Event-related potentials in performance monitoring are influenced by the endogenous opioid system

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A B S T R A C T

Recent research suggests that not only the dopamine neurotransmitter system but also the endogenous opioid system is involved in performance monitoring and the generation of prediction error signals. Heightened performance monitoring is also associated with psychopathology such as internalizing disorders. Therefore, the current study investigated the potential link between the functional opioid peptide prodynorphin (PDYN) 68 bp VNTR genetic polymorphism and neuronal correlates of performance monitoring.

To this end, 47 healthy participants genotyped for this polymorphism, related to high-, intermediate-, and low-expression levels of PDYN, performed a choice-reaction task while their electroencephalogram (EEG) was recorded. On the behavioural level, no differences between the three PDYN groups could be observed. EEG data, however, showed significant differences. High PDYN expression individuals showed heightened neural error processing indicated by higher ERN amplitudes, compared to intermediate and low expression individuals. Later stages of error processing, indexed by late Pe amplitudes, and stimulus-driven conflict processing, indexed by N2 amplitudes, were not affected by PDYN genotype.

The current results corroborate the notion of an indirect effect of endogenous opioids on performance monitoring, probably mediated by the mesencephalic dopamine system. Overall, enhanced ERN amplitudes suggest a hyper-active performance monitoring system in high PDYN expression individuals, and this might also be an indicator of a higher risk for internalizing disorders.

\textsuperscript{1} DMP and JP contributed equally to this manuscript.

1. Introduction

To successfully navigate in our social world, we have to be able to adapt our behaviour to a (sometimes persistently) changing environment. Recent research assumes that this kind of cognitive and behavioural adaptation (i.e., performance monitoring) involves learning processes that are driven by so-called (reward) prediction error signals—RPEs (Friston, 2010). RPEs indicate the discrepancy between expected and received outcomes and account for internal expectation updates of stimulus-outcome relations (Rescorla and Wagner, 1972). Mesencephalic dopamine transmission is considered as their neuronal correlate (Schultz, 2007; Schultz et al., 1997). It has been posited by Holroyd and Coles (2002) that phasic dips in mesencephalic dopamine transmission signal the need for adaptation after error commission to the anterior cingulate cortex (ACC). Thereby, ACC neuron activity is disinhibited to allow modification of task performance which leads to a sharp negative deflection on the scalp (the so-called Error-Related Negativity component; Falkenstein et al., 1991; Gehring et al., 1993) – thus reflecting RPE signals. However, recent animal research on fear conditioning suggests the involvement of another neurotransmitter system, which is the opioid system, in generating RPEs (Cole and McNally, 2007; Matzel et al., 1988; McNally and Cole, 2006). These studies showed that fear blocking was prevented after administration of an opioid antagonist in mice. Moreover, Pecina et al. (2014) suggested that opioid-mediated placebo responses can also be seen as prediction error signals. In these studies manipulating the opioid system, the expected outcome (i.e., the predicted pain signal) did not match the actual outcome (i.e., the actually perceived nociceptive stimulus) – which describes exactly the discrepancy between expected and received outcomes as reflected by prediction error signals. Although previous research assumed that endogenous opioids are primarily involved in nociception and analgesia, but also in hedonic control and reward processing (Le Merrill et al., 2009), the mentioned...
studies support the assumption that opioids are also involved in the generation of RPE, possibly also during performance monitoring.

The aim of the current study was to investigate RPEs and their relation to genetic variation affecting the function of the human endogenous opioid system. We measured event-related potentials (ERPs) in individuals with a naturally occurring polymorphism of the opioid peptide prodynorphin (PDYN). PDYN is the precursor of the endogenous opioid peptide dynorphin which is involved in locomotor activity, stress response, food consumption, sexual and anxiety-related behaviour, and drug intake (Bodnar, 2011; Bruijnzeel, 2009). Dynorphin acts as moderately selective agonist for the kappa (κ) opioid receptor (Chavkin et al., 1982). Importantly, dynorphin-like peptides and κ-opioid receptors are expressed and localized, among others, in mesolimbic-mesocortical systems (i.e., ventral tegmental area, nucleus accumbens, and prefrontal areas (Shippenberg, 2009)) and are assumed to exert tonic inhibitory control over striatal dopamine release (Bruijnzeel, 2009; Kreek et al., 2002; Lutz and Kieffer, 2013; Margolis et al., 2006; Steiner and Gerfen, 1998). In particular, dynorphin peptides lower basal, but also drug-induced dopamine levels in these systems (Kreek et al., 2005). Dopamine neurons of the ventral tegmental area (the starting point of the mesolimbic dopamine system) receive input from neurons innervated by dynorphin and also express κ-opioid receptors. Activation of these κ-opioid receptors via dynorphin decreases dopamine release in the respective areas (Knoll and Carlezon, 2010; Margolis et al., 2006; Wee and Koob, 2010).

The dynorphin and κ-opioid receptor system is widely distributed within the central nervous system. It constitutes a sort of *neuro-modulatory master system* within the brain together with other opioid receptor types and ligands (Chavkin et al., 1982; Corbett et al., 1982). Its main role is to function as a negative feedback inhibition within the circuits it modulates (for a review see Steiner and Gerfen, 1998). The dynorphin and κ-opioid receptor system is setting the threshold for the initiation of the negative feedback inhibition loop and is thereby able to fine-tune the excitability of these neuronal circuits. Whereas the dampening of dopaminergic effects by increased dynorphin function may act as a compensatory mechanism at the cellular level (Steiner and Gerfen, 1998), increased negative feedback will also affect activity in the pathways it is contained in, and consequently alter behaviour. For example, the dynorphin and κ-opioid receptor system is also involved in regulating the excitability of the reward system (Bruijnzeel, 2009). Therefore, imbalance of the negative dynorphin-dopamine feedback loop in the reward system might be associated with clinical pathologies characterized by either hyper- or hyposensitivity to rewards, such as substance abuse or affective disorders (Knoll and Carlezon, 2010; Shippenberg, 2009; Tejeda et al., 2012).

Based on previous research on gene expression (Zimprich, et al., 2000), we targeted participants with a functional polymorphism in the promoter region of the PDYN gene (68 bp VNTR). This 68 bp repeat has been the subject of several functional analyses studies showing that one- and two-repeat haplotypes have lower inducibility in vitro as well as a lower PDYN expression level in vivo than three- and four-repeat haplotypes (Nikoshkov et al., 2008). Individuals with high, intermediate, and low PDYN expression rates were distinguished in the current study. We assumed that group-specific dynorphin expression would influence striatal dopamine release and that the differential modulation of dopamine release should become evident in amplitude variation of ERPs related to prediction error signals and performance monitoring.

As measures of performance monitoring, three ERPs were assessed: Error-Related Negativity (ERN; Falkenstein et al., 1991; Gehring et al., 1993), Error Positivity (Pe; Falkenstein et al., 1991, 2000), and conflict N2. The ERN component is a negative deflection peaking within the first 100 ms after erroneous responses at fronto-central electrodes and is assumed to be generated in the anterior midcingulate cortex (aMCC), i.e. a brain area important for conflict monitoring and behavioural regulation (Debener et al., 2005; Hoffmann and Falkenstein, 2010; Vogt, 2005), see also Bauer et al. (2003) and Pfabigan et al. (2013). The Pe component is a positive deflection indicating conscious error processing (Nieuwenhuis et al., 2001) or affective responses after errors (Falkenstein et al., 2000). The Pe is sometimes differentiated in an early and a late component since two distinct peaks can be temporally discriminated (Tops et al., 2013; Van Veen and Carter, 2002) - as in the current study. The early Pe is often assumed to reflect rebounding ERN activity (Falkenstein et al., 1995). Thus, investigating later Pe aspects might be more informative. Indeed, it is in particular the late Pe component (around 300–600 ms after error commission) which shows enhanced amplitudes for aware compared to unaware errors (Endrass, et al., 2007). The late Pe component has been hypothesized to originate from inferior frontal gyrus and anterior insula (Ullsperger, et al., 2010). The current study focused on the late Pe component. The conflict N2 component is a negative-going stimulus-locked ERP indicating visual template mismatch. It peaks within 200–300 ms after stimulus onset over fronto-central electrodes, and the aMCC is also assumed to be its neuronal generator (Folstein and Van Petten, 2008; Nieuwenhuis et al., 2003; Yeung et al., 2004). Whereas ERN and Pe amplitudes are assumed to be modulated by phasic mesencephalic dopamine release reflecting RPEs (Holroyd and Coles, 2002), the conflict N2 is believed to be unaffected by changes in mesencephalic dopamine, and has been linked to noradrenergic functions (Warren and Holroyd, 2012). The link between ERN amplitude variation and dopamine is further corroborated by psychopharmacological studies. Administration of dopamine agonists increased ERN amplitudes (Barnes et al., 2014; De Bruijn et al., 2004), whereas administration of dopamine antagonists attenuated ERN amplitudes in comparison to placebo (De Bruijn, et al., 2006; Zirnheld et al., 2004). Moreover, investigations of several functional polymorphisms in dopamine-related genes showed variation in ERN amplitudes (Agam et al., 2014; Biehl et al., 2011; Kramer et al., 2007), as well as interactions between the functional polymorphisms and dopamine antagonists (Mueller et al., 2011, 2014). Variation of Pe amplitudes after administration of dopamine agonists/antagonists or in relation to functional dopamine polymorphisms is not consistently reported (but see Althaus et al., 2010).

The present study aimed to investigate the influence of the PDYN 68 bp VNTR polymorphism on RPEs reflected in ERN amplitude variation. In particular, we expected amplitude differences between high and low PDYN expression participants. Since high levels of PDYN exert tonic inhibitory control over dopamine release, we expected decreased ERN amplitudes in high PDYN expression individuals compared to low PDYN expression ones since the tonic inhibitory control might act in a similar way as the administration of dopamine antagonists, shown to affect ERN amplitudes in previous studies (De Bruijn et al., 2006; Zirnheld et al., 2004). We had no directional hypotheses concerning the late Pe component as previous results were not consistent, or the intermediate PDYN expression group and ERP amplitude variation, but included it for exploratory reasons. The dopamine-independent N2 component served as control condition to explore potential influences of the PDYN polymorphism on aMCC activity unrelated to the link between opioid and dopamine systems (e.g., Bruijnzeel, 2009). Therefore, we only expected effects of stimulus congruency on N2 amplitudes. Furthermore, we explored behavioural indices of performance monitoring (reaction times, error rates, post-error slowing) and personality characteristics associated with reward.
sensitivity, impulsivity, and risk for substance abuse in the three PDYN groups.

2. Material and methods

2.1. Participants

Initially, 286 healthy Caucasian volunteers were genotyped on the prodynorphin (PDYN) 68 bp variable nucleotide tandem repeat (VNTR) polymorphism. This functional polymorphism is located in the PDYN promotor region with one to four repeats of a 68 bp segment with one binding site per repeat for the transcription factor AP-1 (c-Fos/c-Jun) (Zimprich et al., 2000). Alleles with three or four repetitions of the 68 bp VNTR (denoted by H) are associated with higher levels of mRNA and consequently higher levels of PDYN peptides (i.e., equatable to enhanced dopamine inhibition), compared to alleles with one or two repetitions (denoted by L) (Nikoshkov et al., 2008; Zimprich et al., 2000).

After genotyping, all volunteers were assigned to one of three groups with high (HH), intermediate (HL or LH, in the following denoted by L), or low (LL) PDYN expression. Genotypes were distributed as follows in the screening sample: HH (n=142), LL (n=113), LH (n=31). The allelic distribution of LL, LH, and HH PDYN polymorphism was in Hardy-Weinberg equilibrium, $\chi^2(2) = 1.39, p=0.500$, demonstrating that the screened population was genetically homogeneous for the PDYN genotype distribution. Twenty participants per group (matched for age, sex, education; tobacco, alcohol, coffee, and energy drink consumption) were invited to the EEG experiment using a double-blind procedure.

Several participants had to be excluded from further analyses because of technical problems ($n=7$), too high or too low error rates ($n=8$; error rates higher than 30% or less than six errors in total (Olvet and Hajcak, 2009)), and outliers exceeding the interquartile range of the ERP amplitudes in question ($n=2$). The final sample consisted of 16 participants in the HH group (nine women, mean age 24 ± 5.34 years), 16 participants in the LH group (eight women, mean age 23 ± 3.29 years), and 15 participants in the LL group (nine women, mean age 25 ± 7.65 years). All participants were right-handed (Oldfield, 1971), had normal or corrected-to-normal vision, and reported no past or recent neurological or psychiatric disorders. All gave written informed consent prior to the experiment, which was conducted in accordance with the Declaration of Helsinki (7th revision, 2013, http://www.wma.net/en/20activities/10ethics/10helsinki/) and local guidelines of the University of Vienna. It was further approved by the ethics board of the Medical University of Vienna.

Participants were administered several questionnaires prior to the experimental session to assess psychological constructs related to reward and error processing. The BIS/BAS Scale (Carver and White, 1994) was administered to assess sensitivity of behavioural inhibition and approach systems, i.e., reward and punishment sensitivity. The Barratt Impulsiveness Scale (BIS-11; Patton, et al., 1995) was administered to assess impulsive personality traits. The Substance Use Risk Profile Scale (SURPS; Woicik et al., 2009) was administered to assess individual risk for substance abuse on four dimensions (hopelessness, anxiety sensitivity, impulsivity, sensation seeking).

2.2. Genetic analyses

Using a self-collection kit for collection and storage, saliva samples were collected to determine DNA sequences (Oragene DNA, DNA Genotek, Ottawa, Canada). DNA was extracted using a commercial kit (Qiagen, Hilden, Germany). PDYN genotyping was performed according to established procedures at the DNA laboratory of the Department of Neurology at the Medical University in Vienna. In detail, purified DNA was diluted into a PCR reaction mix consisting of 20 mM Tris–HCl (pH: 8.8), 50 mM KCl, 1.5 mM MgCl₂, deoxynucleotide triphosphates each at 0.4 mM, 10 pmol of each primer, and 0.6 U of Taq polymerase in a total volume of 30 μL. Amplification conditions were 30 s at 94 °C, 45 s at 62 °C, and 45 s at 72 °C for 30 cycles using the following primers (which are flanking the entire promotor region): upstream (P1), 5′−AGC AAT CAG AGG TTG AAG TTG GCA GC; downstream (P2), 5′−GCA CCA CCC GGT TAG GIA CAG TTT TC. The resulting amplification products were resolved on a 2.5% agarose gel stained with ethidium bromide.

2.3. Task

The stimuli were presented using Cogent 2000 v1.32, developed by the Cogent 2000 team at FIL and ICN and Cogent Graphics developed by John Romaya at the LON at the Wellcome Department of Imaging Neuroscience (on a Pentium IV, 3.00 GHz). Participants were seated about 70 cm in front of a 19 in. cathode ray tube monitor (Philips 201 P; 75 Hz refresh rate) in a shielded chamber. Participants performed an arrowhead version of the Eriksen Flanker task (Eriksen and Eriksen, 1974). Five-arrow strings were presented centrally on the screen. Half of the trials comprised of congruently aligned arrays \(< < < < < \); \(> > > > > \); the other half of incongruently aligned arrays \(< < < > > \); \(> > > > > \). Participants’ task was to indicate the left- or right-hand orientation of the middle arrow via a button press with the right hand on a keyboard as quickly and accurately as possible. When the middle arrow pointed to the left, the left arrow key had to be pressed with the index finger. When the middle arrow pointed to the right, the right arrow key had to be pressed with the middle finger.

The experiment started with 20 training trials to familiarize participants with the task. Each trial started with a white fixation cross on a black screen presented for 2000 ms. Next, the four flanking arrows of the five-arrow string were presented for 100 ms in white colour on black background. Subsequently, the middle arrow was blended into the string for another 35 ms. This sequential presentation order was introduced by Kopp et al., (1996) to enhance interfering effects and thereby increase error rates. Immediately afterwards, the screen turned black for 600 ms and participants responded via button press to indicate the orientation of the middle arrow. Button presses were followed by an intertrial-interval presenting a fixation cross for the respective 2000 ms. The same number of congruent (160) and incongruent (160) flanker arrays was presented pseudo-randomly mixed. Participants were given a short rest after 160 trials. The task took about 15 min to be completed. All participants received a fixed amount of monetary remuneration at the end of the experiment.

2.4. Data acquisition and analyses

Reaction times were defined as the interval from middle arrow onset until button press. In line with recently proposed procedures, trials with reaction times faster than 200 ms were discarded from all analyses (Hajcak et al., 2005; Wiswede et al., 2009a, b). Individual mean reaction times were calculated participant- and condition-wise. Analysis of the mean reaction times was performed using two two-way repeated measures ANOVAs with the between-subject factor group (HH vs. LH vs. LL) and either the within-subject factor response type (correct vs. error) or stimulus type (congruent vs. incongruent). Furthermore, error percentage was calculated per participant and analysed via a two-way repeated-measures ANOVA with the factors group and stimulus type. Additionally, to assess post-error slowing (PES; Rabbitt, 1966),
reaction times of correct trials were extracted participant-wise before and after erroneous trials, i.e., applying the so-called PES\textsubscript{robust} method (Dutilh et al., 2012). This procedure was chosen to avoid problems of the traditional PES calculation in which post-correct and post-error trials are not evenly distributed over the time series of the experiment which could cause spurious effects (Dutilh et al., 2012). These mean reaction times were analysed via another two-way repeated-measures ANOVA with the factors group and sequence (pre-error trials vs. post-error trials). Questionnaire data were compared by one-way ANOVAs with group as between-subject factor for each subscale.

EEG data were recorded from 61 Ag/AgCl ring electrodes with a DC amplifier (NeuroPrax, neuroConn GmbH, Ilmenau, Germany). Online EEG recordings were referenced to an electrode on the forehead. Additional electrodes were placed above and below the left eye and on the outer canthi to assess eye-movements. Two pre-experimental eye-movement calibration tasks were performed for subsequent artefact correction. Electrode impedances were kept below 2 k\textOmega using a skin scratching procedure (Proctor and Hillyard, 1972). Signals were sampled at 500 Hz for digital storage. Offline EEG data analyses were performed using EEGLAB 6.03b, implemented in Matlab 7.5.0 (The MathWorks, Inc., Natick, MA). EEG data were re-referenced to averaged linked mastoids. A high-pass filter (cut-off frequency 0.1 Hz) and a low-pass filter (cut-off frequency 30 Hz, roll-off 6 dB/octave) were applied subsequently. Independent component analysis (ICA; Bell and Sejnowski, 1995; Lee et al., 1999) was performed on the concatenated continuous pre-experimental calibration tasks and all experimental trials. Independent components reflecting eye-movement activity were discarded separately for each participant. Epochs were either time-locked to participants’ responses to assess ERN and late Pe amplitudes, starting 400 ms prior to the button press and lasting for 1000 ms (baseline: −200 to −100 ms prior to the button press), or time-locked to the onset of the flanking arrows to assess conflict-N2 amplitudes, starting 200 ms prior to the onset of the flanking arrows (baseline: −100 to 0 ms prior to stimulus onset) and lasting for 1000 ms. A semi-automatic artefact correction was applied to all epoched data. Artefact-affected trials meeting the criteria of voltage values exceeding ±75 μV or voltage drifts larger than 50 μV were labelled automatically. These trials were eventually rejected in case visual inspection also indicated artefact affliction. Subsequently, artefact-free trials were averaged separately for each participant for the response-locked conditions: (1) trials including correct responses after congruent and incongruent stimulus arrays − correct-response, (2) trials including incorrect responses after congruent and incongruent stimulus arrays − error-response. On average, 32.28 errors (SD=21.76), with a minimum of seven errors per participant (Olivet and Hajcak, 2009), were subjected to analyses. For stimulus-locked data, the following artefact-free trials were averaged: (3) trials including congruent stimulus arrays with subsequent correct responses, and (4) trials including incongruent stimulus arrays with subsequent correct responses. ERN, late Pe, and N2 amplitudes were assessed at midline electrode sites Fz, Cz, and Pz, which is consistent with previous literature (Gehring et al., 1993; Pfabigan et al., 2013; Wiswede et al., 2009a,b). ERN amplitudes were assessed as the difference between the most negative peak within -100 to 100 ms in relation to the response and the preceding positive peak (De Brujin et al., 2006; Kramer et al., 2007). Moreover, to gain a measure of neural activity specific to errors for the response-locked data, ΔERN amplitudes were calculated by subtracting averaged correct from averaged error trials per participant to remove processes common to both error and correct trials (Luck, 2005; Meyer et al., 2012). These difference waves were baseline-corrected in the time interval -100 to 0 ms to allow better comparisons of the three groups since the positive peak preceding the ΔERN peak was now included in the baseline interval. Subsequently, peaks of ΔERN (in the interval −100 to 100 ms) and the preceding positive peak were assessed individually at electrodes Fz, Cz, and Pz; and then subtracted from each other. Late Pe amplitudes were assessed as the most positive peak 200–600 ms after the response. N2 amplitudes were assessed as the difference between the most negative peak within 300–500 ms after the onset of the flanking arrows and the preceding positive peak.

ERPs were analysed separately for all participants with three-way repeated measures ANOVAs with the within-subject factors electrode site (Fz, Cz, Pz) and type (error vs. correct for ERN and late Pe components; congruent vs. incongruent for the N2) and the between-subject factor group (HH vs. LH vs. LL). Additionally for ΔERN, a two-way ANOVA with the between-subject factor group and the within-subject factor electrode site was calculated to assess error specific activity in the three groups. Furthermore, a Pearson correlation was calculated between the PDYN allele number per participant and ΔERN values to corroborate potential group differences also on a dimensional level. Spearman correlations were calculated for HH and LL participants to investigate the association between the ERP components per condition (at Cz for ERN and Pe components; at Fz for the N2 component) and the sub-scales of the BIS/BAS, BIS-11, and SURPS (which included mostly not normally-distributed data).

If not stated otherwise, significant interaction effects were explored with Tukey’s HSD post-hoc tests. Group effects were analysed with a priori planned linear contrasts. If indicated by Mauchley’s test of sphericity, degrees of freedom were adapted applying the Greenhouse–Geisser (GG) correction. Partial eta\textsuperscript{2} is reported for significant results to demonstrate effect sizes of the current ANOVA model (Kirk, 1996). All statistical analyses were performed using PASW Statistics 18.0 (IBM SPSS Statistics, Somer, NY) and Statistica 6.0 (StatSoft Inc., Tulsa OK). The alpha level was set at p ≤ 0.05. For the correlation analyses between the questionnaire data and the ERPs, we corrected the significance threshold because of multiple testing using the Bonferroni correction (p\textsubscript{corr} ≤ 0.004).

3. Results

3.1. Behavioural results

Mean reaction times were faster for incorrect compared to correct responses (F(1,44)=474.32, p < 0.001, η\textsuperscript{2}p=0.92). No group (F(2,44)=1.92, p=0.159) or interaction effects (F(2,44)=0.90, p=0.413) were observed. Mean reaction times were also faster for congruent compared to incongruent stimulus arrays (F(1,44)=724.62, p < 0.001, η\textsuperscript{2}p=0.94). No group (F(2,44)=2.17, p=0.128) or interaction effects (F(2,44)=0.44, p=0.649) were observed. Error percentage was higher for incongruent than congruent stimulus arrays (F(1,44)=25.42, p < 0.001, η\textsuperscript{2}p=0.36). No group (F(2,44)=0.76, p=0.474) or interaction effects (F(2,44)=1.06, p=0.356) were observed. A significant post-error slowing effect was observed (F(1,44)=75.41, p < 0.001, η\textsuperscript{2}p=0.64) − correct responses were given slower in trials following error occurrence as compared to trials preceding them. Again, no group (F(2,44)=1.44, p=0.249) or interaction effects (F(2,44)=0.13, p=0.876) were observed. The questionnaire analyses revealed no significant group differences for the BIS/BAS Scales (all p-values > 0.317), the BIS-11 Scales (all p-values > 0.189), or for the SURPS (all p-values > 0.063). Table 1 provides behavioural and questionnaire results per group.
were found for groups for response-locked and stimulus-locked ERPs. For the ERN for high, intermediate, and low PDYN expression participants, the questionnaire data (BIS/BAS scales, BIS-11, and SURPS) are depicted separately.

Table 1

<table>
<thead>
<tr>
<th>HH PDYN group</th>
<th>LH PDYN group</th>
<th>LL PDYN group</th>
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<tr>
<td><strong>M</strong></td>
<td><strong>SD</strong></td>
<td><strong>M</strong></td>
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<td><strong>Reaction times</strong></td>
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<td>409.21</td>
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<tr>
<td>BAS Total</td>
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3.2. ERP results

Means and standard deviations for all ERP measures are provided in Table 2. Fig. 1 depicts amplitude courses of the three groups for response-locked and stimulus-locked ERPs. For the ERN ANOVA, significant main effects were observed for electrode site (F(2,88) = 19.42, p < 0.001, ηp² = 0.31) and type (F(1,44) = 102.29, p < 0.001, ηp² = 0.70). Moreover, significant interaction effects were found for electrode site × type (F(2,88) = 18.11, p < 0.001, ηp² = 0.29) and group × type (F(2,44) = 3.34, p = 0.045, ηp² = 0.013). The main effect for group (F(2,44) = 2.09, p = 0.136) and the remaining interactions (all p-values > 0.557) were not significant.

Tukey post-hoc tests showed that ERN amplitudes were comparable at electrode sites Fz and Cz after errors (p = 0.999) and at electrode sites Fz, Cz, and Pz after correct responses (all p-values > 0.340). In general, errors led to more negative ERN amplitudes than positive responses (all p-values < 0.001). Concerning the group × type interaction, correct responses yielded comparable ERN amplitudes in all three group (all p-values > 0.999). Erroneous responses yielded enhanced ERN amplitudes compared to correct ones in the three groups (all p-values < 0.001). Although ERN amplitudes after errors were most pronounced in HH participants, the comparison with LL (p = 0.288) and LH participants (p = 0.166) did not reach significance. LL and LH participants did not differ regarding their ERN amplitudes after errors (p = 0.999).

When assessing the ERP component as the difference between incorrect and correct trials to extract error-specific processes (i.e., ΔERN), the ANOVA model resulted in significant main effects for electrode site (F(2,88) = 325.77, p < 0.001, ηp² = 0.37) and group (F(2,44) = 4.47, p = 0.017, ηp² = 0.17). ΔERN amplitudes were more pronounced at Fz and Cz compared to Pz (both p-values < 0.001). Importantly, HH participants had more pronounced ΔERN amplitudes than LL (p = 0.019) and LH (p = 0.010) participants. Amplitudes of LL and LH participants did not differ from each other (p = 0.828) – see Fig. 2. No interaction effect was found (F(4,88) = 0.57, p = 0.683).

The late Pe ANOVA model showed significant main effects for electrode site (F(2,88) = 51.12, p(ζGC) < 0.001, ηp² = 0.54) and type (F(1,44) = 122.18, p < 0.001, ηp² = 0.74), but no significant effect for group (F(2,44) = 1.63, p = 0.208). The electrode site × type interaction was significant (F(2,88) = 38.74, p < 0.001, ηp² = 0.47), the others did not reach significance (all p-values > 0.541). Tukey post-hoc tests showed that again error commission yielded largest Pe amplitudes at all electrode sites (all p-values < 0.001). Errors lead to comparable late Pe amplitudes at electrodes Fz and Cz (p = 0.709), which were in turn more positive than all other conditions (all p-values < 0.001).

The N2 ANOVA model showed significant main effects for electrode site (F(2,88) = 22.51, p(ζGC) < 0.001, ηp² = 0.34) and type (F(1,44) = 34.47, p < 0.001, ηp² = 0.44), and a significant interaction of electrode site × type (F(2,88) = 7.41, p = 0.001, ηp² = 0.14). The factor group (F(2,44) = 0.32, p = 0.730) and the remaining interaction effects were not significant (all p-values > 0.112). Post-hoc tests of the significant interaction effect showed that N2 amplitudes were
most pronounced at Fz during incongruent stimulus arrays (all p-values < 0.004). At all electrode sites, incongruent stimulus arrays elicited larger N2 amplitudes than congruent ones (all p-values < 0.001).

3.3. Correlation analyses

Supporting the observed group differences for ΔERN amplitudes, a significant positive correlation was found between averaged ΔERN values at Fz and Cz and individual allele numbers (r = -0.340, p = 0.019) – higher ΔERN amplitudes were associated with higher allele numbers.

We did not find significant correlations between the ERPs and the BIS-11 scales (all pcorr-values > 0.020) or the BIS/BAS scales (all pcorr-values > 0.006) in HH or LL participants. For the SURPs, we observed a significant correlation between N2 amplitudes for incongruent stimulus arrays and the Hopelessness scale in HH participants (r = -0.745, pcorr = 0.001), no other correlation reached the significance level (all pcorr-values > 0.006).

4. Discussion

This is the first study investigating whether or not genetic variation related to endogenous opioids is implicated in the modulation of electrophysiological correlates of reward prediction error signals (RPEs) and performance monitoring in humans. Although all groups showed comparable behavioural performance, high PDYN expression individuals showed enhanced error-related neural activity (ΔERN amplitudes) in comparison to intermediate and low PDYN expression ones – which was observable in significant group differences, but also when using a dimensional approach to assess the relation between the PDYN polymorphism and RPEs. In contrast, no group differences were observed for late Pe and N2 amplitudes.

4.1. PDYN group differences

The enhanced ΔERN amplitudes in individuals with high PDYN expression in comparison to those with intermediate and low PDYN expression rates corroborate the assumption of an indirect

Fig. 1. Grand average waveforms separately for high, intermediate and low PDYN expression participants of response-locked correct and error trials at Cz (upper panel) and of stimulus-locked congruent and incongruent trials at Fz (lower panel). Time point zero indicates participants’ button press (upper panel) and the onset of the four flanking arrow stimuli (lower panel). Negative is drawn upwards per convention.
The effect of PDYN on electrophysiological correlates of performance monitoring and RPEs via its relation to the dopaminergic system – in particular during error commission. Nevertheless, the observed effects were not in the expected direction since individuals with the high PDYN expression polymorphism showed enhanced ΔERN amplitudes and not diminished ones, as hypothesized.

ERN amplitude variation is mostly seen as a reinforcement learning signal (Holroyd and Coles, 2002) or as a signal of motivational error significance (Gehring and Willoughby, 2002; Inzlicht and Al-Khindi, 2012; Yeung et al., 2005). The influential reinforcement-learning-theory (RL-theory; Holroyd and Coles, 2002) relates ERN amplitude variation to changes in phasic dopamine transmission in the mesencephalon. Events worse than expected are assumed to yield phasic mesencephalic dopamine decrease which is associated with enhanced ERN amplitudes (and concurrently reflecting a negative RPE); whereas events better than expected are assumed to yield phasic mesencephalic dopamine increase which is associated with diminished ERN amplitudes (reflecting a positive RPE). Recent theories on prediction errors suggest that ERN or Feedback-Related Negativity (FRN) amplitude variation rather reflects an absolute reward prediction error signal without a numerical sign (Alexander and Brown, 2011; Chase et al., 2011; Hauser et al., 2014; Talmi et al., 2013) than outcomes better or worse than expected. These unsigned prediction error signals are assumed to represent the surprise which is accompanying unexpected outcomes (Hayden et al., 2011). Thus, the current results of error-specific ΔERN enhancement point towards the assumption that high PDYN expression individuals showed enhanced RPE signals in comparison to low and intermediate PDYN expression ones. Regarding performance monitoring ERPs, the effects of different PDYN polymorphisms do not seem to be comparable to the effects of psychopharmacological studies directly manipulating dopamine neurotransmission. Assuming that PDYN expression levels modulate mesencephalic dopamine levels differently, the theory of tonic vs. phasic dopamine effects (Grace, 1991, 1993; Moore et al., 1999) might help to explain the results. The theory claims that two processes are involved in regulating the dynamics of dopamine transmission in limbic and striatal areas. On the one hand, there is transient phasic dopamine release with high amplitudes which is mediated by burst firing of dopamine neurons. On the other hand, there is constant low-level tonic dopamine release which is mediated by baseline firing of dopamine neurons and corticostriatal glutamatergic afferents. The phasic dopamine release is supposed to transmit prediction error signals. Importantly, it was proposed that tonic dopamine release regulates the amplitude of the phasic dopamine bursts by stimulating autoreceptors located on the dopaminergic neurons. Thus, tonic dopamine release determines the responsivity of the dopamine system per se and serves to suppress phasic dopamine release via the activation of these autoreceptors (see Floresco et al., 2003; Grace, 1991). Consequently, low tonic levels of dopamine – caused by high levels of dynorphin, as suggested in high PDYN expression individuals (Zimprich et al., 2000) – might, for a given prediction error signal, result in a relatively higher dopamine release and thereby could explain enhanced ΔERN amplitudes in high PDYN expression individuals compared to intermediate and low PDYN expression ones.

However, other authors argued that phasic dopamine dips should be more pronounced in case of high tonic dopamine levels because there is a larger range available for the phasic dip (Kemnens and Kahkonen, 2011). More precisely, when tonic dopamine levels are high, even small phasic changes will result in

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2 The Feedback-Related Negativity component (FRN; Miltner et al., 1997) is an indicator of external performance monitoring, whereas the ERN component reflects internal performance monitoring processes.
relatively large ERN differences. This assumption is in line with ERN amplitude enhancement observed after administration of dopamine agonists (Barnes et al., 2014; De Bruin et al., 2004), but cannot be reconciled with the current results. Future studies are therefore needed which clarify the interaction of the variants of the functional PDYN 68 bp VNTR polymorphism, resulting dynorphin levels, and dopamine transmission in mesencephalic structures, and how this affects ERN amplitude variation.

Further linking RPE, opioid and dopamine systems, recent studies using electrophysiological, functional, and structural imaging methods reported neuronal generators for ERN/FRN components in dopamine- innervated striatal areas (Becker et al., 2014; Carlson et al., 2015; Carlson et al., 2011; Foti et al., 2011). In particular Becker et al. (2014) suggested that the ventral striatum exerts a modulating contribution to the FRN scalp topography. Also dynorphin peptides and κ-opioid receptors are accumulated in ventral striatal areas (Fallon and Leslie, 1986; Svingos et al., 1989, 2001) – thereby suggesting a shared contribution to scalp-recorded ERPs reflecting RPEs.

Importantly, no group differences were observed for late Pe amplitudes, i.e., more elaborate stages after error commission. Therefore, we assume that conscious error awareness (Nieuwenhuis et al., 2001) or affective error responses (Falkenstein et al., 2000) were comparable in the three PDYN groups. Thus, despite initial hyperactivity of the performance monitoring system in high PDYN expression individuals, they were nevertheless able to regulate later processing stages to be comparable with the activation patterns of the two other groups.

### 4.2. Implications of ERN enhancement

There is an ongoing debate as to whether enhanced ERN/ΔERN amplitudes functionally indicate a positive or a negative adaptation of the performance monitoring system – either heightened awareness of cognitive conflict or an overactive performance monitoring system (Larson et al., 2014). Increased ERN amplitudes have been reported to be an individual trait marker, but have also been observed after experimental state manipulations. For example, ERN amplitudes were enhanced in individuals scoring high on negative affect scales compared to low-scoring individuals (Hajcak et al., 2004; Luu et al., 2000). Concerning state manipulations, ERN enhancement was observed after the presentation of derogatory feedback (Wiswede et al., 2009b), after the induction of self-relevant failure (Unger et al., 2012), after the induction of feelings of helplessness (Pfabigan et al., 2013), and after punishment threat (Riesel et al., 2012). In this context, ERN amplitude enhancement has been mostly interpreted as an indicator of motivational significance (Gehring and Willoughby, 2002; Inzlicht and Al-Khindi, 2012; Yeung et al., 2005). Errors might be more salient events for negative affect-prone individuals or after the induction of negative affective states. Thus, one could speculate that high PDYN expression participants – with high levels of PDYN and consequently low levels of dopamine as continuous/chronic state-belongs to the group of this negative affect-prone individuals in which ERN amplitude enhancement could indicate heightened risk for internalizing disorders (Olvet and Hajcak, 2008), which are characterized by anxious, fearful, and depressive symptoms and behavioural tendencies (Krueger, 1999; Vollebergh et al., 2001). Recent research suggests indirectly that the dynorphin and κ-opioid receptor system is implicated in different aspects of psychopathology (Tejeda et al., 2012). For example, McLaughlin et al. (2005) summarized that κ-opioid receptor agonists induce “pro-depressive” effects while κ-opioid receptor antagonists induce “anti-depressant” effects. Furthermore, dynorphins are also implicated in the maintenance of substance abuse disorders (Margolis et al., 2008; Wee and Koob, 2010). Relating prediction error signals and these disorders, studies investigating performance monitoring in substance-use disorder individuals observed decreased ERN amplitudes in comparison to healthy controls (Franken et al., 2007; Morie et al., 2014; Sokhadze et al., 2008); even in high-risk groups (Euser et al., 2013) – thereby indicating a hypo-active performance monitoring system in these individuals. Thus, the dynorphin and κ-opioid receptor system might be indirectly implicated in either hyper- or hypo-reactivity to errors. Research assessing the relation between different PDYN polymorphism variants, performance monitoring, and clinical symptoms might be a promising research avenue in the future.

Moreover, our interpretation of enhanced motivational significance of erroneous events in high PDYN expression individuals is in line with recent findings suggesting that they also display enhanced sensitivity for upcoming rewards (Votinov et al., 2014). Importantly, in the current study, all participants received the same financial bonus after task completion to avoid confounding effects of external incentives on performance monitoring ERPs (Van den Berg et al., 2012). Performance-based external incentives might have induced additional motivation in particular in individuals more sensitive to expected rewards (Votinov et al., 2014). Thus, both the current data and the study by Votinov et al. (2014) point towards a hyper-active performance monitoring system in high PDYN expression individuals.

### 4.3. Conflict N2 results

As expected, N2 amplitude variation differentiated between congruent and incongruent stimulus arrays (Nieuwenhuis et al., 2003), thereby further validating the current paradigm. Both ERN and conflict N2 amplitudes are assumed to be generated within the aMCC (Debener et al., 2005; Gruendler et al., 2011; Hoffmann and Falkenstein, 2010; Van Veen and Carter, 2002; Yeung et al., 2004) and Yeung et al. (2004) even proposed that both reflect similar cognitive processes related to conflict monitoring (either response- or stimulus-driven). However, in contrast to ERN amplitudes, conflict N2 amplitudes were not affected by PDYN genotype. Differences in PDYN availability might only affect particular stages of performance monitoring such as the automatic evaluation of the error information (reflected in ΔERN amplitude differences), and not the initial perceptual processing of stimulus-driven conflict as reflected in N2 amplitudes.

### 4.4. Behavioural and questionnaire results

The behavioural results are in line with previous results, replicating findings of faster reaction times for error responses, lower error rates for congruent trials (Eriksen and Eriksen, 1974), and post-error slowing effects (Rabbitt, 1966) – thus generally validating the administered experimental paradigm. However, the three PDYN groups did not differ in any behavioural measure. This is in line with Weinberg et al., (2012) who summarize that ERN amplitude enhancement is rarely accompanied by behavioural effects. Individuals might be able to compensate behaviourally for the observed neuronal alterations when performing simple stimulus–response tasks as in the current study (Miller, 1996). Importantly, the comparable error rates in all groups strengthen the current results since ERN amplitude variation is also influenced by error frequency (Hajcak et al., 2003; Yeung et al., 2004). Thus, the observed group effects are not related to group-wise error frequency. Interestingly, when confronting high PDYN expression individuals with more demanding cognitive control tasks such as a reversal learning paradigm, enhanced error rates in high compared to low PDYN expression individuals were observed. The more complex performance monitoring and behavioural adaptation processes seem to be less flexible in high compared to low PDYN expression.
individuals. This assumption is also reflected in fewer cortical activation patterns and decreased functional connectivity in brain areas associated with cognitive control (Votinov et al., 2015).

Since the current groups were matched for age, sex, and education, and since the applied questionnaires did not reveal reliable group differences, the current ERP group differences can also not be explained by these variables. Limitations of the current study pertain to the small sample size investigated. Although the effect sizes of the ERN/ΔERN ANOVA results can be considered as medium to high (according to effect size classifications based on partial eta-squared; Kirk, 1996), future studies should nevertheless strive for larger group size when investigating the effects of functional polymorphisms on ERP correlates. Moreover, the current results only demonstrate indirect evidence for effects of the endogenous opioid system on performance monitoring. Our hypotheses were based on findings by Zimprich et al. (2000) and Nikoshkov et al. (2008). Contrary to these authors, recent evidence claims that alleles with fewer repeat copies of the 68 bp VNTR polymorphism are associated with higher transcriptional activation and not those with more repeat copies (Rouault et al., 2011). Further pharmaco-genetic studies are therefore necessary to investigate the link between functional polymorphisms and PDYN expression rates.

5. Conclusion

The current results indicate an impact of genetic variation in the endogenous opioid system on specific electrophysiological correlates of performance monitoring and reward prediction error signals. In particular, we observed a hyper-active performance monitoring system probably based on enhanced prediction error signals in individuals with the high PDYN expression polymorphism (Zimprich et al., 2000) compared to those with intermediate and low PDYN expression. It was the automatic response evaluation stage, but not earlier perceptual conflict or later conscious error processing stages, which differentiated high expression PDYN participants from the other participants. This hyper-reactivity to committed errors despite successful behavioural compensation might be a characteristic of the probable association between PDYN, dynorphin and dopamine levels, and internalizing disorders.

Conflict of interest

All authors declare that this research project was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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References


