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Cardiac modulation of startle is altered in depersonalization-/derealization disorder: Evidence for impaired brainstem representation of baro-afferent neural traffic

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ABSTRACT

Patients with depersonalization-/derealization disorder (DPD) show altered heartbeat-evoked brain potentials, which are considered psychophysiological indicators of cortical representation of visceral-afferent neural signals. The aim of the current investigation was to clarify whether the impaired CNS representation of visceral-afferent neural signals in DPD is restricted to the cortical level or is also present in sub-cortical structures. We used cardiac modulation of startle (CMS) to assess baro-afferent signal transmission at brainstem level in 22 DPD and 23 healthy control individuals. The CMS paradigm involved acoustic startle stimuli (105 dB(A), 50 ms) elicited 0, 100, 200, 300, 400 and 500 ms after a cardiac R-wave. In healthy control individuals, we observed lower startle responses at 100 and 300 ms than at 0 and 400 ms after an R-wave. In DPD patients, no effect of the cardiac cycle on startle response magnitude was found. We conclude that the representation of visceral-afferent neural signals at brainstem level may be deficient in DPD. This effect may be due to increased peripheral sympathetic tone or to dysregulated signal processing at brainstem level.

1. Introduction

Depersonalization-/derealization disorder is a severe mental disease, which is characterized by a subjective detachment of bodily sensations (disembodiment), emotional numbing and experiencing the external world as strange or unreal (Sierra and David, 2011; American-Psychiatric-Association, 2013). The lifetime prevalence of DPD is about 1% in western societies (Hunter et al., 2004; Lee et al., 2012) and its course it typically chronic (American-Psychiatric-Association, 2013). There are several psychobiological correlates of the core feature of emotional numbing in DPD, for example altered activation and connectivity of brain regions involved in affective stimuli processing (Medford et al., 2006; Lemche et al., 2008, 2013) and abnormal responses in electrodermal activity (EDA) to affective stimuli. However, previous studies have yielded mixed results with some studies finding higher amplitudes of EDA responses (Michal et al., 2013), more spontaneous EDA fluctuations (Schoenberg et al., 2012) and slower EDA recovery in DPD (Giesbrecht et al., 2010), while others report lower EDA responses and response probability (Sierra et al., 2002) or even no differences between DPD patients and controls (Sierra et al., 2006). In contrast to emotional numbing, the psychobiology underlying the core symptom of disembodiment remains unclear.

Previous findings suggest altered cortical representation of visceral-afferent neural signals in DPD patients as assessed by heartbeat-evoked brain potentials (HEPs) (Schulz et al., 2015), in that the pattern observed for healthy individuals (i.e. higher HEPs during the heartbeat perception task than during rest) was not present in DPD patients. DPD patients also exhibited higher salivary alpha-amylase than healthy controls (Schulz et al., 2015), indicating higher peripheral sympathetic tone (Schneyer and Hall, 1991; Nater et al., 2005). It remains unclear, therefore, whether the altered HEP pattern reflects either a selective impairment of the

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cortical representation of visceral-afferent neural signals or if it is a result of altered peripheral activation. The aim of the current study was to clarify whether impaired CNS representation of visceral-afferent neural signals in DPD is restricted to the cortical level or may also be present in sub-cortical structures.

The cardiac modulation of startle (CMS) has been used previously as pre-attentive methodology to assess baro-afferent signal transmission (Schulz et al., 2009a, 2011). Baro-afferent neural signals represent one important neural correlate of conscious cardiac interoception (Dworkin, 2007). In this CMS paradigm acoustic startle probes are presented in different time intervals after an R-wave of the ECG. As repeatedly demonstrated, acoustic startle stimuli presented in the early cardiac cycle phase (ca. 100–300 ms after an R-wave) provoke lower startle responses than stimuli presented during the late cardiac cycle phase (0 ms, 400–500 ms) (Richter et al., 2009; Schulz et al., 2009a, 2009b, 2009c, 2011), which is presumably caused by the arrival of baro-afferent neural signals during the early cardiac cycle phase (Elbert and Rau, 1995; Nyklicek et al., 2005). Since this CMS effect was largely diminished in individuals with degeneration of afferent autonomic nerves (Schulz et al., 2009a), this methodology may reflect intact baro-afferent signal transmission. Consequently, the CMS pattern is modulated after a strong combined social and cold pain stressor (Schulz et al., 2011), which was repeatedly demonstrated to induce an autonomic stress response and thus an increase of amplitude and shift of timing of baro-afferent signal transmission. We, therefore, expect that chronically increased sympathetic activation as previously observed in DPD patients (Schulz et al., 2015), will also result in altered baro-afferent signal transmission and thus in a modified CMS pattern.

Although the precise neural structures involved in the CMS effect remain unclear, it is likely that the primary acoustic startle circuit (i.e. cochlear root neurons, nucleus reticularis pontis caudalis, facial motor center) (Koch, 1999; Davis, 2006) and the arterial baroreflex circuit (nucleus tractus solitarius, nucleus ambiguus, RVLM, CVLM, cardiac pacemaker) (Jänig, 2006), which are both located in the brainstem, are involved. As summarized by Edwards et al. (2009), the maximal stimulation of baro-afferent signal transmission is expected approx. 250 ms after a cardiac R-wave, while it does not reach cortical structures until 400 ms after an R-wave (Fagius and Wallin, 1980; Gray et al., 2007). As we found evidence for lower startle responses already at 200–230 ms after an R-wave, one may speculate that baro-afferent signals may affect startle via a fast and direct pathway, which may operate without the involvement of higher (e.g., limbic or cortical) areas. Therefore, CMS can be seen as indicator of intact representation of baro-afferent neural signals at a lower brain level, possibly at the brainstem (Schulz et al., 2009a, 2009b, 2009c, 2011).

The objective of this study was to determine whether the CMS as indicator of baro-afferent signal transmission at brainstem level is altered in DPD patients as compared to a healthy control group. Participants of both groups (DPD; healthy controls) received acoustic startle stimuli of 105 dB(A) intensity, which were elicited 0, 100, 200, 300, 400 and 500 ms after the detection of an R-wave in the ECG. We expected (I) to find the previously described CMS effect, implying lower startle responses during the early compared to the late cardiac cycle phase in healthy individuals. As we previously found evidence for altered activation of the periphery (increased alpha-amylase) and changed representation of visceral-afferent signals (insensitivity of HEPs) in DPD (Schulz et al., 2015), we also expected the brainstem representation of bodily signals to be altered in DPD. In particular, and in analogy to what we observed with regard to the HEP pattern, we hypothesize (II) that the overall amplitude of startle responses are not different between DPD patients and healthy controls, but that (III) the startle response is insensitive to baro-afferent signal transmission in DPD.

2. Methods

2.1. Participants

The study was approved by the Ethics Committee of the State Board of Physicians of Rhineland-Palatinate (Germany), and was part of an extended protocol, which is in parts reported elsewhere (Michal et al., 2014; Schulz et al., 2015). All participants provided written informed consent. 27 DPD patients were recruited from the DPD clinic of the Department of Psychosomatic Medicine and Psychotherapy (Mainz, Germany) and 27 healthy controls (HC) via advertisement, who participated in the study. Five DPD patients and four healthy control individuals were later excluded from analysis due to (a) incomplete data, (b) less than 50% of valid startle responses, (c) response magnitudes that differed more than ± 3 SD from the average, (d) a resting heart rate of >85 bpm preventing startle stimuli from extending into the following cardiac cycle, or (e) occurrence of multiple ectopic heartbeats, resulting in a final sample of 22 DPD patients and 23 healthy individuals. Sample characteristics are described in Table 1. The diagnosis of DPD was established by M.M. according to the German version of the Structured Clinical Interview for Dissociative Disorders (Gast et al., 2000). Participants fulfilled the criteria for DPD according to DSM-5 (300.6) and the criteria for depersonalization-derealization-syndrome according to ICD-10 (F48.1), with all DPD patients experiencing chronic and persistent depersonalization. The mean age at onset was 17.2 (SD=6.9) years, with an average duration of 8.9 (7.1) years. Lifetime diagnosis of psychotic disorder, brain damage or cardiovascular disease and current intake of benzodiazepines or antipsychotics were exclusion criteria. Current mental disorders other than DPD were as follows: Major depression ($n=13$), dysthymia ($n=7$), social phobia ($n=5$), agoraphobia ($n=5$), generalized anxiety disorder ($n=3$), obsessive-compulsive disorder ($n=3$), bruxism ($n=1$). There were 9 patients with personality disorders, with 6 from the fearful cluster, 1 histrionic and 2 Borderline personality disorders. In the DPD group, 7 patients took antidepressants (5 SSRIs, 1 venlafaxine, 1 mirtazapine, 1 tricyclic). Medication in this inpatient sample was low, as there is no evidence-based pharmacological treatment regime for DPD (Sierra, 2008; Michal et al., 2012).

2.2. Questionnaires

Depersonalization was assessed with the Cambridge Depersonalization Scale (CDS) (Sierra and Berrios, 2000; Michal et al., 2004). The CDS consists of 29 items and measures frequency and duration of depersonalization over the last 6 months. Scores range from 0 to 290. DPD patients typically score above 70 (Sierra and Berrios, 2000). Further, the state version of the CDS (S-CDS) was applied after the experiment. The S-CDS comprises 22 items and reflects intensity of state depersonalization. Scores range from 0 to 2200. Depression was assessed with the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996) and anxiety with the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970). Adverse childhood experiences were measured using the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003).

2.3. Experimental procedure

Experimental sessions took place between 1300 h and 1700 h. Before the experimental procedure, participants completed trait questionnaires. Participants were seated in front of a LCD computer display in a comfortable chair. Electrodes for ECG-measurement were placed according to an Einthoven lead II configuration. EEG electrodes (Fz, Cz, Pz, mastoids) were placed for later calculation of heartbeat-evoked potentials (reported elsewhere:

Table 1
Sample characteristics with regard to socio-demographic data, BMI, resting heart rate and questionnaire measures.

		Healthy control group (n=23)		DPD patient group (n=22)				
Measure	**Unit**	M	SD	M	SD	t/χ^2	df	*p*
Sex[a]	m/f	11/12		13/9		0.57	1	0.45
Age[a]	years	26.2	1.9	26.1	5.5	0.06	25.50	0.95
BMI[a]	kg/m^2	21.7	2.3	23.6	3.5	**−2.14**	**36.55**	**0.039**
Resting heart rate[a]	bpm	74.5	7.3	74.0	8.5	0.17	43	0.86
Questionnaire measures[a]								
CDS trait		5.9	8.4	143.7	49.0	**−13.02**	**22.19**	**<0.001**
CDS state		35.0	61.0	1045.5	417.0	**−11.25**	**21.90**	**<0.001**
BDI-II		3.4	3.4	26.2	11.9	**−8.67**	**24.32**	**<0.001**
STAI trait		36.5	7.6	62.6	8.8	**−10.56**	**42**	**<0.001**
Childhood Trauma Questionnaire[a]								
CTQ total score		37.2	8.0	53.0	13.5	**−4.74**	**33.92**	**<0.001**
CTQ emotional abuse		7.0	3.0	11.3	4.9	**−3.60**	**34.49**	**<0.001**
CTQ physical abuse		5.2	0.4	6.1	1.9	**−2.29**	**22.61**	**0.032**
CTQ sexual abuse		5.3	0.9	5.8	1.7	−1.36	31.83	0.19
CTQ emotional neclect		8.7	3.4	13.6	5.0	**−3.85**	**43**	**<0.001**
CTQ physical neclect		5.9	1.7	8.0	2.3	**−3.59**	**39.30**	**0.001**

BMI=body mass index; CDS=Cambridge Depersonalization Scale; BDI=Becks Depression Inventory; STAI=State Trait Anxiety Inventory.

[a] Please note that sociodemographic sample characteristics, BMI, HR, and questionnaire data in an overlapping sample have already been published elsewhere (Michal et al., 2014; Schulz et al., 2015).

Schulz et al., 2015). Glasses were removed and electrodes were attached below the left eye with an inter-electrode distance of 1.5 cm to assess EMG-activity of the M. orbicularis oculi. Earphones (Creative Labs EP-630) were placed, and participants were informed about the experimental procedures on the computer display.

Psychophysiological data was first assessed during a resting period (300 s), which was divided into two 150 s-periods, one with eyes open and one with eyes closed. The order was counterbalanced across participants. Thereafter, participants completed the following 3 experimental paradigms in counterbalanced order across subjects: (1) a cardiac modulation of startle session. Six startle probes preceded the session without any relationship to the participants' heartbeats, which served as habituation trials and were not analyzed further. The startle session consisted of 60 startle probes with a jittering inter-stimulus-interval of 6–10 s, identical to an earlier applied protocol (Schulz et al., 2011), in which startle stimuli were presented with six different latencies after a detected R-wave (0 ms, 100 ms, 200 ms, 300 ms, 400 ms, and 500 ms). The total duration of the startle session was about 8 minutes. Participants were asked to relax, to neither speak nor move, to avoid longer periods of eye closure, and to listen carefully to all acoustic stimuli during the startle session. (2) A Whitehead-based heartbeat perception task, in which participants were requested to judge whether a sequence of auditory stimuli occur simultaneously or delayed with their own heartbeats (Whitehead et al., 1977). (3) A Schandry heartbeat perception task (Schandry, 1981). Participants were instructed not to take their own pulse or try any other manipulations facilitating the perception of heartbeats. The length of the experimental session was approx. 40 min. Data on heartbeat perception accuracy was reported elsewhere (Michal et al., 2014).

2.4. Recording parameters

Physiological data were collected via a Biopac MP150 amplifier system (Biopac Systems, Inc.) at 16-bit resolution and 1 kHz sampling rate. EMG-responses to acoustic white noise startle probes (105 dB, 50 ms duration, instantaneous rise time, binaural stimulation) were recorded on hard disk via Tyco Healthcare H124SG electrodes (diameter: 24 mm). Hardware band-pass filter settings were 10–500 Hz, followed by a 28 Hz software high-pass filter (van Boxtel et al., 1998). The raw signal was rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994). The ECG signal was assessed via ECG Tyco Healthcare H34SG Ag/AgCl electrodes (diameter: 45 mm), high-pass filtered (0.5 Hz) and stored to disk (1 kHz) as well. R-waves were identified online by an AccuSync 41 ECG detection device (AccuSync Medical Research Corp., Milford, CT). Accuracy of R-wave detection in sinus rhythm was higher than 99.8%, with a latency below 3 ms (internal lab report).

2.5. Analysis of psychophysiological data

Interbeat intervals were calculated from the ECG and manually corrected, with a normal cycle RR-interval time series as output signal, from which mean heart rate data was derived. A customized C++ based semi-automated PC program was used on a WinXP platform to analyze EMG responses offline. The algorithm identified response peaks in the rectified and integrated signal in the time interval of 20–150 ms after the startle probe onset. The baseline period was defined by a 50 ms interval prior to acoustic stimulation. All response data were manually inspected. Signals with electrical and physiological artifacts, such as coinciding blinks or excessive noise from other facial muscular activity, were rejected from analysis and defined as missing. If responses were not visible in the typical response latency range of a particular subject, response amplitude was set to zero. Zero response data were included in the averaging procedure, with startle response magnitude as the final output measure (Blumenthal et al., 2005). Averaging was done per participant and according to the six latency conditions. To reveal possible differences between the DPD patient and the healthy control group in overall startle response magnitude, raw values were used.

2.6. Statistical analysis

A-priori group differences (age, sex, BMI, resting heart rate)

were calculated using chi-square test for categorical variables and *t*-tests for independent samples for metric variables. In case of inhomogeneity of variances (Levene test), degrees of freedom were corrected accordingly. To inspect the difference between DPD patients and healthy control individuals in cardiac modulation of startle, we conducted a 2 × 6 mixed design ANOVA with the between-subjects factor 'group' (DPD patients; healthy controls), the within-subjects factor 'cardiac cycle phase' (R+0, 100, 200, 300, 400, and 500 ms), and the dependent variable 'startle response magnitude'. As we previously observed lower startle magnitudes at 100, 200 and 300 ms (=early cardiac cycle phase) as compared to 0, 400 and 500 ms after the R-wave (=late cardiac cycle phase) (Schulz et al., 2010, 2011), we compared each startle magnitude of the early cardiac cycle phase against each startle magnitude of the late cardiac cycle phase using a-priori contrasts (one-tailed). In subsequent exploratory analyses we compared all startle magnitudes against all others using Bonferroni-corrected *t*-tests (two-tailed) for dependent samples. All *p*-values of within-subjects factors with more than two conditions are reported after Huynh-Feldt correction. Critical α-level was set to .05 for all analyses. All statistics were conducted with SPSS 19.0 (IBM, Inc.).

3. Results

3.1. A-priori group differences

Depersonalization, depression, anxiety and childhood trauma scores, as well as heart rate data has previously been reported in an overlapping sample (Michal et al., 2014; Schulz et al., 2015). One participant did not complete the STAI questionnaire. Groups did not differ with regards to sex distribution, age or resting heart rate (see Table 1). DPD patients had higher BMI, state and trait depersonalization scores, depression and anxiety scores, as well as childhood trauma scores in all scales, except for the scale 'sexual abuse', than the healthy control group.

3.2. Startle response magnitudes

Average startle response magnitudes did not differ between DPD patients (M=40.2; SD=61.0 μV) and healthy controls (M=55.4; SD =59.7 μV; main effect 'group': $F[1,43]=1.418$; $p=0.24$; $\eta^2=0.032$). There was a main effect for 'cardiac cycle phase' ($F[5,215]=3.158$, $p=0.012$; $\eta^2=0.068$). Furthermore, we observed a significant 2-way interaction 'group' × 'cardiac cycle phase' ($F[5,215]=2.562$; $p=0.034$; $\eta^2=0.056$), suggesting that effects of 'cardiac cycle phases' on startle response magnitudes were present only in the healthy control group, but not in the DPD group. A-priori comparisons contrasting startle magnitudes of the early (100, 200, 300 ms) against those of the late cardiac cycle phase (0, 400, 500 ms) revealed that in the healthy control group startle response magnitudes at 'R+0 ms' were higher than at 'R+100 ms' ($p=0.010$) and 'R+300 ms' ($p=0.014$), and startle response magnitudes at 'R+100 ms' were lower than at 'R+400 ms' ($p=0.034$; see Fig. 1). In addition, our exploratory post-hoc analyses showed that startle magnitudes at 'R+200 ms' were higher than at 'R+100 ms' ($p=0.004$) and 'R+300 ms' ($p=0.028$). In the DPD group neither the a-priori nor the exploratory comparisons showed any significant differences between startle magnitudes across the cardiac cycle.

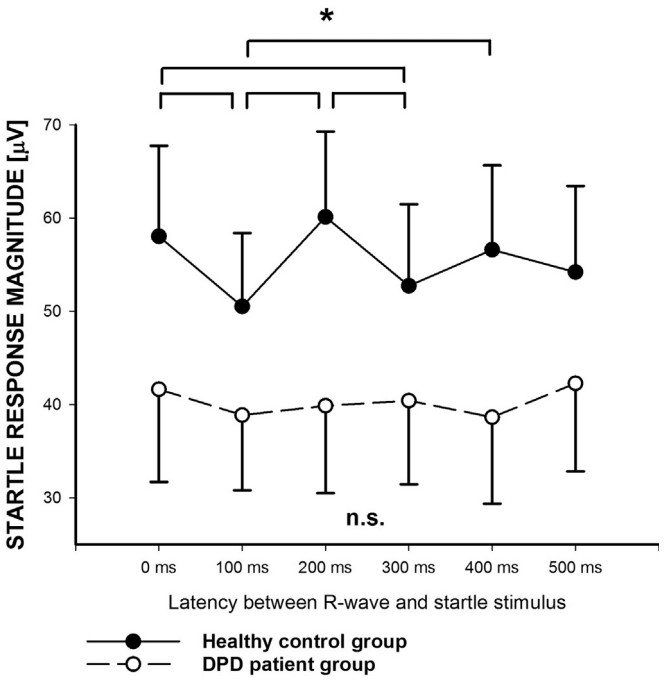

Fig. 1. Startle response magnitudes were modulated by the cardiac cycle phases in the healthy control group, but not in the DPD group (significant 2-way interaction). There was no overall difference in startle magnitude between groups.

4. Discussion

The main aim of the current study was to clarify whether baro-afferent signal transmission at brainstem level is altered in DPD patients as compared to a healthy control group. To address this yet unanswered question we investigated cardiac modulation of startle (CMS) in a group of 22 DPD patients and a group of 23 healthy control individuals. Based on previous observations that DPD patients show altered cortical processing of afferent signals from the cardiovascular system and increased peripheral sympathetic tone (Schulz et al., 2015), the main hypothesis (III) was that CMS is altered in DPD patients as compared to healthy control individuals. In partial support of hypothesis (I), in the healthy control group we observed lower startle response magnitudes at cardiac cycle phases, which are associated with higher baro-afferent signal transmission (R-wave+100 ms and +300 ms) than at phases, which are associated with lower baro-afferent signal transmission (R+0 ms and +400 ms). Interestingly, in the DPD group there were no effects of cardiac cycle phase on startle response magnitude. The absence of any CMS effect in the DPD group supports our main hypothesis.

The mutual evaluation of all data derived from the extended study setup of the current project results in a more complete picture of interoception in DPD: previously we found that, in contrast to healthy controls, DPD patients exhibit insensitivity of HEPs for attention focused on heartbeats, which suggests altered processing of visceral-afferent signals at cortical level (Schulz et al., 2015). This altered processing at cortical level is also reflected in a modified accuracy in heartbeat perception in that DPD patients show a decrease of accuracy over time, while healthy control individuals improve their accuracy due to training effects (Michal et al., 2014). The present study involving a largely overlapping sample addresses CMS and, therefore, an indicator of baro-afferent neural transmission at brainstem level. The fact that we did not find any CMS effect in DPD patients suggests that the representation of visceral-afferent signals from the cardiovascular system may already be deficient at sub-cortical (i.e. brainstem) level. This deficiency of interoceptive signal processing at an early, presumably pre-attentive stage is in line with existing studies on visual and verbal memory, demonstrating that only early perceptual and attentional processes may be impaired in DPD, while delayed recall seems unaffected (Guralnik et al., 2000; Guralnik et al., 2007). From a clinical point of view, it may be concluded that treatment should aim at modifying early information processing stages via top-down mechanisms (e.g., biofeedback training to improve baroreflex signal processing), and which could be monitored with CMS methodology as applied in the current study.

The question remains whether altered visceral-afferent signal processing in DPD is limited to the central nervous system (i.e. brainstem and cortex) or whether the peripheral activation of visceral organs is dysregulated in DPD, which may also account for

the observed effects. Indeed, we found higher alpha-amylase concentrations in DPD patients than in healthy individuals based on an overlapping sample (Schulz et al., 2015), which indicates increased peripheral (beta-adrenergic) sympathetic tone (Nater et al., 2005). The possibly large amplitude of changes in baroreceptor stimulation due to chronic peripheral sympathetic hyperactivation in DPD may induce insensitivity for the small amplitudes of natural variation as to be expected during the cardiac cycle and could, therefore, be responsible for alterations of CMS and HEPs in DPD. This notion implies that in the treatment of DPD special attention should be paid on physiological stress axes activity, and potentially stress coping and stress resilience.

Nevertheless, acute sympathetic activation by a laboratory stress test (cold pressor test) induces a time shift in CMS pattern (Schulz et al., 2011) but does not abolish a CMS effect, as observed in the current study. Furthermore, acute sympathetic activation provoked by a cold pressor test may also increase the amplitude of HEPs (Shao et al., 2011), which was not observed in DPD (Schulz et al., 2015). One may argue, therefore, that the current pattern of insensitive HEP and absent CMS is not simply due to increased sympathetic activation, but specific for DPD. Future studies should investigate whether this pattern can also be found in individuals with chronic peripheral hyper-activation, but without DPD, to establish the specificity of this effect for DPD.

This is the second study to apply the 'extended' CMS paradigm comprising latencies between R-waves and acoustic startle stimuli of 0, 100, 200, 300, 400 and 500 ms. In a previous study, we observed lower startle response magnitudes at 100, 200 and 300 ms than at 0, 400 and 500 ms after an R-wave (Schulz et al., 2011). As the CMS effect only occurs in individuals with intact baro-afferent signal transmission (Schulz et al., 2009a), we proposed that this effect is due to increased neural feedback from arterial baroreceptors caused by the arterial pulse wave, which is expected between 90 and 390 ms after the R-wave (Edwards et al., 2009). We could partially replicate these findings by observing lower startle responses at 100 and 300 ms than at 0 and 400 ms after an R-wave. In contrast to our expectations, however, at 200 ms after the R-wave the startle response magnitude was higher than at 100 and 300 ms.

One possible reason for this finding could be the higher baseline heart rate of the control group in the present study (74 bpm) as compared to earlier studies, which ranged from 68 (Schulz et al., 2009a), over 70 (Schulz et al., 2009b) to 72 (Schulz et al., 2011). While sympathetic activation increases heart rate and decreases stroke volume (SV) and pre-ejection period (PEP) (Eichler and Katkin, 1994; Schachinger et al., 2001), orthostatic manipulation during rest (e.g., upright tilt, sitting vs. supine position, etc.) induces an increase of heart rate and a decrease of SV, but a prolongation of PEP (Brener et al., 1990; Ring et al., 1994). The same relationship was found for inter-individual differences in heart rate, SV and PEP (Brener et al., 1990). Since in the present and in previous studies CMS was measured without stimulating the sympathetic nervous system, it is plausible that higher heart rate may be accompanied by lower SV and prolonged PEP than in the previous studies. The processing of afferent cardiac sensations in the central nervous system (e.g., perception of heartbeats) is strongest about 230 ms after a cardiac R-wave (Ring and Brener, 1992; Brener and Ring, 1995), but intra- and inter-individually lower SV and longer PEP leads to delayed CNS processing of cardiac sensations (Brener et al., 1990). It is possible, therefore, that higher heart rate (and possibly longer PEP) in the current study caused later CNS processing of afferent signals from the cardiovascular system, which resulted in a CMS effect, which was not visible before 300 ms after the R-wave. Earlier modulatory effects of the cardiac cycle (i.e. 100 ms) could still be attributed to other sources than the arterial pulse wave, such as stimulation of atrial mechanoreceptors (Schulz et al., 2011). However, this only explains why the CMS effect was not present at 200 ms as expected, but it does not explain why startle amplitudes were even higher than at other latencies. A possible explanation for this latter finding would be that over and beyond the natural variation in arterial baroreceptor stimulation there are other effects that can be observed within the cardiac cycle. In particular, for the modulation of reaction time and signal detection across the cardiac cycle, an oscillation between 8 and 12 Hz was reported (Velden and Juris, 1975; Wölk and Velden, 1987). These results have been interpreted as evidence for a time-lock between the cardiac cycle and alpha rhythm oscillation in the brain. An assessment of startle every 100 ms (=10 Hz), such as in the current study, may be too low to reflect these oscillations and could result in an artificial frequency with one of its peaks in signal detection around 200 ms after the R-wave (Wölk and Velden, 1987). Unexpected effects of the cardiac cycle on startle responses may eventually result from an interaction of arterial baroreceptor feedback and a possible alpha rhythm that interferes with the CNS processing of stimuli related to the cardiac cycle.

To the best of our knowledge, the present study is the first to investigate startle eye-blink responses in DPD as compared to a healthy control group. In line with hypothesis (II), we did not find differences between groups in their average startle response magnitude. The majority of DPD patients had an additional diagnosis of major depression (59%) or of at least one anxiety disorder (59%), and they exhibited higher scores of childhood trauma in all but one CTQ scale compared to controls. On the one hand, patients with major depression show lower baseline startle responses than healthy control individuals and a negative relationship of severity of depressive symptoms and startle response magnitude (Allen et al., 1999; O'Brien-Simpson et al., 2009). On the other hand, studies on anxiety disorders have yielded mixed findings. While alterations of startle responses in specific phobias may be restricted to the effect of a phobia-associated stimulus, there is no generally increased startle response in phobias (Hamm et al., 1997; Muhlberger et al., 2006), generalized anxiety disorder (Grillon et al., 2009) or panic disorder (Cuthbert et al., 2003). Furthermore, there is a tendency for increased overall startle response magnitudes in PTSD (Morgan et al., 1995, 1996; Orr et al., 1995; Jovanovic et al., 2009). McTeague and Lang (2012) provide an explanation for these mixed findings on startle magnitude in anxiety disorders by distinguishing between fear vs. pervasive anxiety/sadness disorders and their respective psychopathological and pathophysiological pattern. Disorders of the fear class, such as social and specific phobias or single traumatic experiences, are rather associated with increased defensive responsivity (e.g., startle response), whereas disorders of the anxiety and sadness class, such as depression, generalized anxiety disorder, or multiple traumatic experiences, are associated with a normal or blunted defensive responsivity. In the current study, all but one participant would have qualified for the anxiety/sadness disorder class. The descriptively lower, but statistically comparable absolute startle amplitudes in DPD as compared to healthy control individuals could, therefore, be explained by the specific startle pattern (normal or decreased amplitude) as to be expected in anxiety/sadness disorders. Alternatively, comparable startle amplitudes in DPD and healthy controls may represent a specific feature for this disorder. It remains for future studies to show whether DPD patients also show a specific pattern of startle modulation by other factors, such as affective stimuli.

4.1. Limitations

This is the first study to investigate CMS in individuals with a severe mental disorder and the second study to apply the

extended design of the CMS paradigm including the latencies of 0, 100, 200, 300, 400 and 500 ms. We suggest that future research should investigate the specificity of the current results for DPD and further examine the unexpected effect of the highest startle magnitude at 200 ms after the R-wave. Comparing results from DPD patients with chronic sympathetic hyper-activation and from healthy individuals under conditions of experimentally induced activation of the autonomic nervous system, as described here, has to be done with caution, since the processes involved in chronic dysregulation of physiological stress axes are complex and not yet fully understood. The investigation of baro-afferent neural traffic through the CMS paradigm is limited to individuals who show a sufficient number of valid startle responses. It cannot be ruled out that the exclusion of startle non-responders had a systematic effect on the results. However, given that altered processing of visceral-afferent signals was also reported for a slightly larger number of individuals from the same sample (Michal et al., 2014; Schulz et al., 2015), such an effect would be implausible. Finally, it has to be acknowledged that the precise neurophysiology behind the CMS effect remains unclear, as there are currently no systematic lesion studies in animals.

4.2. Conclusion

The present results suggest that the representation of visceral-afferent neural signals at the level of the brainstem may be deficient in DPD. This effect may be due to increased peripheral sympathetic tone or to dysregulated signal processing at brainstem level.

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