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ORIGINAL ARTICLE

Mammalian cadherins DCHS1-FAT4 affect functional cerebral architecture

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Abstract Cortical development is a complex process where a multitude of factors, including cadherins, plays an important role and where disruptions are known to have far reaching effects in neural development and cortical patterning. Cadherins play a central role in structural left–right differentiation during brain and body development, but their effect on a functional level remains elusive. We addressed this question by examining functional cerebral asymmetries in a patient with Van Maldergem Syndrome (VMS) (MIM#601390), which is caused by mutations in *DCHS1-FAT4* cadherins, using a dichotic listening task. Using neurophysiological (EEG) data, we show that when key regulators during mammalian cerebral cortical development are disrupted due to *DCHS1-FAT4* mutations, functional cerebral asymmetries are stronger. Basic perceptual processing of biaurally presented auditory stimuli was unaffected. This suggests that the strength and emergence of functional cerebral asymmetries is a direct function of proliferation and differentiation of neuronal stem cells. Moreover, these results support the recent assumption that the molecular mechanisms establishing early left–right differentiation are an important factor in the ontogenesis of functional lateralization.

Keywords Functional cerebral asymmetries · Lateralization · Dichotic listening · Left–right differentiation · Van maldergem syndrome

Introduction

Cortical development is a complex process and neurons undergo several transitions before they form the layers of the cerebral cortex. In the process of proliferation and differentiation of stem cells, cadherins are important for cell adhesion (Zakaria et al. 2014; Bystron et al. 2008). Disruptions in these processes are known to have far reaching effects in neural development and cortical patterning (Capello et al. 2013), but their effects on functional brain architecture remain elusive. Interestingly, cadherins play a central role in structural left–right differentiation during brain and body development (Mendes et al. 2014; Petzoldt et al. 2012). Since a recent genome-wide association study (Brandler et al. 2013) showed that genes involved in early left–right differentiation affect functional cerebral asymmetries (FCA's), i.e., functional differences between the left and the right hemispheres of the mammalian brain, it can be assumed that cadherin dysregulation may affect FCA's. FCA's are a major organizational principle of mammalian functional cerebral architecture (Vallortigara and Rogers 2005). The most well-known example for FCA's is probably the strong dominance of the left hemisphere for most speech tasks (Hugdahl 2000). Interestingly, FCA's in the language system have been shown to be associated with structural left–right asymmetries in certain brain regions, e.g., the planum temporale

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(Dorsaint-Pierre et al. 2006) to a larger extent than FCA's in other cognitive systems. Thus, they might be particularly affected by molecular mechanisms involved in structural left–right differentiation such as the mechanisms mediated by cadherins.

Mutations in the genes encoding the receptor-ligand cadherin pair *DCHS1* or *FAT4* lead to dysregulated proliferation and differentiation of neuronal stem cells before the generation and migration of neurons in the cortex (Capello et al. 2013) and cause Van Maldergem Syndrome (VMS; MIM#601390), a rare autosomal-recessive disorder with only a few known cases world-wide (Capello et al. 2013). Patients with VMS syndrome (also the case investigated) show facial dysmorphism including telecanthus, epicanthus, short palpebral fissures as well as dental anomalies and dysplastic ears. Neurologically, the phenotype comprised neonatal hypotonia, hyperkinetic behaviors and mental retardation (van Maldergem et al. 1992). We examined VMS as a disease model to evaluate the effects of Fat and Dachsous cadherins on functional organization of the mammalian cerebral cortex. By means of bootstrap statistics (Crawford and Garthwaite 2012), we compare a single patient with VMS to a group of controls in a dichotic listening task using EEG recordings to examine neurophysiological correlates of cortical information processing and functional asymmetries during the task.

Materials and methods

Subjects and statistics

In this study, we examined one right-handed (LQ = 80) patient with genetically confirmed Van Maldergem Syndrome (VMS) (11 years of age) in comparison to a sample of healthy controls ($N = 12$) in the age range between 10 and 14 years of age (mean age 12.5 years ± 3.4). Details describing the VMS case examined in this study can be found in Neuhann et al. (2012). The boy carried a homozygous frameshift mutation in the *FAT4* gene—NM_024582.4(FAT4):c.[14512_14513delTC];[14512_14513delTC], NM_024582.4:p.[Ser4838Leufs*3];[Ser4838-Leufs*3] (Capello et al. 2013). The controls were right-handed. Their mean laterality quotient as assessed using the Edinburgh Handedness Inventory (Oldfield 1971) was 90 ± 5. To compare the VMS case with the control cohort, we used specialized single-case statistics (Crawford and Garthwaite 2012). The *t* value together with the 95 % confidence interval (CI) is given. This method offers the best possible method to compare a single case against a control cohort (Crawford and Garthwaite 2012). For comparisons within the control cohort, paired-samples *t* tests were applied and Bonferroni corrected when necessary. These methods have been shown to be reliable with EEG data (e.g., Beste et al. 2014; Beste and Saft 2015).

Task

The task used to assess asymmetries of evoked potential latency to speech sounds was similar to that used by Eichele et al. (2005), but with an additional control condition. Stimuli were digitally recorded consonant–vowel syllable pairs (e.g., "BA," "DA," "GA," "KA," "PA," and "TA") spoken by an adult German male baritone voice. Stimuli had a mean duration of 350 ms and were presented using Presentation software (Neurobehavioral Systems, Inc., Albany, USA) at 80 dB. The stimuli have undergone extensive pretesting and were used and validated in previous studies (Ocklenburg et al. 2011, 2013). Differences between the voice onset times of voiceless ("KA," "PA," and "TA") and voiced consonants ("BA," "DA," and "GA") were controlled for, and the spectral temporal envelopes of the syllables were matched. After 10 training trials, there were three blocks of experimental 90 trials each, 270 trials in total. In each block, there were three conditions of 30 trials each. In the "dichotic condition," two different syllables (e.g., "BA" and "DA") were presented simultaneously to the two ears. All possible CV pairs were presented counterbalanced to both the ears in this condition to avoid any possibly confounding effects of syllable-type on the EEG data. In the "homonymous condition," the same syllable was presented to both ears (e.g., "BA" and "BA"). Moreover, a "noise condition" was included, in which white noise was presented instead of verbal stimuli. The inter-stimulus interval was jittered between 3150 and 3650 ms to avoid stimulus habituation effects.

EEG recording and analysis

The EEG was recorded from standard scalp positions using an electrode grid of 60 Ag/AgCl electrodes (filter band-width 0.5–100 Hz). The primary reference during data acquisition was located at FPz. The data were filtered off-line using an IIR filter with the filter band-width of 0.5–20 Hz. Before filtering, the data were inspected visually, and occasionally occurring technical artifacts were removed. Eye-movement artifacts (blinks and saccadic activity) as well as pulse artifacts were removed using independent component analysis (ICA; infomax algorithm). Afterward, the EEG epoch was stimulus-locked according to the different experimental conditions, i.e., separate segments were built for the "noise stimuli condition," "homonymous condition," and "dichotic condition." Afterward, an automated artifact rejection procedure was applied, using a maximal value difference of 200 μV in a

100-ms interval as well as an activity below 0.5 μV in a 200 ms period as rejection criteria. Then, the data were re-referenced using CSD transformation (Nunez and Pilgreen 1991) after which the resulting CSD values were stated in $\mu V/m^2$. The CSD transformation was used to eliminate the reference potential from the data. It also works as a spatial filter making it easy to identify electrodes that need to be analyzed for the different ERP components. Afterward, before averaging, a baseline correction was applied in the time interval from −100 ms till stimulus presentation. Electrodes were selected on the basis of the scalp topography of the N1 ERP component. This choice was validated using a statistical approach outlined in Mückschel et al. (2014). This revealed the same electrodes as previously chosen by visual inspection of the data. Importantly, we controlled the signal-to-noise ratio (SNR) of the data to obtain an estimate about the reliability of the neurophysiological data. This is important when comparing to a single subject. We calculated the signal-to-noise (SNR) in the VMS case and controls as implemented in the Brain Vision Analyzer II software package (BrainProducts Inc.) (see also Beste et al. 2014). The SNRs did not differ between controls and the VMS case in all experimental conditions ($p > 0.6$). The data presented are thus reliable.

Results

The results in the dichotic listening condition are shown in Fig. 1a.

We examine the N1 amplitude in response to verbal stimuli, a technique that has been shown to reliably indicate functional cerebral asymmetries in the language system (Eichele et al. 2005). Within the control group, the amplitude was higher at electrode C5 (left hemisphere) (24.1 $\mu V/m^2 \pm 1.8$) than at electrode C6 (right hemisphere) (16.8 $\mu V/m^2 \pm 3.5$) ($t = 3.73$; $p = 0.003$). Latency effects were also observed showing that the latencies were shorter at electrode C5 (181 ms ± 2.2) than at electrode C6 (198 ms ± 3) ($p < 0.001$). The mean difference within the control group was 17 ms ± 5. For the VMS case, it is shown that the difference in N1 latency between the left and right lateralized (90 ms) cluster was larger than in controls ($t = 14.02$; $p < 0.000001$; 95 % confidence interval 8.52–20.62). Moreover, there was a general prolonging of the latencies compared to healthy controls ($t = 7.55$; $p < 0.0001$; 95 % CI 4.85–11.85) (refer Fig. 1). There was no difference in the amplitude of the N1 in VMS, compared to the control group ($p > 0.5$).

As described in the methods section (see above), a condition where noise stimuli and a condition where the same syllables (homonymous condition) were presented to both the ears. The ERPs of these conditions are shown in

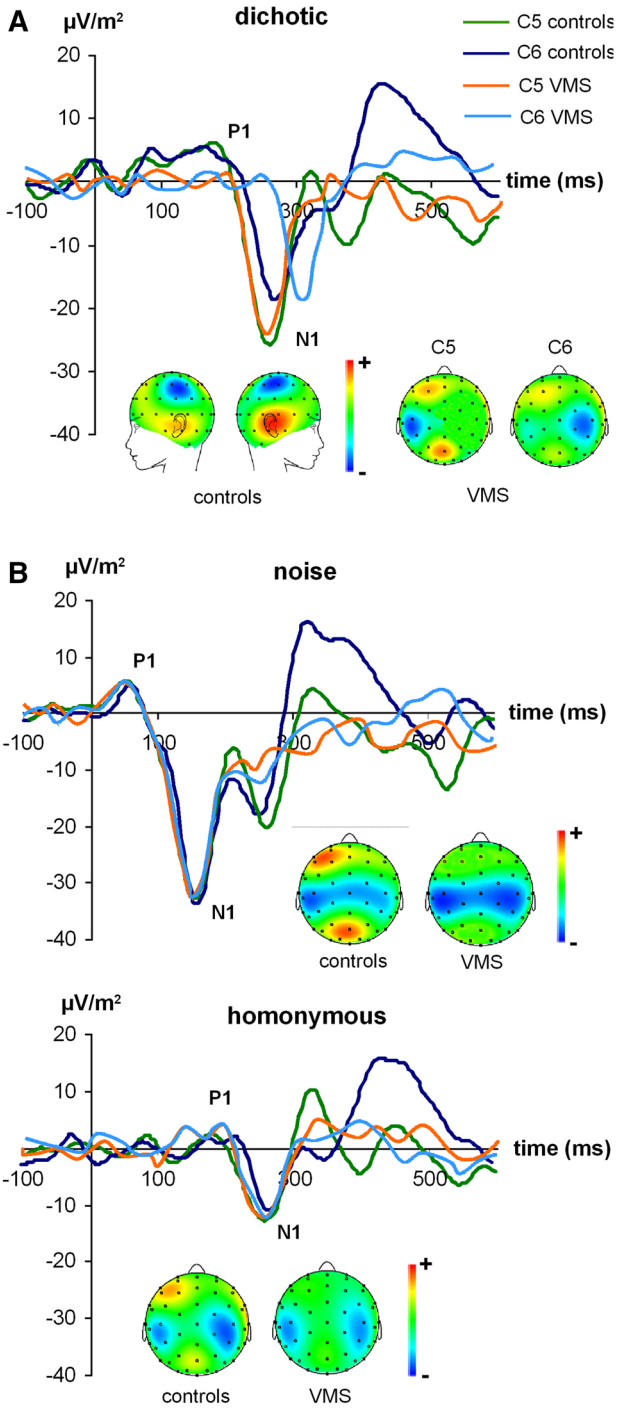

Fig. 1 **a** Event-related potentials (ERPs) on the dichotic listening stimuli for electrodes C5 and C6. As can be seen in the maps, these electrode sites best reflected the N1 on the dichotic listening stimuli. Electrode C5 reflects the potentials for stimuli presented to the *right* ear and electrode C6 for stimuli presented to the *left* ear. *Green* and *dark blue lines* denote the control group, *orange* and *light blue lines* the VMS case. There are strong latency differences between the N1 peak at electrode C5 and C6 in the VMS case, which is also reflected in a strongly lateralized activation in the scalp topography *plots*. In the scalp topography *plots*, *cold colors* denote negativity, *warm colors* denote positivity. **b** ERPs for noise (*top*) and homonymous (*bottom*) stimuli. No differences between controls and the VMS are seen

Fig. 1b. Figure 1b suggests that there are no differences in amplitudes and latencies of the P1 and N1 ERP, neither between electrode sites nor between controls and the VMS case. This is underlined in the statistical analysis of the data (all $p > 0.5$). This pattern did not change when the mean of a cluster of electrodes (i.e., FC5, C5, C3, T7 for the left side and FC6, C6, C4, T8) was used to for data analysis. Also here, there were no significant differences (all $p > 0.7$). The results from these control conditions therefore underline the specific importance of DCHS1 or FAT4 for highly lateralized processes and show that changes in VMS are not due to purely perceptual deficits.

Discussion

In the current study, we examined aspects of a potential molecular basis for the emergence of functional cerebral asymmetries. We focused on the possible importance of Dchs1-Fat4 cadherins because cadherins play a central role in structural left–right differentiation during brain and body development (Mendes et al. 2014; Petzoldt et al. 2012), but their effect on a functional level remained elusive. We assessed the role of Dchs1-Fat4 cadherins by examining a case with Van Maldergem Syndrome (VMS; MIM#601390), which is caused by mutations in DCHS1 or FAT4 (Capello et al. 2013). Capello et al. (2013) showed that in VMS, abnormal neuronal positioning in the developing cerebral cortex does not occur because the migrational capability is affected, but because of an increase in proliferation of early progenitor cells that is followed by insufficient subsequent differentiation which lead to heterotopic positioning of neurons in the cortex.

The results suggest that a dysregulation of these processes leads to stronger FCAs, suggesting that functional cerebral asymmetries emerge as a direct function of proliferation and differentiation of neuronal stem cells. Therefore, complementary to their role in structural left–right asymmetry establishment (Mendes et al. 2014; Petzoldt et al. 2012), cadherins also affect functional brain organization. One interesting finding in this regard is the fact the patient investigated in the present study showed hypoplasia of the corpus callosum (Neuhann et al. 2012). The corpus callosum is the largest commissure in the human brain and has been shown to serve excitatory and inhibitory functions during interhemispheric transfer of information (Bloom and Hynd 2005). Importantly, Gadea et al. (2009) found that in patients with multiple sclerosis, a significant progressive loss of posterior corpus callosum areas was related to enhanced lateralization in a behavioral dichotic listening task. Thus, the corpus callosum seems to play an at least partly inhibitory role during this task, which might explain why the patient in our study showed enhanced lateralization.

Identifying the proteins and cellular processes underlying the development of FCAs may prove useful in helping to clarify the complex relation between brain structure and FCAs. On the other hand, it helps by providing candidate genes for future studies investigating the genetic and epigenetic determinants of the functional brain architecture. This field of research is important due to findings that patients with neurodevelopmental or psychiatric disorders often show atypical lateralization, implicating that genes involved in asymmetrical organization of function might be relevant for the ontogenesis of these disorders (Brandler and Paracchini 2014). While it was not possible to attain reliable behavioral data for the patient with VMS, N1 amplitude asymmetries have been shown to highly correlate with behavioral measures of language lateralization (Eichele et al. 2005), making additional behavioral testing unnecessary. Moreover, electrophysiological measures of functional language asymmetries are a more direct measure of the underlying neuronal processes than behavioral measures and are less confounded by cognitive control processes than behavioral measures (Hugdahl et al. 2013). A clear limitation of the study is that only a single case could be examined, which is due to the extreme rareness of the disease, with only about nine patients known worldwide (Capello et al. 2013). However, despite of the SNR, data obtained from the VMS patient are as reliable as from the control cohort, and the pathogenesis of a rare disease may act as a paradigm of a dysfunctional brain architecture. The genetic defect investigated may be an interesting model to study effects in other lateralized domains, such as prosody, or emotional processing in general, as well.

In summary, the study suggests that cadherins, important in the process of proliferation and differentiation of stem cells during neural development strongly, influence the strength of functional cerebral asymmetries in the language domain. This finding strikingly supports the recent assumption that the molecular mechanisms establishing early left–right differentiation also are an important factor in the ontogenesis of functional lateralization (Brandler et al. 2013).

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