (1.7)	29.9 (1.9)	555.8 (119.3)	4.8 (0.7)
controls	14	9:5	58.8 (3.3)	5.8 (1.5)				

B

	DART*	MMSE	BIS total	BDI*	FAB		Digit span		Block completion		Number cancellation	
					on	off	on	off	on*	off*	on*	off*
patients	79.3 (3.1)	28.6 (0.4)	62.7 (1.5)	8.5 (1.0)	17.4 (0.2)	16.5 (0.3)	6.0 (0.3)	5.7 (0.2)	108 (6)	112 (7)	315 (7)	314 (10)
controls	90.3 (2.4)	28.6 (0.3)	60.9 (1.7)	3.7 (0.9)	17.2 (0.2)		6.2 (0.3)		81 (8)		260 (16)	

a series of associations between affectively neutral stimuli and reward or punishment. In the adapted version, reward and punishment values were assigned to the outcomes only after learning and prior to testing. The implication of this adaptation is that any valence-specific effect of medication on approaching rewards versus avoiding punishments must be due to an effect of medication on value-based choice rather than on reinforcement learning. If value-based choice on our adapted task is not altered by dopaminergic medication, then this would suggest that previously observed valence-specific effects on probabilistic selection tasks (Frank, et al., 2004) are more likely due to modulation of learning rather than choice. However, if we do find an effect of dopaminergic medication on value-based choice, then this would support a role for DA in driving behavior independent of reinforcement learning. Such a result would help refine current models of DA by emphasizing a role in behavioral control that is independent of learning. We expect to find that dopaminergic medication improves performance on trials involving reward while at the same time impairing performance on trials involving punishment. Withdrawing medication should have the opposite effect: low dopamine impairs performance on trials involving reward, but improves performance on trials involving punishment.

2. Methods

2.1 Subjects

Eighteen patients with PD and 13 age- and education-matched healthy controls participated in the study. The clinical and demographic characteristics of the subjects are shown in table 1, the in- and exclusion criteria are shown in S1 (see online edition for supplementary material).

All patients were diagnosed by a neurologist (BRB or Dr R. Esselink) at the general neurology clinic at the Department of Neurology of the Radboud University Nijmegen Medical Centre as having idiopathic PD, according to the UK Brain Bank criteria. Clinical disease severity was assessed at the start of each session using the motor subscale (part III) of the Unified Parkinson's Disease Rating Scale (Fahn, Elton, & Others, 1987). All patients were taking dopaminergic medication (levodopa and/or DA receptor agonists; details, including the levodopa equivalent daily dose (Wenzelburger et al., 2002) are summarized in S2 (see online edition)).

2.2 General procedure

Patients were tested both on and off their dopaminergic medication, at least seven days apart. The order of on/off testing was counterbalanced (9

patients were tested ON medication first). In the ON condition patients took their regular second dose of the day (around 12pm) 45 minutes before the start of the experiment. Prolonged release medication was taken at the regular time, commonly early in the morning. In the OFF condition, all dopaminergic medication was withheld for at least 21 hours (or 51 hours for prolonged release medication such as Requip Modutab, Sifrol, and Sinemet controlled release) at the time of the experiment. Healthy controls were tested twice to estimate test-retest (e.g. practice) effects.

All subjects provided informed written consent prior to their participation in the study. All procedures were approved by the Committee for the Protection of Human Subjects (CMO region Arnhem Nijmegen; protocol number 2008/159).

The experiment was performed as part of a larger study for which patients and controls were scanned using functional magnetic resonance imaging (results reported elsewhere). Disease severity, demographics and background neuropsychological performance were assessed prior to scanner entrance. The experimental paradigm of interest for the current paper was administered approximately 60 minutes after subjects were scanned.

2.3 Background neuropsychological tests

A battery of tests was used to probe a range of neuropsychological functions. For details of the tests, readers are referred to the appropriate references. Depression using the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), dementia using the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), frontal executive function using the Frontal Assessment Battery (Dubois, Slachevsky, Litvan, & Pillon, 2000) and cognitive processing speed using the box completion task (Salthouse, 1994) were assessed to provide background data. The Dutch version of the National Adult Reading Test (DART) (Nelson & O'Connell, 1978; Schmand, Bakker, Saan, & Louman, 1991) provided a measure of premorbid intelligence levels. The BIS-11 (Barratt, 1985) was used to assess cognitive impulsiveness, motor impulsiveness and non-planning impulsiveness. A number cancellation task was used to assess sustained attention and concentration. The results were analysed using independent samples t-tests. Except for the DART, the neuropsychological profile was consistent with the mild pattern of cognitive impairments seen in previous studies.

2.4 Task

A modified version of the probabilistic selection (PS) task was used (Frank, et al., 2004). The task was programmed using Presentation 14.1 (Neurobs, Inc., Albany, California, USA; www.neurobs.com) and presented on a Windows XP SP2 computer. Subjects sat in front of a computer screen in a lighted room. The instructions as given to the subjects are described in S5 (see online edition).

Like the original task, the current task consisted of two phases: an initial training phase and a subsequent testing phase. Unlike the original learning phase, our learning phase required subjects to learn a series of associations between affectively neutral cues and affectively neutral outcomes, rather than a series of associations between affectively neutral stimuli and reward or punishment. Here, reward and punishment values were assigned to the outcomes only after learning and prior to testing (see Figure 1).

In the learning phase, three different pairs of Hiragana characters (AB, CD, EF) were presented in random order, with the assignment of Hiragana characters to elements A-F randomised between subjects. Each character represented a cue and was associated, stochastically, with one of two affectively neutral outcomes, represented by two coloured shapes. For example, cue (Hiragana character) A was associated with outcome (coloured shape) 1 on 80% of trials, while cue B was associated with outcome 1 on 20% of trials. Cue A was thus associated with outcome 2 on 20% of trials, while cue B was associated with outcome 2 on 80% of trials (Fig. 1). Hiragana characters were presented in white (8 cm height on-screen) and outcomes in colour on a black background (5 cm height on-screen), ~ 80 cm from the subject.

During the learning phase, the subject's task was to learn the associations between cues and affectively neutral outcomes through observational learning. In a single trial, a pair of cues was presented (right/left location randomized). Subjects had eight seconds to respond with the left or right key—to choose between the two cues—and to observe the outcome. Upon choosing a cue, a white box was shown around that cue and the outcome was shown in between the two cues for 0.5 s. After a jittered fixation period of 500 to 1500 ms, a new pair of cues was shown. Subjects learned to associate cues with outcomes by keeping track of the outcomes of their choices. Subjects were informed that the cue-outcome contingency was reversed between the two cues of each pair.

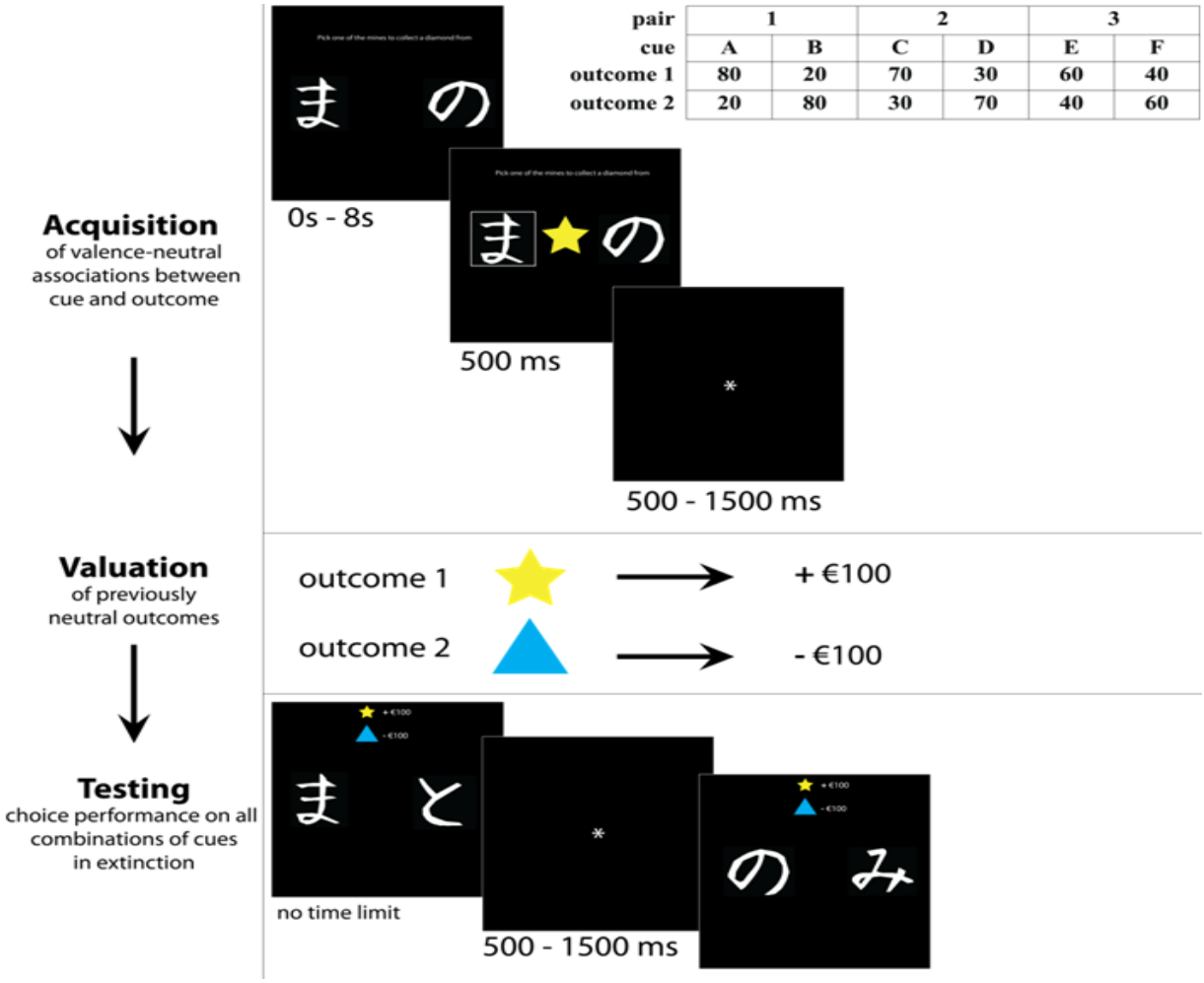

Fig. 1 Description of the probabilistic task with delayed valuation. During the acquisition phase subjects gradually acquire an association between cues, represented by Hiragana characters, and two possible outcomes, represented by coloured shapes. During each acquisition trial, participants are shown one of the pairs, which are presented in pseudorandom order. After choosing one of the cues within 8 seconds, one of two possible outcomes is shown, chosen randomly based on the contingencies shown in the table (in %). Over the course of acquisition, participants learn to associate cue A, C and E with neutral outcome 1, and cue B, D and F with neutral outcome 2. Note that acquisition of these associations does not involve reinforcement learning, as the participants have not yet been told the value of both outcomes. After 60 trials with each pair, subjects are then explicitly told what the value of each outcome is: outcome 1 yields money, whereas outcome 2 will lead to loss of money. This money is not actually paid, but participants are informed they will see their score at the end of the experiment. During the testing phase, all 15 possible combinations of cues are shown 6 times and the participant is instructed to maximise their gains, using their experience from the acquisition phase. No feedback is given to prevent on-going learning, but a reminder of outcome values is shown at the top of the screen.

The learning phase consisted of 4 blocks, each block consisting of 60 trials. Each pair was shown 20 times per block, providing 80 trials per pair during the whole learning phase. The order of pairs was pseudo-randomised per three trials within each block of 60 trials, the outcome was pseudo-randomised per ten trials of each pair.

During the learning phase there is no way to assess learning performance from the choice pattern because there is no 'right' or 'wrong' choice. Learning was assessed using a visual analogue scale (VAS) questionnaire after every 60 trials (yielding 4 measurements over the course of learning) and once after the testing phase. Using the arrow keys, subjects first estimated the ratio of the two outcomes for each cue in 10% steps, and then indicated how certain they were of this estimate (for illustration, see S3 in the online edition). The cues were shown one by one, in random order. Learning performance was determined for each questionnaire as error on the cue A and B estimates, expressed in arbitrary unit length distance on the scale. This allowed a comparison of learning performance between medication states, and between patients and controls. After the VAS questionnaire, subjects had a 15-second break and text on the screen reminded them to learn the cue-outcome associations.

After completion of the learning phase, the

outcomes were assigned values; subjects were instructed that outcome 1, most strongly associated with cue A, would yield €100, while outcome 2, most strongly associated with cue B, would yield a loss of €100 (for literal instructions, see S6 in the online edition). During the test phase, which was identical to that used by Frank et al. (2004), subjects were shown novel combinations of the previously learned cues, with a reminder of the outcome values at the top of the screen (see S4). They were instructed to maximize their profits by choosing the best cue in each pair, based on the outcomes associated with them and their instructed values (for literal instructions, see S6 in the online edition). In total, 15 pairs were shown in random order 6 times. There was no limit on response time, and testing was conducted in extinction; i.e. no feedback was provided. Subjects were informed that the score was recorded and would be shown to them after task completion. There was a 500 to 1500 ms jitter between response and presentation of the next pair.

All subjects, with the exception of 2 control subjects, were tested on two separate occasions. Both sessions used a unique set of stimuli for the cues and outcomes.

2.5 Data analysis

All statistical tests were performed in SPSS for Windows (2007). The first set of analyses was aimed at revealing any test-retest effects on performance (log-transformed reaction times (RTs) and arcsine-transformed accuracy). Group (PD versus controls) was included as a between-subject factor, and testing session (first versus second) as a within-subject factor. These analyses revealed that there were no significant test-retest effects, nor was there any difference in test-retest effects between groups (see results). This enabled us to conduct the second set of analyses, aimed at elucidating effects of dopaminergic medication on performance, irrespective of session order.

We performed two repeated measures ANOVAs on the patient data. In the first model we examined the effect of the within-subject factors medication status (ON versus OFF) and valence (approach-A versus avoid-B) on test phase accuracy. In this model, the critical dependent measure was the number of times the reward-associated cue A was chosen (approach-A) when a pair was shown during the test phase that included A (but not B). Conversely, avoid-B represented the number of times B was avoided (i.e. not chosen) when a pair was shown that included loss-associated cue B (but not A). In the second model we examined the effect of within-subject factors medication status and valence on RTs of successful approach-A and avoid-B trials. Any RTs <200 ms were excluded as these could not reflect a value-based choice.

Both models included a covariate that captured a bias for choosing visual cue A or B during the learning phase. We reasoned that if subjects happened to choose one of the two cues more often, then this might lead to improved learning of that specific cue-outcome relation. This would add variance of no interest to the data, which in turn would reduce power to detect effects of interest. The covariate was calculated by taking the proportion of trials in which cue A was chosen in AB-pairs during the learning phase of each session (a value between 0 and 1). The covariate represented the difference in bias between the on and off session (a value between -1 and 1).

Post-hoc simple tests of accuracy and RT measures were conducted to compare controls with PD patients ON and OFF medication respectively. For these analyses, data was averaged over both sessions for 12 controls or duplicated for 2 controls who participated only once, such that all comparisons between controls and patients involved identical control data. The learning bias covariate was also included.

3. Results

Medication significantly decreased UPDRS scores in patients ($F(1,17) = 35.79$, $p < .0001$). One patient did not make any successful avoid-B trials when ON medication and was therefore excluded from RT analyses involving avoid-B.

Supplementary analyses were performed on a subset of the patients and controls, excluding all subjects that did not choose cue A in the A-B test pair >50% of the time in either of the sessions. This was the criterion used in the original task(Frank, et al., 2004). The results are described in S7 (see online edition).

3.1 Background neuropsychological tests

Data from the neuropsychological tests are shown in table 1B. Independent samples t-tests revealed a significant main effect of group for the following background neuropsychological tests: controls scored higher on the DART, indicating higher verbal IQ ($t(30) = 2.66$, $p = .01$); patients scored higher on the BDI ($t(30) = 3.41$, $p = .002$); patients were slower on the box completion task both ON (t(30)

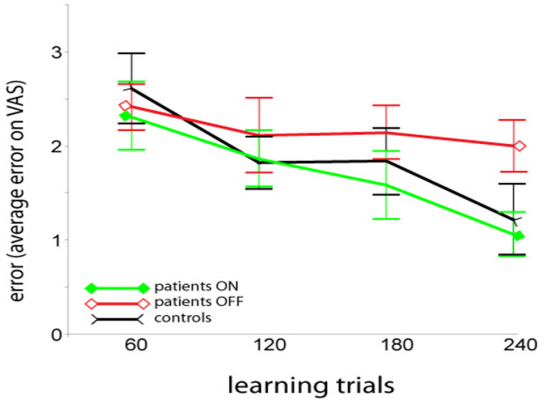

Fig. 2 Inaccuracy of cue-outcome estimates on the VAS questionnaires during the learning phase. Lower scores on the y-axis indicate a smaller error in the estimate. There was no significant difference in learning over time between groups. Error bars indicate twice the standard error of the mean.

= 2.82, p = .008) and OFF (t(30) = 2.92, p = .007) medication; and patients were also slower on the number cancellation tasks both ON (t(30) = 2.59, p = .02) and OFF (t(30) = 2.93, p = .01) medication. All other scores were matched between patients and controls. Elevated depression scores and slowing on cognitive tasks are common in PD. A difference in pre-morbid IQ as measured by the DART was not expected, but it is not obvious how differences in IQ could explain any valence-specific effects in this task

3.2 Learning of cue-outcome relationships

Both patients and controls understood the task and learned the cue-outcome associations. We assessed learning performance based on VAS accuracy on cue A and B estimates (Fig. 2).

In terms of the VAS ratings, there was a main effect of medication (F(1,16) = 10.56, p = 0.01) and a main effect of time (F(3,48) = 3.80, p = 0.02). Medication did not improve learning rate, as shown by the non-significant medication x time interaction (F(3,48) = 1.13, p = 0.35). Control subjects showed no difference in learning over time compared to patients ON medication (F(3,87) < 1) or OFF medication (F(3,87) = 1.32, p = .27). Learning differences between groups or medication conditions most likely influenced overall performance in the test phase. However, impaired or improved learning does not discriminate against cue A or B, so any general effect will not invalidate valence-specific effects (i.e. differences between approach and avoid conditions). Also, whereas learning rates are not influenced by dopaminergic medication, medication does improve observational learning of stimulus-stimulus associations.

3.3 Session effects

All patients and most of the controls were tested twice on the same task. For a fairly complex task like the adapted PS task, subjects might improve in the second session compared to the first. This was not the case: over all subjects, there was no effect of session on test accuracy (F(1,28) = .10, p = .76) and there was no session x valence interaction on accuracy (F(1,28) = .15, p = .70). These findings allowed us to 1) average control data over both sessions and 2) examine medication effects regardless of whether patients were tested ON or OFF their medication first.

3.4 Accuracy on approach-A and avoid-B

The classic behavioral measures of the PS task are approach-A and avoid-B accuracy. In our adaptation of the PS task, we set out to test whether dopaminergic medication affects value-based choice accuracy, independent of the incremental learning of reward-related associations (Fig. 3). There was a main

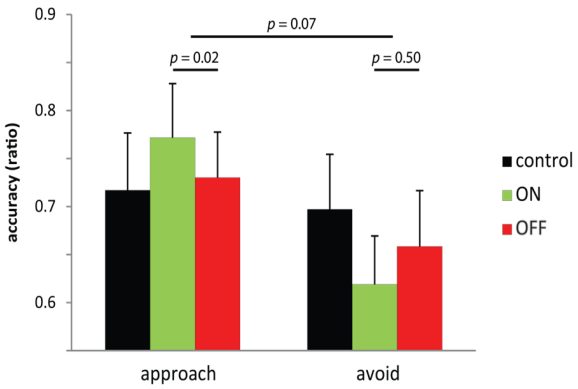

Fig. 3 Dopaminergic medication improves accuracy on approach, but not avoid trials. Medication did not improve overall performance (main effect of medication: F(1,16) = 2.66, p = 0.12). Patients did perform better on approach trials than avoid trials (main effect of valence: F(1,16) = 5.75, p = 0.03). The crucial medication X valence interaction was not significant; however, a trend is evident (F(1,16) = 3.78, p = 0.07). This trend is mainly driven by a medication-induced improvement on approach trials (effect of medication on approach accuracy: F(1,16) = 7.19, p = 0.02), similar to previous studies involving value-based choice (e.g. Frank et al. 2004). However, unlike previous studies, medication had no effect on accuracy on avoid trials (main effect of medication on avoid accuracy: F(1,16) = 0.48, p = 0.50). Although these results are not conclusive due to the lack of a significant interaction, they provide a strong indication that dopaminergic medication in PD has a similar valence-specific effect on accuracy as reported in previous studies (Frank et al., 2004, 2007, Cools et al., 2006). All F-tests included a covariate for acquisition bias

effect of valence on accuracy in PD patients ($F(1,16) = 5.75$, $p = .03$), but no main effect of medication ($F(1,16) = 2.67$, $p = .12$). The crucial interaction, medication x valence, trended towards significance ($F(1,16) = 3.78$, $p = .07$). Simple effects analyses revealed that medication in PD patients significantly improved approach-A accuracy ($F(1,16) = 7.19$, $p = .02$), but had no effect on avoid-B ($F(1,16) < 1$). The trend in the interaction and significant simple effect resemble results from the original PS task in which DA could influence performance through both RL and value-based choice (Frank, et al., 2004). Control subjects had similar accuracy on approach-A and avoid-B ($F(1,12) = 2.08$, $p = .18$). Approach-A accuracy in controls versus patients ON medication was not significantly different ($F(1,30) < 1$). These results indicate that dopaminergic medication affects value-based choice only for the approach-A condition, but not for avoid-B. However, because the interaction was not significant, and approach-A was not different between PD patients ON medication and controls, caution is warranted in interpreting this result.

3.5 Reaction times on approach-A and avoid-B

Dopamine not only affects accuracy, but also response vigor (Niv, Daw, Joel, & Dayan, 2007; Salamone & Correa, 2002). We examined RTs on successful approach-A and avoid-B trials (Fig. 4).

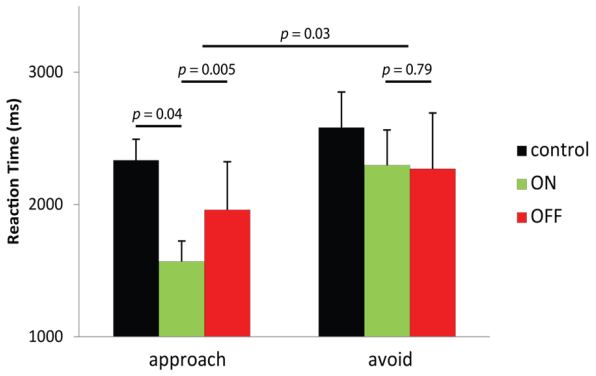

Fig. 4 Medication speeds up responses to obtain rewards, but does not affect reaction speed on avoid trials. Reaction times on successful test trials showed a significant medication X valence interaction ($F(1,15) = 5.84$, $p = 0.03$). This interaction was driven by medication speeding up approach trials (main effect of medication on approach RTs: $F(1,16) = 10.68$, $p = 0.005$), but not avoid trials (main effect of medication on avoid RTs: $F(1,15) = 0.07$, $p = 0.79$). This result indicates medication speeds up responding to appetitive, but not aversive, stimuli. Patients on medication were also faster than controls on approach trials ($F(1,29) = 4.86$, $p = 0.04$). Note that this speed increase did not lead to reduced accuracy (see figure 3). Error bars indicate standard error of the mean.

There was a main effect of valence on RTs ($F(1,15) = 12.83$, $p = 0.003$), but no main effect of medication ($F(1,15) = 3.20$, $p = 0.09$). A significant medication x valence interaction for RTs was found ($F(1,15) = 5.48$, $p = .03$). This interaction was driven by faster responses of patients ON medication compared to OFF medication in approach-A trials ($F(1,16) = 10.68$, $p = .01$). There was no such difference between avoid-B RTs ON versus OFF medication ($F(1,15) < 1$). There was a valence x disease interaction between controls and patients ON medication ($F(1,28) = 10.76$, $p = 0.003$) but not between controls and patients OFF medication ($F(1,29) < 1$). These results show that dopaminergic medication in PD patients causes faster RTs to obtain reward without affecting RTs on trials in which punishment must be avoided.

4. Discussion

4.1 Summary of results

The adapted version of the original probabilistic selection task (Frank, et al., 2004) presented in this article specifically examines the role of DA in value-based choice independent of DA's role in reinforcement learning. Dopaminergic medication in PD patients was associated with a valence-specific speeding of reaction times. Specifically, medication decreased RTs on trials to obtain reward, whereas trials to avoid punishment were unaffected. The results also suggest that dopaminergic medication increases accuracy in choosing cues associated with reward. However, the crucial interaction between medication and valence was trending, but not significant.

In the last decade DA's role in motivation, vigor, and effort has gained increased attention in the literature. The current debate concerns the distinct role of DA in 1) reinforcement learning, i.e. the gradual acquisition of stimulus-response associations based on appetitive/aversive outcomes, and 2) a collection of constructs (e.g. motivation, effort, activation) all related to performance and behavioral output. This distinction has been called learning vs. 'wanting'(Berridge, 2007; Robinson, Sandstrom, Denenberg, & Palmiter, 2005). Other theorists have attempted to bridge the gap between RL and motivational accounts of DA (Niv, et al., 2007). Our results emphasize a role of DA in performance and motivation. First, we will relate our findings to theories that consider a causal relationship between DA and motivation, vigor and effort. Second, implications of our findings for

previous work on dopaminergic modulations of choice will be discussed.

4.2 DA and response vigor

The finding that dopaminergic medication leads to faster responding for reward-related cues is in line with empirical and theoretical work on performance effects of DA (Berridge, 2007; Berridge & Robinson, 1998; Niv, Daw, & Dayan, 2006; Niv, et al., 2007; Salamone, et al., 2005). Despite the fact that subjects did not have a response deadline, or were in no other way extrinsically motivated to make fast responses, medication still induced reward-specific speeding.

In recent years, a number of hypotheses have been put forward to capture DA's diverse role in reward-driven behavior. Niv et al. have proposed that striatal tonic DA represents opportunity cost—the cost of doing nothing—and controls response vigor (Niv, et al., 2007). Given a high-reward environment, characterized by many positive prediction errors, the cost of doing nothing is high (Niv, Daw, et al., 2006). Niv et al. argue that in such a situation, it would be advantageous to expend energy and effort to work fast, so as to maximize rewards. Such a 'running average of reward' would also explain why animals sometimes work hard even for non-relevant outcomes (Niv, Daw, et al., 2006; Niv, Joel, & Dayan, 2006). In the adapted PS task, there is no direct reinforcement to alter vigor. However, such changes in vigor can also be caused by other manipulations such as dopaminergic drugs, which is the case in this paper. Indeed, we find that a medication-induced increase in striatal DA levels is associated with increased response vigor. However, based on the opportunity cost hypothesis and associated results (Niv, Daw, et al., 2006; Niv, et al., 2007; Niv, Joel, et al., 2006), it is surprising that dopaminergic medication only speeds up responses to cues associated with reward, but not cues associated with punishment. This implies that response vigor is modulated on a trial-by-trial basis dependent on midbrain DA firing (Berridge, 2007; Satoh, Nakai, Sato, & Kimura, 2003), rather than over larger timescales as suggested by Niv et al. (2007). The incentive salience hypothesis (Berridge, 2007; Berridge & Robinson, 1998) does explain an approach-specific speeding of RTs for PD patients ON medication versus OFF (Peciña, Cagniard, Berridge, Aldridge, & Zhuang, 2003). However, the incentive salience hypothesis almost exclusively concerns salience attribution to rewarding cues, without making specific predictions on incentive salience attribution to aversive cues. Ikemoto et al. (1999) suggested that DA mediates both aversive and appetitive motivation, but they do not provide a clear explanation for the valence-specific effect presented in this paper.

Robbins and Everitt (1982, 2007) proposed that DA mediates an 'activational state' which modulates behavioral output. Similar to incentive motivation, activation of target structures—such as the striatum—acts to 'enhance behavior' and increase responsiveness to cues paired with reinforcement and to cues paired with aversive outcomes. In our results, we do not find such a general activation due to increased DA levels in the striatum: the decrease in RT is restricted to the appetitive cue, and does not extend to the aversive cue. The authors do mention that high DA levels can have deleterious effects on performance, but make no mention of such a deleterious effect being specific to approach or avoid behaviour (Robbins & Everitt, 2007).

If one assumes that 'aversive salience' is not modulated by DA, then a valence-specific effect such as the one presented in this article can be explained by the incentive salience hypothesis. Regarding vigor in avoid behavior, a number of authors have suggested that serotonin mediates punishment-related behavior much in the same way as DA mediates reward-related behaviour (Daw, Kakade, & Dayan, 2002). Serotonin may act to inhibit actions when punishment is likely to occur (Cools, Nakamura, & Daw, 2011). Applying a tryptophan depletion manipulation to causally reduce serotonin to the adapted PS task, RTs on avoidance of punishment-related cues should become faster, as response inhibition is lifted.

Localisation of the drug effect on approach RTs is inherently difficult because of the use of drug administration, which is a brain-wide manipulation. The nucleus accumbens has been suggested to mediate effects of DA on motivation and vigor (Ikemoto & Panksepp, 1999; Niv, et al., 2007; Salamone & Correa, 2002) and this view is supported by work in the animal literature (Robbins & Everitt, 1982; Salamone et al., 1996; Salamone et al., 1991; Satoh, et al., 2003; Taylor & Robbins, 1984).

4.3 Accuracy on the PS task

Our finding that dopaminergic medication seems to differentially affect approach and avoid accuracy, although only based on a significant simple effect and a trending valence x medication interaction, could provide additional insight into other studies examining approach and avoid accuracy. In the original PS task it was also found that medication improves approach performance (Frank, et al., 2004).

This finding, based on a significant medication x valence interaction, was interpreted as a DA-induced bias in learning from positive versus negative feedback. This bias in learning then carries over to the testing phase, during which these associations are 'probed' and the bias in learning is revealed. In our study, however, there can be no valence-specific effect of dopaminergic medication on learning the positive cues, because subjects are not informed of the values of the (previously neutral) outcomes until after learning. However, we still see a simple effect of medication on approach accuracy and an – admittedly trending – differential effect of dopaminergic medication on approach and avoid accuracy, despite no RL taking place. This indicates that at least part of the effect of DA on the original PS task might not be on RL, but on value-based choice, as is acknowledged in computational models of DA function (Frank, 2005).

Many studies show an essentially similar effect to Frank et al. (2004) (Bodi et al., 2009; Cools, Altamirano, & D'Esposito, 2006; Cools, et al., 2009; Frank, et al., 2007; Shohamy, Myers, Kalanithi, & Gluck, 2008; Voon et al., 2010) and have supported the hypothesis that DA in the striatum underlies learning from positive and negative feedback. More specifically, low levels of DA in the striatum, caused by a low baseline, PD, DA antagonists, or DA depletion, are associated with improved learning from negative feedback. High levels of DA in the striatum, caused by a high baseline, dopaminergic medication, DA agonists, drugs of abuse, or microstimulation, are associated with improved learning from positive feedback. However, a caveat in these types of RL studies is that RL is assessed by subsequent choice accuracy, as is the case in the studies mentioned above. This paper shows that choice accuracy itself might be under the influence of DA in much the same pattern that was found in studies on RL, i.e. dopaminergic medication seems to improve value-based choice on rewarded cues, but not on cues associated with punishment. The implications of this finding for a number of RL paradigms warrant further investigation into DA-dependent modulation of accuracy in value-based choice.

So what might be the underlying neural mechanism causing a valence-specific effect in our adapted PS task? Since our adaptation excludes a valence-specific modulation of learning, dopaminergic medication must be directly biasing value-based choice. According to the incentive salience hypothesis, the incentive value of cues is carried by DA, such that when a cue predicting a reward is shown, midbrain DA neurons dynamically generate the motivation or 'wanting' signal that drives behavior to obtain the reward (Berridge, 2007; Berridge & Robinson, 1998). For example, when a cue is shown that predicts a small reward, the corresponding DA burst will be small. But when a cue is shown that predicts a large reward, the corresponding DA burst will be large. This corresponds to electrophysiological work which shows that midbrain dopaminergic neuron firing rates at the onset of a conditioned stimulus code the predicted reward associated with the CS (Hollerman & Schultz, 1998; Satoh, et al., 2003; Zaghloul, et al., 2009). Through this mechanism, midbrain DA might act to translate stored cue-reward associations into appropriate behavior. PD patients ON medication have increased levels of DA in the striatum, most notably the ventral part (Cools, et al., 2001; Gotham, Brown, & Marsden, 1988; Kish, Shannak, & Hornykiewicz, 1988; Maruyama, Naoi, & Narabayashi, 1996). Increased levels of DA in the striatum may further increase incentive salience attribution to cues associated with reward (e.g. cue A) without affecting incentive salience of cues associated with punishment (e.g. cue B). Increased striatal DA levels when ON medication may cause PD patients to be strongly motivated to obtain rewards compared to when patients are OFF medication. This is reflected in their accuracy scores and RTs on the adapted PS task.

Concluding, the increase in accuracy for approaching cues associated with reward for patients on dopaminergic medication lines up with theories that posit a motivational role for DA. Specifically, DA in the striatum might increase motivation to obtain rewards without affecting accuracy on avoidance of cues to avoid punishment. However, a closer examination of DA's distinct roles in approach and avoidance behavior, specifically regarding value-based choice, is warranted as our results are inconclusive. Also, given the overwhelming evidence in favour of DA's role in incremental acquisition of stimulus-response associations based on reward and punishment, we think the elucidation of DA's role in value-based choice will complement, rather than substitute, current theories on DA and RL.

4.4 Observational learning and PD

Our data provide evidence for a role of DA in observational learning, i.e. incremental strengthening of stimulus-stimulus associations, by showing that PD patients performed significantly more poorly on the learning phase of the task when they were OFF relative to ON their medication. Previous work

has shown that PD is associated with decreased performance in learning from feedback (Knowlton, Mangels, & Squire, 1996; Shohamy et al., 2004), possibly caused by a disruption of dopaminergic transmission by levodopa medication (Cools, et al., 2001; Shohamy, Myers, Geghman, Sage, & Gluck, 2006). This effect of levodopa on learning from feedback has been contrasted with a lack of effect of levodopa on observational learning (Shohamy, et al., 2004). Based on these results, Shohamy et al. have suggested that observational learning is most likely mediated by the medial temporal lobe (MTL), which is classically involved in explicit memory (Poldrack et al., 2001). This conflicts with our finding that dopaminergic medication affects observational learning. A recent fMRI study confirmed MTL-basal ganglia interactions for explicit learning (Sadeh, Shohamy, Levy, Reggev, & Maril, 2010), which could explain a dopaminergic modulation of stimulus-stimulus learning.

5. Conclusion

To tease apart DA's role in reinforcement learning from its role in value-based choice we tested PD patients ON and OFF their dopaminergic medication on a probabilistic selection task. Specifically, the task was adapted in such a way that medication could not cause a valence-specific bias in learning cue-outcome associations. Using this adapted task, we have shown that medication in PD patients increases accuracy on trials involving approach of reward, but not on trials involving avoidance of punishment. In the original PS task, improved accuracy on approaching cues associated with reward was attributed to a medication-induced bias in learning in favour of learning from positive feedback. We now show that such a bias might be caused by an effect of dopaminergic medication on value-based choice, rather than on RL. However, further experiments should confirm these findings, as the results on accuracy presented in this paper do not provide conclusive evidence on the matter.

Dopaminergic medication also caused patients to respond faster to cues associated with reward compared to cues associated with punishment. This is in line with findings that DA mediates response vigor, incentive salience or motivational aspects of behavior. Although a number of recent theories provide subtly different explanations for this phenomenon, it is clear that DA influences the expression of learned associations in a valence-specific manner. Future experiments will need to show under what conditions DA is responsible for response vigor in punishment conditions and to what extent associated systems, such as the serotonergic system, are involved.

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BDNF Val66Met polymorphism interacts with sex to influence bimanual motor control in healthy humans

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BDNF plays a critical role in brain development. A common single nucleotide polymorphism in the gene encoding BDNF (rs6265, Val66Met) affects its release and has been associated with altered brain morphology, disease vulnerability and learning and memory performance. Research shows that these effects of BDNF and BDNF Val66Met activity might be modulated by sex and female sex hormones like estrogen. BDNF has recently also been shown to influence motor learning and performance. However, the relationship between BDNF and sex in the motor domain remains uninvestigated. In the current study we investigate the relationship between BDNF genotype and sex in the motor system. Because of the role of BDNF in brain development, we used a bimanual motor control task to include contributions of both processes related to motor function and inter-hemispheric connectivity. Seventy-six healthy participants were genotyped and performed a task in which the participant drew lines at different angles of varying difficulty. Subjects controlled the horizontal and vertical movement of the line on a computer screen by rotating two cylinders (Preilowski's task). We found that BDNF genotype interacts with sex to influence the motor performance of healthy participants in this bimanual motor control task. The BDNF genotype by sex interaction was present in the more difficult trials only, which is in line with earlier findings that genetic effects may become apparent only when a system is challenged. Our results emphasize the importance of taking sex into account when investigating the role of BDNF genotype in the motor system.

Keywords: BDNF, Val66Met, single nucleotide polymorphism, motor, bimanual, Preilowski's task, genetics, rs6265

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1. Introduction

Brain – Derived Neurotrophic Factor (BDNF) plays an important role in the development and maintenance of neurons and neuronal connections in the central and peripheral nervous system (Cohen-Cory, Kidane, Shirkey, & Marshak, 2010). Activity-dependent secretion of BDNF is a necessary component for long term potentiation (LTP) and depression (LTD) processes, which are regarded as key-elements of neural plasticity underlying learning and memory (Minichiello, 2009). A common functional single nucleotide polymorphism (SNP) in the gene (rs6265), leading to an amino-acid change in the pro-domain of BDNF at codon 66 (Val66Met), occurs in about 30% of the human population of Caucasian ancestry (Egan et al., 2003; Hariri et al., 2003; Sen et al., 2003). The substitution of Val to Met in BDNF affects the intracellular trafficking and secretion of the BDNF protein and impairs the ability of BDNF to undergo activity-dependent release (Chen et al., 2004; Egan et al., 2003; Hariri et al., 2003). Most research has focused on the effects of BDNF Val66Met on memory processes and related brain structures. Here Met-carriership has been associated with smaller hippocampal volumes (Bueller et al., 2006; Frodl et al., 2007; Pezawas et al., 2004; Karnik, Wang, Barch, Morris, & Csernansky, 2010), decreased hippocampal activity and lower declarative memory performance (Egan et al., 2003; Hariri et al., 2003).

Research on the effects of BDNF in the brain has recently been extended into the motor system and motor learning. Using Transcranial Magnetic Stimulation (TMS) it was shown that BDNF Met-carriers do not show the expansion of motor cortex surface area that is typically observed after a motor learning episode (Kleim et al., 2006). Cheeran, Ritter, Rothwell, and Siebner (2009) further elaborated on this study by showing that the LTP/LTD-like motor excitability induced with various TMS protocols is modulated by BDNF genotype, with Met-carriers showing less motor cortex excitability. Met-carriers were also shown to be more error-prone when learning new motor skills during a delayed driving task (McHughen et al., 2010). Together these TMS and behavioral studies provide strong evidence that BDNF genotype indeed affects motor performance and motor learning.

In addition to studies on the association of BDNF Val66Met with brain structure and function, there is emerging evidence that the effects of BDNF genotype may be influenced by the activity of sex hormones like estrogen. It has been shown that estrogen can induce BDNF gene expression but also that estrogen can interact with BDNF signal transduction. This interaction is possible due to the convergence of estrogen and BDNF-related signal transduction pathways (Scharfman & MacLusky, 2006). Thus, the effects of BDNF Val66Met on brain structure, function and plasticity may be modulated by sex, as has been found in for example disease vulnerability (Fukumoto et al., 2010; Verhagen et al., 2010).

In the current study we tested such an interaction between BDNF and sex in the motor domain. As BDNF Val66Met has been shown to influence brain connectivity as observed with resting-state fMRI (Thomason, Yoo, Glover, & Gotlib, 2009), we use a bimanual motor task to capture possible contributions from both primary motor and inter-hemispheric motor connectivity-related processes.

2. Materials and Methods

2.1 Subjects

Seventy-six highly educated (bachelor student level or higher) subjects between 18 and 35 years of age (mean = 23.3, standard deviation= 3.2, 39 females) of Caucasian origin participated in this study. All participants reported no history of psychiatric or neurological disorders and had normal or corrected-to-normal vision. All participants gave written informed consent and the study was approved by the local ethics committee. This study is part of the Brain Imaging Genetics (BIG) project running at the Radboud University Nijmegen (Medical Centre) (Franke et al., 2010).

2.2 Genotyping

Saliva samples were collected from all subjects using Oragene (DNA Genotek, Kanata, Canada) and DNA extracted from these samples was used for genotyping of the BDNF (rs6265, Val66Met) SNP as described by Franke et al., (2010). The experiment leader was blinded for the genotype of the participants until after data analysis.

2.3 Experimental procedure

We used a digital adaptation of Preilowski's Task (Preilowski, 1972) conceptually similar to the task used by (Mueller, Marion, Paul, & Brown, 2009). In this task participants have to draw a line at a

predetermined angle by simultaneously rotating two cylinders. The ability to accurately draw these lines depends on the coordination of the rotation speed of both cylinders by the participant. Participants were seated in a dimly lit room in front of a computer screen and the task controller. Following instruction, the experiment consisted of 15 trials (3 blocks of 5 trials) in which the participant had to draw a right-bound line at one of five possible angles (20, 30, 45, 60 and 70 degrees). To indicate the predetermined angle at which the participants had to draw, a 10 pixel wide example line was shown on the computer screen during each trial. The order of the angles was pseudo-randomized, such that each angle was shown once randomly in a block of 5 consecutive trials and the same angle never appeared twice in a row. The order of the angles was the same for each participant. In order to make the task more challenging for healthy participants (the original Preilowski's task was designed for patients) we included a strict time limit of 25 seconds in which the 800-pixel line had to be completed, after which a 5 second break followed. Subjects were instructed to finish drawing in time (see Figure 1 for example data).

2.4 Data processing

First, any line drawing data located outside of the endpoints of the example line was removed. Subsequently, the area under the curve (AUC) for each line was calculated using MATLAB (MATLAB 2009a, Seattle: The Mathworks Inc., USA) by summation of the differences between the example line and the line drawn by the subject for each point on that line. The resulting AUC scores were analyzed in SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Because it has been shown that the 45 degree angle requires less bimanual motor control compared to the other angles (Mueller et al., 2009), we used these lines as a baseline measure of performance. To exclude possible learning effects and to keep the number of trials with a particular angle equal, the first 5 trials of the experiment were removed from the analysis. In the remaining 10 trials results of the two occurrences of the same angle were averaged. Because of the symmetry of the rotation movements necessary for the 60 & 30 and 70 & 20 degree angles the scores for these angles were combined into a single score. Finally, all scores were divided by the baseline score of that subject. This resulted in two baseline corrected measures for each subject, one

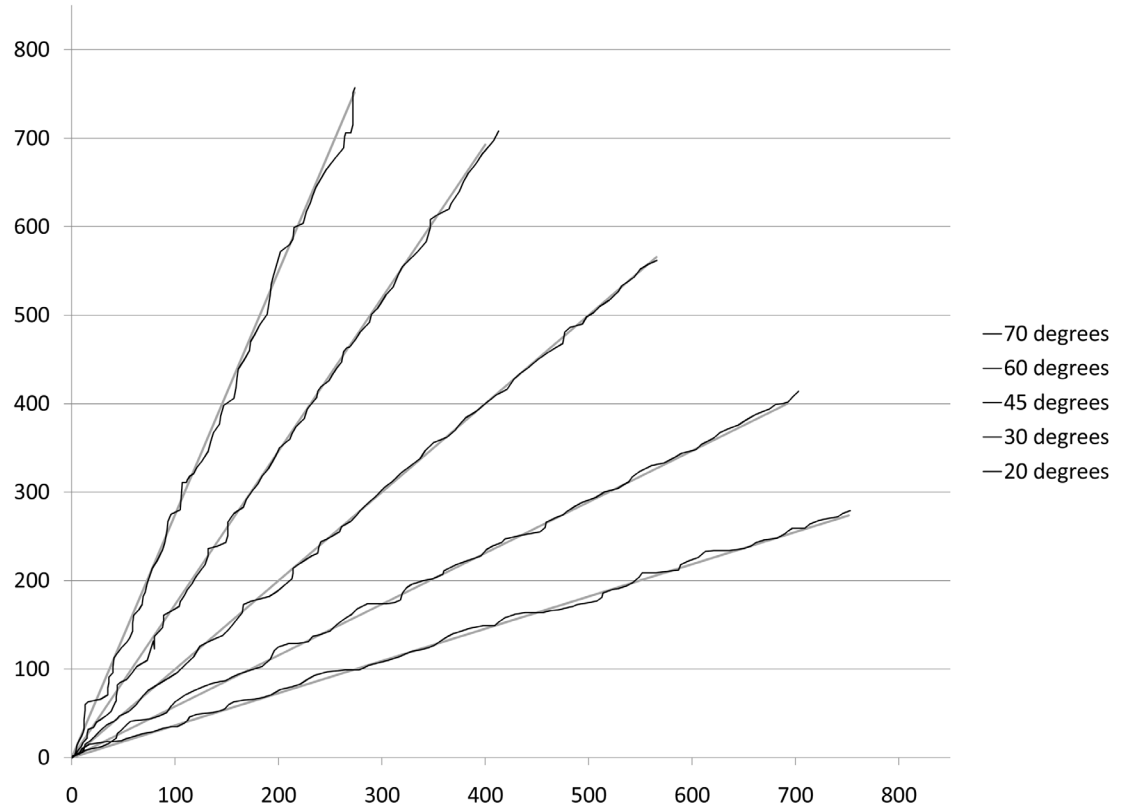

Fig. 1 Example data of a representative subject. Data is shown for each of the angles (20, 30, 45, 60, and 70 degrees) present in the experiment. The graph represents the computer screen with the pixels in horizontal and vertical direction indicated on the x and y axis. The gray lines are the example lines the subject had to mimic by simultaneously rotating two cylinders that controlled the horizontal and vertical movement.

measure for accuracy on trials of the easier (60 & 30 degree angles) and one measure for the more difficult angles (70 & 20 degree angles).

In this experiment we used the baseline-corrected performance on the easier and more difficult angles as within-subject variables with BDNF genotype and sex as between subject factors. This resulted 2 x 2 x 2 mixed within-subject design. The between subject factors together resulted in 4 experimental cells, male and female homozygous for the BDNF Val-allele and male and female Met-carriers. For post-hoc testing a split-file procedure from SPSS was used which organized the output according to sex.

Data quality was ensured by applying the following procedure. Participants who failed to pass an average completion of 90% of all the lines were rejected. In contrast to the participants who had finished the lines in time, these participants may have focused more on accuracy. In order to remove outlier trials, AUC scores more than 4 times the standard deviation away from the mean of that trial over all subjects were rejected as unreliable data. Visual inspection of the resulting data showed that all trials suffering from these outlier artifacts were successfully removed by this procedure. Subsequently, trials in each of the experimental cells whose scores differed by more than 2.5 times the standard deviation from the mean for that trial within that genotype group were removed from the analysis.

3. Results

Of the 76 individuals entering the experiment, four subjects that had completed less than 90% of the lines and three other subjects with too many outlier data had to be excluded from the analysis. In the resulting sample of 69 participants (age 18 - 35; 34 females): 17 were males, homozygous for the Val-allele, 16 were Val-homozygous females and there were 18 Met-carrier males and females.

Because of the role of BDNF in brain maturation we controlled for age by using age as a covariate. The between-groups BDNF by sex interaction was borderline significant ($F(1, 65) = 3.95$, $p = 0.05$. The BDNF genotype by sex by angle interaction in the mixed within and between subjects 2x2x2 repeated measures ANOVA however was significant ($F(1, 65) = 4.01$, $p < 0.05$). To explore this interaction further, the split-file analysis revealed a significant between group difference between Val-homozygous females and Met-carrier females ($p < 0.05$; see Figure 2). Such effects were not observed in the male groups. Furthermore, there was no main-effect of BDNF genotype on angle (F (1,

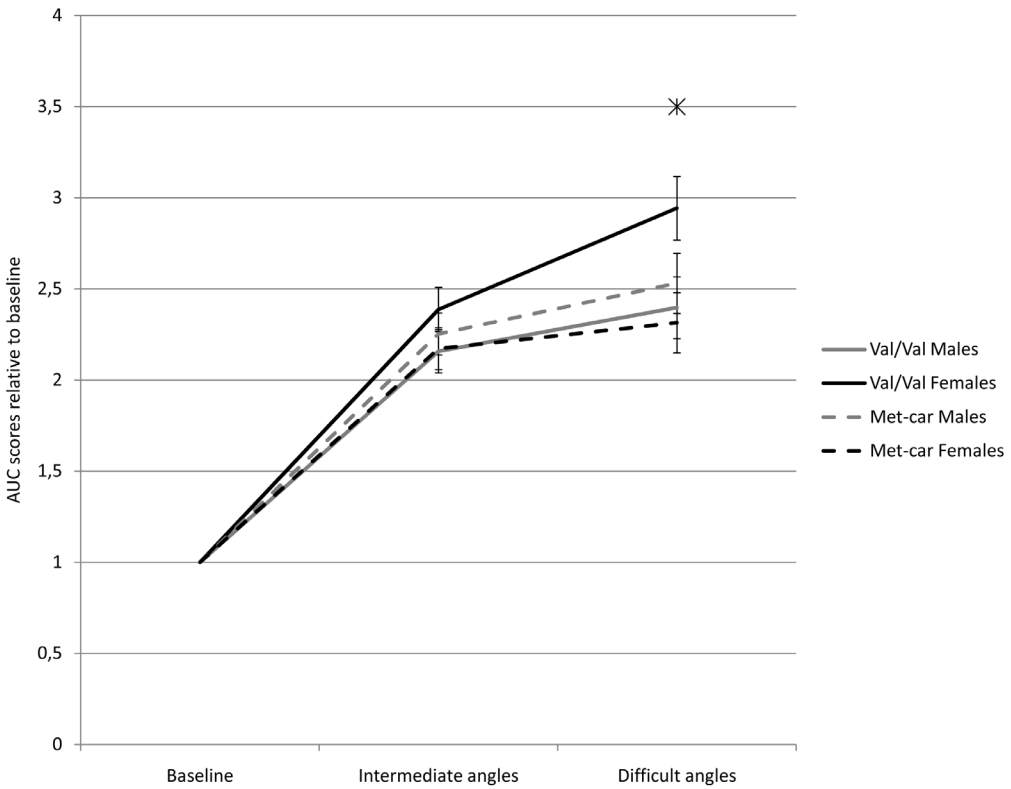

Fig. 2 Relative Area under the Curve (AUC) compared to baseline. The AUC relative to the baseline is shown for 45 (baseline), 60 & 30 (intermediate difficulty), and 70 & 20 (difficult) degree angles. A higher score indicates less accuracy relative to baseline. For difficult angles we show a significant interaction of BDNF genotype and sex.

65) = 1.87, p = 0.17) nor did we observe a main-effect of sex (F (1, 65) < 1, p = ns) in the repeated measures ANOVA.

4. Discussion

We show that BDNF genotype and sex interact to influence the motor performance in a bimanual motor control task in females, but not in males. These results are in line with evidence of an interaction of BDNF with estrogen (Scharfman & MacLusky, 2006), which may also affect the motor domain. The current findings show the importance of taking sex into account when investigating the role of BDNF genotype. The BDNF by sex interaction was only apparent in the more difficult conditions of the task. This is particularly interesting considering earlier work by (Cousijn et al., 2010), which showed that genotype effects may only become apparent under circumstances in which the system is particularly challenged.

Currently most of the literature on BDNF and the motor domain consists of various measurements of motor learning, such as cortical map size (Kleim et al., 2006), motor cortex excitability (Cheeran et al., 2009) and long-term motor learning (McHughen et al., 2010). This line of research may have emerged from earlier studies on BDNF and learning and memory processes (Egan et al., 2003; Hariri et al., 2003; Pezawas et al., 2004). However, together with McHughen et al. (2010) we show that BDNF genotype may influence motor performance independent of LTP/LTD-related processes and that these differences may already be present without a learning episode. In line with the finding of McHughen et al. (2010) we show that BDNF genotype influences one's immediate motor performance and not just motor learning.

The present study also fits with findings of BDNF genotype by sex interactions in other areas of research. For example, BDNF genotype effects on various aspects of behavior in female rats are dependent on the phase of the estrus cycle confirming the notion that sex steroid hormones modulate BDNF action in females (Spencer, Waters, Milner, Lee, & McEwen, 2010). BDNF genotype by sex interactions are also found for disease vulnerability. Recently, Fukumoto and coworkers (2009) found that elderly female Met-carriers are more vulnerable to developing Alzheimer's disease in the later stages of life compared to males and Val homozygous females. BDNF genotype also seems to be a risk factor for developing depression, in this case specifically in men (Verhagen et al., 2010). While the precise mechanisms underlying these effects of BDNF on disease vulnerability are currently unknown, the role of BDNF in neuronal development and its interaction with estrogen suggest that changes in brain structure and function may be involved.

A mechanism that could explain both the findings in the present study and the findings of McHughen and coworkers (2010) originates from the idea that individual differences in bimanual motor performance are related to the structural properties of the corpus callosum (CC). The CC is the largest inter-hemispheric communication pathway and plays a central role in the transfer of information from one hemisphere to the other. The integrity of the CC has been shown to be important for a variety of bimanual tasks such as Preilowski's task (Preilowski, 1972), other bimanual tasks (Gerloff & Andres, 2002), and simultaneous finger movements (Bonzano et al., 2008). Individual differences in CC fiber density are also associated with bimanual motor performance (Johansen-Berg, Della-Maggiore, Behrens, Smith, & Paus, 2007). Recently it was shown that there is no main effect of BDNF genotype on CC fiber density (Montag, Schoene-Bake, Faber, Reuter, & Weber, 2010). However findings others have reported indicate a BDNF genotype by sex interaction in the fiber density of the anterior part of the CC (Rijpkema et al., 39th Annual Meeting of the Society for Neuroscience, Chicago, USA, 2009). Thus, the present results may be explained by the BDNF genotype by sex interaction that influences inter-hemispheric connectivity which becomes apparent in bimanual tasks such as the one used here.

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Abstracts

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Motivational Compensation of Cognitive Decline in Parkinson's Disease: Preliminary Results from a Pharmacological fMRI Study

Abraham.A.M. Nusselein, Roshan Cools, Esther Aarts, Ivan Toni

Dopamine (DA) depletion in Parkinson's disease (PD) not only affects motor function, but also cognitive flexibility, associated with the DA-depleted dorsolateral (DL) circuitry, while relatively sparing the separate ventromedial (VM) frontostriatal circuitry necessary for reward processing. We employed a rewarded switching task and fMRI to assess whether early PD patients OFF medication can compensate for switch deficits with anticipated monetary reward. Furthermore, we investigated the effects of DA medication on this motivation-cognition interface. Results showed that PD patients OFF medication exhibit a task-switching deficit in the proportion of errors on low reward trials, but not on high reward trials, accompanied by an increase of switch-related BOLD signal in the dorsal anterior cingulate cortex (dACC) on high relative to low reward targets. PD patients ON medication did not show abnormal cognitive inflexibility, and did not use

anticipated reward to reduce their switch cost. These findings concur with our hypothesis that motivational processing can be used by PD patient OFF medication to overcome cognitive inflexibility, and implicate a crucial role of the dACC in this compensation process.

Effects of aging on cerebral oxygenation during working memory performance: A functional Near-Infrared Spectroscopy study

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Background

Aging is accompanied by a decline in working memory performance in both the verbal and visuospatial domain. Evidence exists that compensatory neural activity is apparent in older adults during working memory tasks, but the underlying neurocognitive mechanisms are unclear. Functional Near-Infrared Spectroscopy (fNIRS), a noninvasive neuroimaging technique, may provide a way to elucidate the neurophysiological mechanisms of compensation. This study examined brain activation by using fNIRS in young and older adults during working memory performance.

Methods

18 healthy young (21-32 years) and 18 older adults (64-81 years, MMSE=29.2±0.9) performed a verbal and spatial 0- and 2-back task. Oxygenated (O2Hb) and deoxygenated hemoglobin (HHb) changes, as indices of brain activation, were registered by two fNIRS channels located over the left and right dorsolateral prefrontal cortex.

Results

High verbal working memory load led to declined accuracy in comparison to the control condition in older adults, while the young had the same level of accuracy in both conditions. fNIRS results demonstrated an increased concentration of O2Hb during the 2-back condition in both groups and a decrease of HHb in older adults. After the beginning of the verbal 2-back task, a significant increase in brain activation was reached earlier in older adults than in the young and the same held true for the maximum level of activation. The spatial n-back task did not induce significant concentration changes of O2Hb and HHb in comparison to the baseline period in either of the groups, although increased working memory load led to declined behavioral performance in both older and young adults.

Discussion.

Older adults showed a stronger recruitment of prefrontal areas during verbal working memory performance in comparison to young adults, suggesting an attempt to compensate for age-related decline. Also, our study indicates that age effects on the time course of hemodynamic processes must be taken into account in the interpretation of neuroimaging studies that rely on blood oxygen levels, such as fMRI.

Using Microelectrode Arrays to Explore the Somatosensory System in the Freely Moving Rat

Han Langeslag, Eric Maris

In this paper we describe a new method of chronically obtaining neurophysiological data in the awake and freely moving rat. The method involves placing a flexible microelectrode array (32 electrodes on a 6 by 5 mm polyimide foil) epidurally onto the rats neocortical somatosensory system. Furthermore, we describe a somatosensory discrimination task which we intend to use to study the neurophysiological mechanism behind pattern recognition in the rat as measured by local field potentials from the surface of the neocortex. The new microelectrode array showed relatively artifact free brain signals and is less correlated then extra-cranial EEG recordings. Moreover, using a multiway decomposition method we can unveil a spatial pattern in the ongoing neurophysiological activity of the awake rat as measured by the microelectrode array. The pattern consists of a phase-amplitude coupling (PAC) between the phase of a 2-4 Hz oscillatory component and the amplitude of a 30-50 Hz oscillatory component. While we are still in the process of optimizing the methods, we think that the use of polyimide microelectrode arrays will provide an excellent opportunity to chronically study large scale neural activity in the awake and freely moving rat.

Emotional Context and Time in a Memory Retrieval Paradigm Different Involvement of Insula and Amygdala

Frauke van der Ven, Atsuko Takashima, Guillén Fernández

Arousing events are usually remembered better than non arousing events. Investigating brain mechanisms underlying this emotional memory enhancement might be fruitful for treatment of patients who are disturbed by recurring retrieval of distressing thoughts. In this study, we wanted to establish the effects of arousing context (at encoding) over time on memory retrieval and in particular on analogous brain activity. Twenty four healthy subjects studied picture-sound pairs on two different time points (72 hr difference), with the picture (item) always being non arousing and the sound (context) being either arousing or non arousing. After the second encoding block, retrieval of the picture-sound pairs took place inside the scanner, where only the non arousing picture items were presented. Pictures associated to an arousing context and arousing contexts themselves were remembered more often and faster, and were accompanied by enhanced
activation of the insula, amygdala and hippocampus. Insula activity reflected the influence of arousing context at encoding, whereas amygdala and hippocampus activity was due to the thought of arousing context. Both the emotional memory enhancement and associated brain activity changes sustained over time. In addition, we found support for lasting involvement of thehippocampus in retrieval of aging episodic memory.

The influence of DHA enriched diets on vascular factors in mouse models for Alzheimer's Disease

Daan van Rooij, Amanda J. Kiliaan, D. Jansen

AD is one of the most common neurodegenerative diseases in the industrialized world, and at the moment there is no available cure for the disease. In recent years preventive interventions have come into focus. In specific, dietary interventions utilizing polyunsaturated fatty acids such as Docosahexanoic acid (DHA) have booked promising results. Given the close relation between AD risk factors and development and other neurovascular disorders, it is hypothesized that the influence of DHA enriched diets works primarily by improving cerebral blood vessel health. In this experiment, the influence of putative diets supplemented with either DHA or cholesterol on cognitive performance and cerebral perfusion are investigated for both the classical AD model, APP/PS1 mice, as well as for two models of vascular factors in AD, the ApoE4 and ApoE KO mice at 10-12 months of age. Our results show the predicted cognitive impairments of APP/PS1 and ApoE4, but not for ApoE KO mice. Cerebral perfusion is found to be impaired in all three transgenic genotypes, most notably in the APP/PS1 and ApoE KO mice. No consistent effects of our dietary intervention are found. From this we conclude that ApoE4 and ApoE KO mice are a valid model for vascular AD factors. We also conclude that the effects of preventive dietary interventions are not yet visible at the early stages of disease progression.

Event related potentials (ERPs) show specificity of psychopathic attention enhancement and externalizing attention deficit associations to different subgroups

Bart Brouns, Inti Brazil, Ellen de Bruijn, Katinka von Borries, Erik Bulten, Robbert Jan Verkes

Previous research has found P3b abnormalities in psychopathy, although findings are inconsistent. Importantly, one study has found an enhanced P3b in psychopathy. In addition, psychopathic abnormalities on other ERPs, such as the CNV, have been found. In the present study, a psychopathic and non-psychopathic group of patients were used to investigate whether enhanced amplitudes of the P3b, NoGo-P3, CNV, and N2pc could be explained by a sensation-seeking account, using the AX-continuous performance task (AX-CPT). However, recent research suggests that psychopathy and externalizing have opposite correlations with the P3b that may suppress group effects. Therefore, additional correlation analyses were performed between ERPs on the one hand and Psychopathy Checklist-Revised (PCL-R) Factor scores and Behavioural Inhibition System / Behavioural Approach System (BIS/BAS) scores on the other hand to investigate whether psychopathy and externalizing had different associations with ERP amplitudes. Psychopathy was operationalized by low BIS and high PCL-R Factor 1 scores, while externalizing was operationalized by high BAS and high PCL-R Factor 2 scores. Although psychopathic patients showed higher N2pc amplitudes than non-psychopathic patients, no P3b group effect was found. However, correlation analyses did reveal group-specific correlations with several ERPs, including the P3b. Psychopathy correlated positively with P3b, NoGo-P3 and CNV amplitudes, but only in the psychopathic group. Externalizing correlated negatively with P3b, CNV, and N2pc amplitudes in the non-psychopathic group, but positively with N2pc amplitudes in the psychopathic group. The results are interpreted in terms of an association between level of psychopathy and generalized attention enhancement, and an association between level of non-psychopathic externalizing and attention deficits. The results stress the importance of studying psychopathy and externalizing in different subgroups.

Leptin induced inhibition and stimulation of action current firing of nonpreganglionic Edinger-Westphal neurons is dependent on Phosphatidylinositol-3 kinase

Goedarz Karimi, Wim J.J.M. Scheenen, Eric W. Roubos

Non-preganglionic Edinger-Westphal (npEW) neurons are involved in stress regulation and adaptation, and are the main source of Urocortin1 (Ucn1) in the central nervous system. Urocortin 1, besides its important function in the stress response, is also known for its potent food suppressing actions. Recent evidence suggests an interaction between the feeding circuitry and the stress axis at the level of the npEW. Confirming this role, functional receptors for leptin, the ObR-b, have been found in npEW-Ucn1 neurons. Leptin is a satiety factor produced by adipocytes. It regulates neurons in the central nervous system through activation of ObR-b, inducing multiple intracellular signal transductions pathways controlling gene expression and membrane excitability. Our previous studies have shown that leptin directly inhibits membrane excitability of npEW neurons. The mechanisms by which leptin regulates excitability of these neurons is not known. Therefore, in the present study, using patch-clamp electrophysiology, we tested the hypothesis that leptin regulates npEW neuron excitability via a phosphatidylinositol-3 kinase (PI3-kinase) dependent pathway. Our results show that treatment of acute npEW brain slices with 100 nM leptin reduces the action current firing frequency of the npEW neuron population by 58%, and that the selective PI3-kinase antagonist wortmannin (200 nM) prevents this inhibition. Surprisingly, at the single neuron level leptin induces an excitation in some npEW neurons, which is also PI3-kinase dependent. Confirming the opposing action of PI3-kinase on excitability of npEW, wortmannin inhibits the majority of leptin-nonresponsive npEW neurons, but induces activation in some cases. Finally, treatment with the Katp channel blocker Tolbutamide (200 μM) activates npEW neurons suggesting the presence of functional Katp channels in these neurons. Taken together our results indicate that leptin induces both excitation and inhibition of npEW neurons through activation of PI3-kinase. We suggest that alternative signaling pathways downstream of PI3-kinase determine whether the leptin action on excitability of npEW is stimulatory or inhibitory.

Genetic and Pharmacological Animal Models of Schizophrenia - Focus On Attention and Vigilance

Nathalie Buscher, Thomas Steckler, John Talpos, Indira Tendolkar

The continuous performance task is a powerful tool for studying schizophrenia, reliably detecting attentional deficits associated with the disorder. This test can be easily adapted for use in a variety of species, including the mouse. Pharmacological (PCP, amphetamine) and genetic (inducible DISC1 – transgene) models of schizophrenia were tested in the continuous performance task for the mouse. Scopolamine was assessed in this same assay to contrast the specific, schizophrenia – related, attention-disrupting effects of amphetamine and PCP. Testing has revealed distinct and dissociable profiles of these models. Amphetamine has resulted in effects on Go trials only, reflecting a possible role of the dopaminergic system in executive function, strategy formation and sustained attention. The profile of PCP was characterized by effects on NoGo trials only, reflecting the probable role of the glutaminergic system in response inhibition, cognitive flexibility and vigilance. Scopolamine resulted in a non-specific profile of full attentional disruption. These results suggest that there is a complex interaction between the contributions of dysfunctional dopaminergic and glutaminergic neurotransmitter systems to the development of the attentional deficits of schizophrenia. Targeting these two systems simultaneously will possibly constitute a new target pathway for drug development.

Age related improvement in visuospatial working memory is associated with increased activity in task relevant areas: corroborating evidence from longitudinal and cross-sectional data

Pär Flodin, Chantal Roggeman, Fiona McNab, Torkel Klingberg

Visuospatial working memory (VSWM) is central for a wide range of cognitive functions and continues to develop throughout childhood and adolescence. The neural processes supporting VSWM development have previously mainly been studied cross-sectionally. A limitation of developmental cross-sectional findings is potential confounds by inter-individual differences unrelated to age. Here we performed both longitudinal, cross-sectional and mixed model analysis of fMRI data to detect developmental changes of the neural underpinnings of VSWM . 138 subjects between 6 and 27 years were scanned while performing a VSWM task. 56 subjects were scanned twice, two years apart. Overlapping results of longitudinal and cross-sectional analyses revealed increased working memory (WM) activity in frontal and parietal regions with increasing age. Additionally,WM capacity correlated with increased activity in a largely overlapping set of regions. Age related WM improvements are associated with increased WM related brain activity in a subset of the areas where both age and WM capacity predict WM activity. These include bilateral superior parietal- and intraparietal cortex, bilateral superior frontal sulci and anterior caudate nucleus. Neither age nor WM capacity were correlated with decreased WM activity anywhere in the brain, supporting the idea that VSWM maturation is associated exclusively with increased WM activity.

Exploiting the relation between users' mental state and performance in a Brain Computer Interface setting

Cecilia Maeder, Benjamin Blankertz, Peter Desain, Claudia Sannelli, Stefan Haufe

High amplitudes in the 8-15 Hz frequency band over sensorimotor areas (Sensorimotor rhythms, SMR) have been correlated with better sensorimotor processing and hypothesized to be due to higher inhibition to external inputs. In this study, we analyze data acquired during motor imagery of the right and left hands in an SMR based BCI setting and show that trials with higher SMR amplitude in the 1000 ms preceding the cue could be better classified than trials with lower amplitude. We also report that this increase in accuracy can be attributed to a higher level of SMR amplitude over the ipsilateral hemisphere. Finally, we conducted an online study in which the pre-stimulus SMR level controlled the timing of cue presentation. Preliminary results from this study are presented here and technical issues for future designs incorporating the monitoring of users ongoing SMR activity are discussed.

The effect of foreign accent on processing morphosyntax: An ERP study

Merel van Goch, Adriana Hanulíková, Petra van Alphen

Previous studies have shown that morphosyntactic errors elicit a P600 effect (Hagoort, Brown & Groothusen, 1993; Osterhout & Holcomb, 1992, 1993). Evidence from several studies in Dutch suggests that nonnative speech contains more (morpho)syntactic errors than native speech, e.g. gender agreement errors (Orgassa & Weerman, 2008; Orgassa, 2009). The current study explored the effect of foreign accent on the online processing of gender agreement, by investigating whether the same gender agreement violations in native and nonnative accents elicit similar ERP responses in listeners. The study showed a difference in the P600 effect for morphosyntactic violations in native speech versus nonnative speech. A semantic control condition revealed that the two accents did not elicit different N400 effects. The results suggest that listeners make inferences about the speaker and about the probability of grammatical errors. Additionally, listeners' morphosyntactic processing is sensitive to different accents and this processing is modulated with respect to the accent of the speaker.

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