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**Neuronal networks of burst suppression EEG as revealed by source analysis  
and renormalized partial directed coherence**

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## Contents

1. Introduction .....	5
1.1. Burst suppression EEG.....	5
1.2. Non-epileptic Encephalopathies.....	8
1.2.1 Hypoxic-Ischemic Encephalopathy (HIE) .....	8
1.3. Epileptic Encephalopathies (EE).....	10
1.3.1 Historical background .....	11
1.3.2. EE of Neonatal period.....	11
1.3.2.1 Early infantile Epileptic Encephalopathy (Ohtahara Syndrome) .....	11
1.3.2.2 Early Myoclonic Encephalopathy (EME) .....	12
1.3.3 EE of Infancy.....	13
1.3.3.1 West Syndrome (WS) .....	13
1.3.3.3 Malignant migrating partial seizures in infancy (MMPSI) .....	14
1.4 Source analysis .....	15
1.5 Aim of the study .....	16
2. Subjects and Methods.....	17
2.1. Subjects .....	17
2.2 EEG recordings .....	21
2.3 EEG analysis .....	22
2.3.1 Selection of EEG epochs .....	22
2.3.2 Spectral analysis .....	22
2.3.3 Source analysis .....	24
2.3.4 Directionality analysis .....	26
3. Results .....	27
3.1 Dynamic imaging of coherent sources (DICS) .....	27
3.1.1 Burst Phases .....	27
3.1.2 Suppression Phases.....	28
3.2 Renormalized partial directed coherence (RPDC) .....	28
3.2.1 Burst Phases .....	28
3.2.2 Suppression Phases.....	29
3.3 Comparison between burst and suppression phases.....	29
4. Discussion .....	33
4.1 Functional and effective connectivity during BS .....	33
4.2. Comparison of the neural networks of epileptic/non-epileptic encephalopathies and West Syndrome.....	37
4.3. Methodological limitations.....	37
5. Conclusion.....	39

6. Summary .....	40
7. Appendix .....	41
7.1 Supplementary figures.....	41
8. References .....	44
9. Acknowledgment.....	51
10. Ethical Publication Statement .....	51
11. Curriculum Vitae .....	52
12. Publications.....	53

# 1. Introduction

## 1.1. Burst suppression EEG

Burst suppression (BS) is an electroencephalographic (EEG) pattern in which burst phases and suppression phases alternate quasiperiodically, with various durations of inter- and intra-burst phases (Niedermeyer et al., 1999). Burst phases represent high voltage EEG activity, with approximately 150 – 350  $\mu$ V amplitude, whereas suppression phases are characterized by periods of electrical silence, with an amplitude of less than 25  $\mu$ V.

BS is considered as a global state of profound brain inactivation (Swank and Watson, 1949). Various conditions can lead to the occurrence of BS such as coma (Young, 2000), cardiac arrest (Soholm et al., 2014), drug-related intoxication (De Rubeis and Young, 2001), hypothermia (Stecker et al., 2001), anesthesia (Akrawi et al., 1996, Brown et al., 2010) but also early infantile epileptic and non-epileptic encephalopathies (Ohtahara et al., 1976, Gloss et al., 2013).

BS pattern is characteristic for Ohtahara Syndrome (Ohtahara et al., 1976), Early Myoclonic Encephalopathy (Aicardi and Goutieres, 1978) and Hypoxic Ischemic Encephalopathy, but can also occur during other epileptic or non-epileptic neonatal and infantile encephalopathies (Aicardi, 2002), usually indicating a severe prognosis (Aicardi, 2002). For a more detailed description of encephalopathies with BS pattern see the following chapters 1.2. and 1.3. .

The fact that different etiologies produce a similar electrophysiological pattern suggests that there might be a common pathophysiological mechanism underlying BS leading to the profound state of brain inactivation. These mechanisms are poorly understood, however, above-mentioned etiologies indicate that BS pattern represents a low-order dynamic mechanism that persists in the absence of higher-level brain activity (Ching et al., 2012).

Steriade and colleagues (1994) studied neuronal correlates of BS in an animal study. Intracellular recordings of cortical and thalamic cells of anesthetized cats indicated a disconnection of thalamocortical networks. Up to 70 % of thalamic neurons were completely silent during suppression phases. Only remaining cells kept on firing rhythmic discharges in delta frequencies, which are generated by the interplay between two of their intrinsic currents. Once BS deepened all thalamic cells ceased discharges and even the remaining cells were silent (Steriade et al.,

1994). These findings are concordant with a later SPECT/PET study of an infant with early myoclonic encephalopathy. It shows a dysfunction in terms of hypometabolism and hypoperfusion of bilateral basal ganglia, thalamus and cerebral cortex during interictal phases with burst suppression EEG, indicating a functional deafferentation of cortex from subcortical structures (Hirose et al., 2010).

Studying BS EEG in vivo, Amzica and colleagues (2007) showed that the cortical network is in a hyperexcitable state. Thus, subliminal stimuli are able to trigger a bursting period, followed by a refractory period which appears as suppression. Bursting periods are accompanied by the phasic depletion of extracellular calcium concentration and thus are limited, because low extracellular calcium levels are equitable to reduced synaptic transmission. During suppression there is no synaptic transmission, until calcium levels and the cortical sensitivity for external or internal stimulation are restored.

In order to investigate central features of BS, Ching and colleagues (2012) developed a neurophysiological-metabolical model that accounts for different etiologies. This model states a causal relationship between reduced cerebral metabolism and BS. According to this model levels of available energetics (adenosine triphosphate – ATP) alter and induce suppression and burst phases. During burst phases the intracellular level of ATP decreases leading to hyperpolarization and thus stabilization of cellular membrane. This prohibits further spiking and a suppression phase occurs. ATP levels then slowly increase, which can be understood as recovery of cellular energy and thus recovery basal cortical dynamics, the next successive burst emerges (Ching et al., 2012).

The perspective that BS is a homogenous state of brain has been strengthened by EEG studies in which burst and suppressions phases have been shown to occur concomitantly across the scalp (Ching et al., 2012, Clark and Rosner, 1973). However, studying local cortical dynamics in the anesthetized brain Lewis and colleagues concluded that local cortical dynamics vary across time and space. Uncoupled BS states might occur potentially across cortex. Thus the underlying dynamics display substantial local heterogeneity (Lewis et al., 2013).

Little is known about the brain structures, which are involved in the genesis of BS. Moreover it is poorly understood which the temporal dynamics among the involved structures are and to what extend these dynamics are responsible for the alternating pattern of burst and suppression

phases.

The high temporal resolution of the electroencephalogram (EEG) is of advantage to the analysis of fast dynamic processes of the human brain. In contrast to other functional imaging procedures like SPECT, PET or functional magnetic resonance imaging (fMRI), the EEG operates in the millisecond range and thus is well suited for the analysis of BS pattern, which shows in fact short transient alternating events (Groening et al., 2009, Vulliemoz et al., 2009, Siniatchkin et al., 2010, Schelter et al., 2009, Astolfi et al., 2007). However, due to the inverse problem, it is difficult to locate the exact position of sources in the brain, especially when there are sources of deep brain structures. For more explanation see the method part (Michel et al., 2004, Holmes, 2008, Holmes et al., 2010, Holmes et al., 2004).

Recent developments in EEG inverse solutions have substantially improved the localization efficiency of EEG. This has enabled the use of EEG data for investigations into neuronal networks, even within deep structures in the brain. Dynamic imaging of coherent sources (DICS) is a method of source analyses, which allows to detect sources even within deep brain structures (Japaridze et al., 2013, Moeller et al., 2013b, Elshoff et al., 2013). However, DICS alone is not able to show the relations of different parts of a network to each other or describe the direction of informational flow between these sources (Gross et al., 2002, Hellwig et al., 2000, Hellwig et al., 2003, Hellwig et al., 2001, Schack et al., 2003, Tass et al., 1998, Volkman et al., 1996). In order to answer questions about the effective connectivity and about the direction of informational flow between sources of a network, renormalized partial directed coherence (RPDC) can be used (Baccala and Sameshima, 2001, Sameshima and Baccala, 1999, Schelter et al., 2009).

The purpose of this study is to acquire knowledge about the mechanisms of BS EEG in neonates and infants with severe encephalopathies of different etiologies. Of particular interest are the components and connectivity of the underlying neuronal network. This may be a step closer to understand the fundamental properties of the brain's arousal circuits.

## **1.2. Non-epileptic Encephalopathies**

According to the definition of the National Institute of Neurological Disorders and Stroke non-epileptic encephalopathy is a term for any diffuse disease of the brain that alters brain function or structure (NIH, 2010). An altered mental state is considered to be the hallmark of encephalopathies and it can be expressed in a broad variety of neurological symptoms (Capovilla et al., 2013).

There is a vast number of underlying etiologies (Young et al., 2011). The most frequent causes of encephalopathy in neonates and infants are hypoxic-ischemic encephalopathy, focal ischemia, cerebral malformations, metabolic disorders, intraventricular hemorrhage and infectious diseases (Vasudevan and Levene, 2013). There are epileptic and non-epileptic encephalopathies with noteworthy differences between them. During non-epileptic encephalopathies the underlying etiological process has a leading role in neurological decline, whereas during epileptic encephalopathies the epileptic activity itself contributes greatly to the neurological deterioration.

A slowing of electrical activity can be seen in EEG recordings during epileptic as well as non-epileptic encephalopathies. The deeper and more severe encephalopathic conditions get, the slower the EEG activity gets, until the EEG becomes discontinuous (burst suppression pattern) and then flat (Gloss et al., 2013).

The following description of different types of encephalopathies will concentrate on hypoxic-ischemic encephalopathy and early epileptic encephalopathies, because in this study we investigated children with these disorders.

### **1.2.1 Hypoxic-Ischemic Encephalopathy (HIE)**

HIE is an acute, nonstatic encephalopathy caused by a perinatal hypoxic-ischemic period, resulting in a brain injury. Moderate to severe neonatal hypoxic-ischemic encephalopathy occurs in approximately 1,5 cases per 1000 term live births (Kurinczuk et al., 2010). The essential diagnostic criteria defined by the American College of Obstetricians and Gynecologists in 2002 are metabolic acidosis, early onset of severe or moderate encephalopathy, signs of global brain damage and exclusion of other etiologies.



Three grades of HIE based on clinical symptoms reaching from mild to severe dysfunction of motor skills, cognitive development, autonomic functions and epileptic seizures have been proposed (Sarnat and Sarnat, 1976) (See table 1). Isolated or repeated seizures are common. Subtle seizures are the most common type of neonatal encephalopathies, which are usually mild paroxysmal alterations in motor, behavior or autonomic function that are not clearly clonic, tonic or myoclonic, for example oral-facial-lingual, limb or ocular movements, autonomic phenomena and others (Sankar et al., 2008).

According to Volpe (2001) the most common evolution of background EEG changes are suppression of frequency and amplitude of electrical brain activity, followed by periodic pattern and/or multifocal or focal sharp activity, afterwards suppression phases interspersed with bursts so called burst suppression pattern and in the end isoelectric background activity.

Therapeutic hypothermia as a protective mechanism to reduce brain damage is used to improve the clinical outcome (Wassink et al., 2014). Another promising approach for the future is to achieve neural recovery of damaged brain areas through stem cell transplantation leading to the initiation of endogenous repair mechanisms. This generates cell recovery of the already damaged regions and thus functional recovery (Gonzales-Portillo et al., 2014).

Table 1: Grades of severity of the HIE

<b>Criteria:</b>	Stage 1	Stage 2	Stage 3
<b>Level of consciousness</b>	Hyperalert	Lethargic or Obtunded	Stuporous
<b>Seizures</b>	None	Common	Uncommon
<b>Neuromuscular control</b>	Normal muscle tone, overactive stretch reflexes, mild distal flexion	Mild hypotonia, overactive stretch reflexes, strong distal flexion	Flaccid, decreased or absent reflexes, intermittent decerebration
<b>Complex reflexes</b>	Strong Moro, others less affected	Weak Suck and Moro, overactive oculovestibular and tonic neck	Fully absent
<b>Autonomic functions</b>	Generalized sympathetic	Generalized parasympathetic	Full depression
<b>EEG</b>	Normal	Low-voltage continuous delta and theta activity, periodic pattern	Isopotential or infrequent periodic discharges
<b>Duration</b>	< 24 hours	2-14 days	Hours to weeks

### 1.3. Epileptic Encephalopathies (EE)

Epileptic Encephalopathies (EE) are a rare, heterogeneous group of syndromes, which are associated with severe cognitive and behavioral disturbances (Sarco and Takeoka, 2013). According to the nomenclature of the International League Against Epilepsy (ILAE) the hallmark of EE is the fact that „the epileptic activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone (e.g., cortical malformation), and that these can worsen over time“ (Berg et al., 2010) .

Generally the characteristics of EE are pharmacoresistant, generalized or focal seizures and persisting EEG abnormalities such as BS pattern or hypsarrhythmia (Noh et al., 2012). There are several neonatal and infantile EE syndromes with different clinical features, which are described below. The clinical and electroencephalographical attributes may vary in time and could evolve into another type of syndrome. Mostly these infants have a poor outcome and die at very young age (Khan and Al Baradie, 2012).

According to the ILAE this category includes the epilepsy syndromes (Berg et al., 2010) as depicted in table 2.

Table 2: Electroclinical syndromes arranged by age at onset

Neonatal period	Infancy	Childhood
Early Myoclonic Encephalopathy (EME)	West Syndrome	Lennox-Gastaut Syndrome (LGS)
Early infantile Epileptic Encephalopathy (Ohtahara Syndrome/OS)	Myoclonic epilepsy in infancy (MEI)	Landau-Kleffner Syndrome (LKS)
	Dravet Syndrome	Epilepsy with continuous spike-waves during slow wave sleep (CSWS)
	Migrating partial seizures in infancy	
	Myoclonic encephalopathy in non-progressive disorders	

### **1.3.1 Historical background**

Dr. West 1841 first mentioned the term of EE and described the syndrome, which was later named after him (West, 1841). During the following years, it was further defined and refined. In 2001, the International League Against Epilepsy (ILAE) Task Force on Classification and Terminology proposed a modified diagnostic scheme for epileptic seizures and epilepsy that, for the first time, recognized epileptic encephalopathies as a distinct category (Engel, 2001). Due to the fact that the concept of EE still remains unclear and it is sometimes difficult to draw the line between these syndromes, the notion of EE is still in the process of evolution and the actual classification is not definitive.

### **1.3.2. EE of Neonatal period**

#### **1.3.2.1 Early infantile Epileptic Encephalopathy (Ohtahara Syndrome)**

Ohtahara Syndrome (OS) (Ohtahara et al., 1976) is a rare EE with onset of symptoms mainly around the first three months of life. The prevailing types of seizures are frequent tonic spasms, which can be isolated or in clusters. Usually the seizures tend to be frequent and do not respond to antiepileptic drugs (Yamatogi and Ohtahara, 2002). The EEG shows a continuous BS pattern, which occurs during wakefulness and sleep (Fusco et al., 2001).

The most common cause is malformation of cerebral development such as hemi-megalencephaly, porencephaly, Aicardi syndrome, olivary-dentate dysplasia, agenesis of mamillary bodies, linear sebaceous nevus syndrome, cerebral dysgenesis and focal cortical dysplasia (Panayiotopoulos, 2005): Also in many cases structural brain lesions have been found in the putamen, thalamus, and hippocampus as well as the tegmentum of the brainstem (Itoh et al., 2001). Rarely metabolic disorders can also be responsible, in addition, a variety of underlying

gene mutations (e.g. STXBP1, ARX, SLC25A22) have been increasingly reported (Kato et al., 2007, Beal et al., 2012).

Over the course of time OS can proceed into West Syndrome and less often from West Syndrome into Lennox-Gastaut Syndrome (Ohtahara and Yamatogi, 2003). As part of this transition, BS pattern evolves into hypsarrhythmia at around 3–4 months of age, and sometimes further to diffuse slow spike-waves (Ohtahara and Yamatogi, 2006). Prognosis seems to be dependent on the evolution of EEG pattern. Patients who have hypsarrhythmia or who change further to diffuse slow spike-waves have a higher mortality rate (Ohtahara and Yamatogi, 2003). Overall the mortality is very high as many patients die during their first years of life. Surviving patients mostly develop multiple disabilities (Ohtahara, 2002). A recent study however showed that, in some cases, a more promising therapeutic approach could be an epilepsy surgery (Malik et al., 2013).

### **1.3.2.2 Early Myoclonic Encephalopathy (EME)**

EME was first described in 1987 (Aicardi and Goutieres, 1978). It is a rare and severe EE with a very poor response to antiepileptic drugs. Onset of the seizures occurs within the first three months of life and mostly within the first month of life (Ohtahara and Yamatogi, 2003).

The essential type of seizure is myoclonia, but tonic spasms and focal seizures also occur (Aicardi, 1992). The most striking feature is the high incidence of familial cases (Aicardi, 1992).

Inborn errors of metabolism are the most frequent cause of EME. In particular, nonketotic hyperglycinemia, propionic aciduria, methylmalonic acidemia, D-glyceric acidemia, sulfite and xanthine oxidase deficiency, Menkes disease, and Zellweger syndrome may all present with EME (Ohtahara and Yamatogi, 2003, Aicardi, 1992). However, structural abnormalities and progressive, diffuse cortical atrophy has also been reported (Murakami et al., 1993). The prognosis is very poor, often the patients die within the first years of life (Ohtahara and Yamatogi, 2003).

The characteristic electroencephalographic pattern is BS pattern, which is predominant and distinct during sleep (Murakami et al., 1993) and can possibly occur after onset of symptoms (Ozyurek et al., 2005). It often evolves into atypical and mostly transient hypsarrhythmia, for

up to two years, and then returns into BS (Panayiotopoulos, 2005).

EME must be considered as a differential diagnoses for OS, although it can be challenging to differentiate between both syndromes due to electro-clinical similarities. See table 3 for a more detailed comparison.

Table 3: Comparison of OS and EME

	<b>Ohtahara Syndrome(OS)</b>	<b>Early Myoclonic Encephalopathy (EME)</b>
<b>Age at onset</b>	Neonatal period	Neonatal period
<b>Main seizures</b>	Tonic spasms	Erratic myoclonia, focal seizures, clusters of spasms
<b>Main cause</b>	Cerebral malformations	Non-structural/metabolic
<b>Interictal EEG</b>	Burst suppression	Burst suppression
<b>Ictal EEG</b>	Desynchronization	Variety of paroxysms with focal onset
<b>Daytime progression of EEG</b>	Sleeping and waking state	Accentuated during sleeping state
<b>Onset of EEG abnormalities</b>	With onset of disease	Variable (1.-5. months)
<b>Evolution</b>	75% → West-Syndrome, 12% → Lennox-Gastaut-Syndrome	Variable, often persistent
<b>Transition of EEG</b>	After appr. 3-4 months: Hypsarrhythmia, evolution into diffuse slow spike-wave also possible	After appr. 3-4 months: Hypsarrhythmia, often return to BS

### 1.3.3 EE of Infancy

#### 1.3.3.1 West Syndrome (WS)

West Syndrome is characterized by the triad of developmental delay, pathognomonic EEG pattern of hypsarrhythmia and infantile spasms, which are a unique seizure type (West, 1841, Mackay et al., 2004). These epileptic seizures are spasms of brief muscle contraction (neck, trunk, and/or extremities) (Fusco and Vigevano, 1993, Mothershill, 2000). The incidence of WS ranges from 2.9 to 4.5 per 100,000 live births, with onset of symptoms mostly in the first year of life (Taghdiri and Nemati, 2014). Interictal EEG registration shows hypsarrhythmia (Fusco

and Vigevano, 1993), which is characterized by chaotic mixture of abnormal, gigantic, arrhythmic and asynchronous slow and sharp waves, multifocal spikes and polyspikes. Moreover, modifications of the originally described pattern can be often seen as well (Hrachovy and Frost, 2003).

Clinical treatment can be challenging (Mackay et al., 2004), although ACTH, corticosteroids, and vigabatrin have conclusively demonstrated effectiveness (Haines and Casto, 1994). WS can have multiple causes, overall, cortical malformations, hypoxic-ischemic, and tuberous sclerosis are the most common associated disorders (Taghdiri and Nemati, 2014).

The pathophysiological mechanism underlying WS are poorly understood. Studies have revealed putamen, brainstem and various cortical regions playing an important role in the pathogenesis of hypsarrhythmia (Chugani et al., 1992, Chugani et al., 1990, Hrachovy and Frost, 2003). An EEG source analysis study showed that ascending projections from basal ganglia to basal ganglia and cerebral cortex may play a key role in the pathogenesis of hypsarrhythmia (Japaridze et al., 2013).

The prognosis is mainly determined by the disorder, which underlies the syndrome and grade of severity. Unaffected development is seldom (5-12%) (Hrachovy and Frost, 2003).

Approximately 17 % of all patients with WS evolve into Lennox-Gastaut Syndrome (Khan and Al Baradie, 2012).

### **1.3.3.3 Malignant migrating partial seizures in infancy (MMPSI)**

MMPSI or migrating focal seizures was first described in 1995 (Coppola et al., 1995). The onset is usually between the first days of life to 7 months of age. Seizures occur after an initial period of normal development, with motor and autonomic symptoms (Panayiotopoulos, 2005). Unilateral eyelid and eye jerking, with eye and head deviations and unilateral tonic or clonic convulsions with alternating sides are the characteristic motor manifestations. Seizures are usually variable in their manifestation, intensity and duration and are highly drug resistant (Coppola, 2013, Yamamoto et al., 2011). Etiology is not known and there is a lack of information regarding the family history of epilepsy. It is associated with multifocal ictal EEG

discharges and profound psychomotor developmental delay as well as acquired microcephaly and evidence of brain atrophy (Coppola, 2013). A lethal outcome in the first year of life is frequent (Wong-Kisiel and Nickels, 2013).

## 1.4 Source analysis

Imaging procedures like PET, SPECT or fMRI have a very high spatial resolution, although very limited temporal resolution. In contrast EEG investigation is characterized by a high temporal resolution and can detect fast dynamic processes of the brain. This fact makes it important for better interpretation of different parts of neuronal networks, especially to separate brain areas with the initial epileptic activity from regions of propagation (Groening et al., 2009, Vulliemoz et al., 2009, Siniatchkin et al., 2010, Astolfi et al., 2007). However, scalp EEG is spatially blurred due to the ambiguity of the underlying static electromagnetic inverse problem, which makes the identification of active brain areas, especially the once in deep structures of the brain, very challenging (Holmes, 2008, Holmes et al., 2004, Holmes et al., 2010).

In this study we used dynamic imaging of coherent sources (DICS), which is an inverse solution model, to improve the localization power of the EEG. DICS depicts neuronal networks even in the deep structures of the brain by using a spatial filter. It works in the frequency domain for EEG and magnetoencephalographic (MEG) data and detects brain regions that are coherent with each other and a reference signal. By imaging power and coherence of oscillatory brain activity it can help to visualize neuronal networks of the brain (Gross et al., 2001). It has been proven by various studies, that DICS is able to characterize networks including deep structures such as the thalamus, cerebellum and brainstem in MEG studies (Gross et al., 2001, Gross et al., 2002, Schnitzler et al., 2006, Sudmeyer et al., 2006b, Timmermann et al., 2003a, Timmermann et al., 2003b) as well as thalamus and brainstem in recent EEG studies (Elshoff et al., 2013, Japaridze et al., 2013, Moeller et al., 2013b, Muthuraman et al., 2013, Muthuraman et al., 2012a, Muthuraman et al., 2012b). However, DICS is not able to describe the interaction between the identified brain sources (Elshoff et al., 2013, Gross et al., 2002, Hellwig et al., 2000, Hellwig et al., 2001, Hellwig et al., 2003, Schack et al., 2003, Tass et al., 1998, Volkman et al., 1996). Therefore another method, called renormalized partial directed coherence (RPDC), has to be utilized (Baccala and Sameshima, 2001, Sameshima and Baccala, 1999, Schelter et

al., 2009) in order to describe the connections i.e. the direction of informational flow between these sources.

### **1.5 Aim of the study**

In this study we investigate the neuronal networks underlying BS EEG in neonates and infants with severe encephalopathies of different etiologies using electrical source imaging. Our aim is to identify the common mechanism leading to the state of brain inactivation and learn more about the fundamental properties of the brain's arousal circuits.



## 2. Subjects and Methods

### 2.1. Subjects

Thirteen infants and neonates at the age from one day to one year old with severe epileptic and non-epileptic encephalopathies with BS EEG pattern were selected for the study. Four patients were recruited from the database of the Department of Neuropediatrics at the University Hospital of Schleswig-Holstein, Campus Kiel and the Northern German Epilepsy Centre for children & adolescents, Schwentinental/Raisdorf, Germany. Nine patients were selected from the Department of Neurophysiology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK. Clinical and demographical data of the patients are presented in table 5.

Four patients had severe hypoxic ischemic encephalopathy, six patients had epileptic encephalopathies, and three patients had neurometabolic disorders. Diagnoses were made according to the ILAE 2001 classification scheme (Commission on Classification and Terminology of the International League against Epilepsy, 2001). (For more detailed description see table 5)

All patients had global developmental delay of various severities, which was evaluated by neurological examinations. Most of the patients had intractable and often therapy-resistant seizures, six patients had no history of clinical seizures (see table 5). The numerous antiepileptic drugs had been given at the time of EEG registration. All of the patients had BS EEG pattern (see figure. 1) (see table 5)

The neurological examination and structural MRI were performed in all cases. Various pathological MRI findings were found in 11 patients. All available information about the medical history of the patients has been summarized in table 5. Routine EEGs (in accordance with the 10–20 system) were recorded in all cases and were independently evaluated by two neurophysiologists who confirmed the type of EEG abnormality.

The study was conducted according to the Declaration of Helsinki (current version, 1996) on biomedical research involving human subjects (Tokyo amendment). The study was registered and approved by the research and development office of the UCL Institute of Child Health, London, United Kingdom.

Table 5: Demographic and clinical Data

Pat.	Diagnose	Age at onset of symptoms	MRI findings	Age at EEG reg.	AED	Type of seizures	Description of EEG/EEG findings	Medical history/ follow up
1 <sup>1</sup>	HIE <sup>a</sup>	From birth	Signal abnormality and swelling of basal ganglia and thalami	1 day	PB	No clinical seizures	Burst suppression	Severe, multiple disabilities, fatal outcome at the age of 12 days
2 <sup>1</sup>	HIE <sup>b</sup>	13 months	Global cerebral, basal ganglia and thalamus infarction	13 mo	MDL	No clinical seizures	Burst suppression	Spasticity
3 <sup>1</sup>	Epileptic Encephalopathy	From birth	Brain malformation, simplified gyration, hypoplastic cerebellum	1 mo	none	No clinical seizures	Burst suppression	Dysmorphic features, spasticity
4 <sup>1</sup>	Neurometabolic disease <sup>c</sup>	2 weeks	Multiple, bilateral hemorrhages	5 days	none	No seizures	Burst suppression	Developmental delay
5 <sup>1</sup>	HIE, IC hemorrhage	First hours of life	Brain atrophy <sup>d</sup>	3 days	PB	Electrographic seizures	Burst suppression	Axial hypotonia with limb spasticity
6 <sup>1</sup>	Migrating epilepsy	12 hours	Normal	7 mo	PHT, CLZ, ZNS, Ketogenic diet	Focal motor and tonic seizures	Burst suppression	Severe developmental delay
7 <sup>1</sup>	Non-ketotic hyperglycaemia	9 days	Mild delay of myelination	2 mo	PB	Tonic seizures	Burst suppression	Severe developmental delay
8 <sup>1</sup>	HIE, ICH	1 day	Intracerebral	6 days	PB,	No seizures	Burst	Mild

			hemorrhage, oedema °		PHT		suppression	developmental delay
9 <sup>1</sup>	Metabolic	6 days	Bilateral perisylvian and insular polymicrogyria	6 weeks	PB	Tonic seizures	Burst suppression	Mild spasticity
10 <sup>2</sup>	Facial epilepsy, later West-Syndrome (CDKL5-gene point mutation)	6 weeks	Delay of myelination, potentially focal cortical dysplasia	8 mo	OXC, VPA, STP	Focal tonic seizures, series of propulsive seizures	Burst suppression, Periodic occurrence of modified Hypsarrhythmia	Global developmental delay, spasticity
11 <sup>2</sup>	EME DD.: Othahara Syndrome	3 months	Delay of myelination, progressive atrophy of white matter	11 mo	PHT, CLB, TPM, CLB	Tonic seizures in series, polytopic myoclonia	Burst suppression, Periodic occurrence of modified Hypsarrhythmia with diffuse spike and waves and high amplitude slow waves and phases of marked EEG depression	spasticity
12 <sup>2</sup>	Focal epilepsy, later West Syndrome,	7 months	No definite pathological finding	1 yr	PHT, CLB	Tonic-clonic seizures	Burst suppression, Periodic occurrence of modified Hypsarrhythmia, with phases of marked EEG depression	Prematurity, global developmental delay, craniofacial dysmorphism, extrapyramidal movement disorder, muscular hypotonia,
13 <sup>2</sup>	Focal epilepsy, later West Syndrome	3 weeks	Focal cortical dysplasia in the left frontal lobe	9 mo	STM, VGB	Infantile spasms in series	Burst suppression, Periodic occurrence of modified	Global developmental delay, muscular hypotonia.

							Hypsarrhythmia with diffuse high amplitude slow waves and multifocal spikes and phases of marked EEG depression	Functional hemispherectomy at age of 2 years
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**List of abbreviations:** AED Antiepileptic drugs; EEG Electroencephalography; PB Phenobarbital; MDL Midazolam; PHT Phenytoin; CLZ Clonazepam; ZNS Zonisamid; OXC – Oxcarbazepin, CLB Clobazam, STP Stiripentol. VGB Vigabatrin, STM Sulthiam. HIE hypoxic ischemic encephalopathy; IC intracranial Hemorrhage;

<sup>1</sup> Patients from the Department of Neurophysiology, Great Ormond Street Hospital for Children, London, UK.

<sup>2</sup> Patients from the Department of Neuropediatrics at the University Hospital of Schleswig-Holstein, Campus Kiel and the Northern German Epilepsy Centre for Children & Adolescents, Schwentinal/Raisdorf, Germany

<sup>a</sup> Severe perinatal hypoxic ischemic encephalopathy

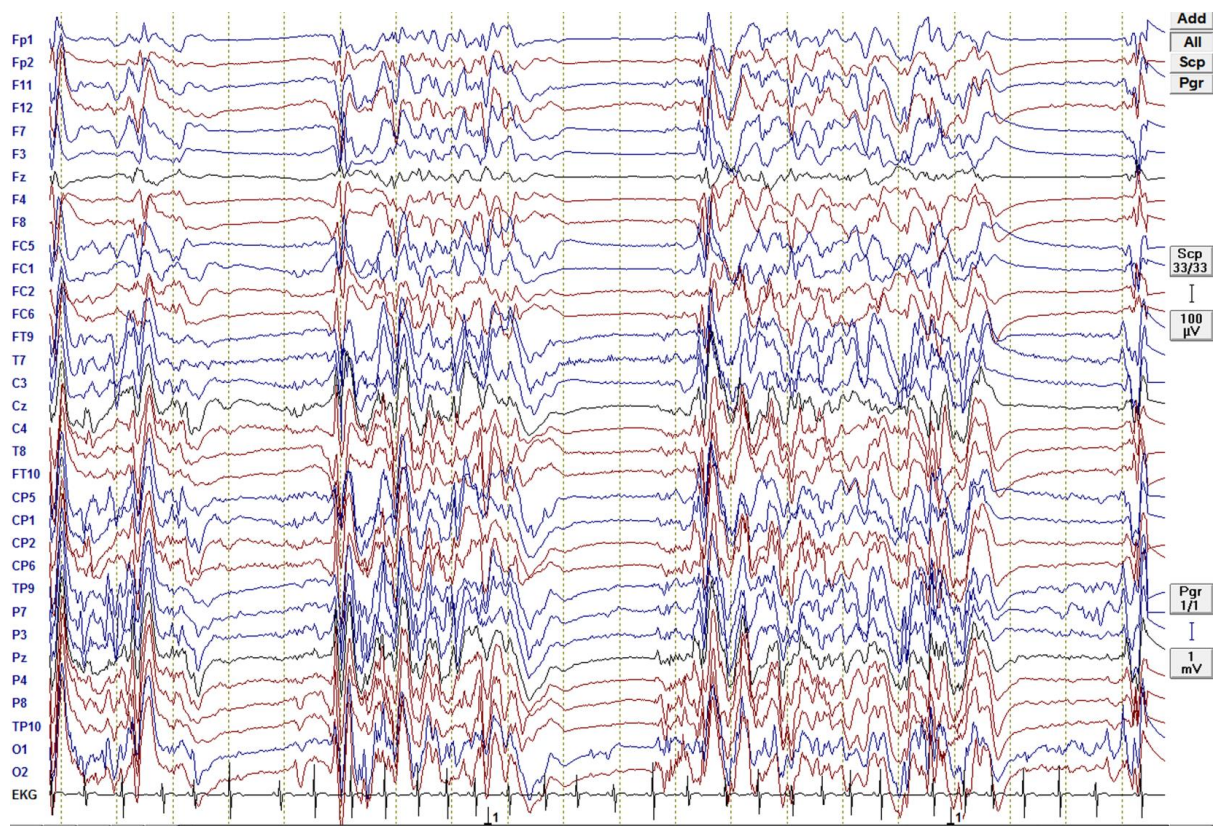
<sup>b</sup> Severe hypoxic ischemic encephalopathy due to an aspiration with the grape and respiratory and cardiac arrest.

<sup>c</sup> DD: thromboembolic disease or genetic / mitochondrial disease.

<sup>d</sup> Marked brain atrophy, almost no remaining occipital parenchymal tissue. No underlying structural cause for the previous hemorrhage.

<sup>e</sup> Intraventricular hemorrhage, hydrocephalus. Diffuse cerebral hemispheric edema. Left cerebellar and infra- and supratentorial subdural hematoma, A small non-compressive intradural hematoma in the upper thoracic spine, subarachnoid hemorrhage.

Fig. 1: Burst Suppression EEG



## 2.2 EEG recordings

Standard EEG recordings, according to the 10/20 system (EEG recording system: Neurofile; IT-med, Bad Homburg, Germany) had been done for the four patients which were recruited from the Department of Neuropediatrics at the University Hospital Schleswig-Holstein and the Northern German Epilepsy Centre for children & adolescents. All EEG recordings were performed during sleeping state. In some cases following additional electrodes were used for the analysis: FC1, FC2, FC5, FC6, CP1, CP2, CP5, CP6, FT9, FT10, TP9, TP10, ECG. Impedance was kept below 10 kOhms, Sampling rate was 512 Hz. Reference was located between Fz and Cz. Further EEG processing for the correction of ECG artifacts was done, if necessary.

For the nine patients recruited from the Department of Neurology, Great Ormond Street Hospital for children, standard EEG recordings, according to a modified 10/20 system (EEG recording system: Natus XLTek, Oakville, Ontario, Canada) were used for the analyses. During the

EEG recordings impedance was kept below 10 kOhms and the sampling rate was 512 Hz. Reference was located between Cz and Pz. If required, EEGs were further processed for the correction of ECG artifacts. As all of the recordings, EEG registration was done during sleep.

## **2.3 EEG analysis**

### **2.3.1 Selection of EEG epochs**

The EEG and EEG segments were visually inspected by two experienced neurophysiologists independently. For gaining valid results of the DICS analysis, the used EEG segments has to be as long as possible in order to achieve acceptable signal-to-noise ratio. Since BS EEG usually contains of short lasting intervals, different segments need to be assembled. Consequently, the whole EEG recording was inspected and suppression phases were selected, cut out and concatenated until a whole length of no less than 60 seconds was achieved. The same process was done for burst phases.

### **2.3.2 Spectral analysis**

The first step of the analysis is to identify the predominant frequencies for both phases separately. For this reason a spectral analysis was performed, which showed the prevalent frequency in Delta range (1-4 Hz) similarly for both burst and suppression phases. Consecutively this frequency was used for further analysis.

To compute the power spectra for all recorded EEG channels a multitaper method (Mitra PP, 1999) was used. The spectra were recorded separately for burst and suppression phases. The multitaper method is a method for highly time-resolved spectral analysis which estimates the spectrum by multiplying the data with different windows ('tapers'). To calculate the power spectrum by discrete Fourier transformation it uses a sliding time window.

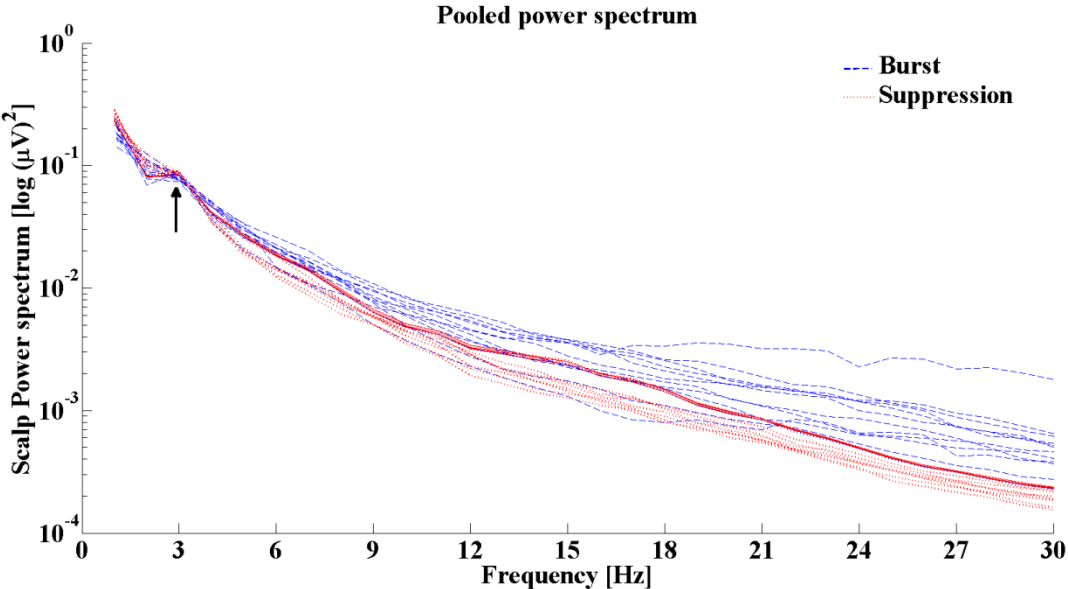
The data epoch of one second length was tapered using a set of discrete prolate spheroidal

sequences. The Fourier-transformation was applied to the tapered data epochs and auto-spectra were computed. In a following step the spectra were averaged and the power spectrum was estimated. Afterwards the main frequency band was defined, which was obligatory for the following source analysis. A detailed description of this method has been given previously (Muthuraman et al., 2010a). The open source package Fieldtrip was used (Oostenveld R, 2011) for the spectral and the source analysis part.

From the original power estimates of the individual EEG electrodes a pooled time frequency power spectrum has to be evaluated (fig. 2). This can be done by pooling the individual second order spectra using a weighting scheme and evaluating the pooled estimate of power as previously described (Amjad et al., 1997, Rosenberg JR, 1989).

In order to evaluate the implications both the burst and suppression phases have on the source analysis because of their different amplitudes in raw EEG data, the relative signal-to-noise ratio (SNR) was estimated. The power at the frequency band 1-4 Hz for the burst as the numerator and the suppression phase as the denominator was calculated in each patient. The relative SNR in all the patients ranged from 35.85 -39.09 dB.

Fig.2: Pooled power spectrum showing a clear peak (black arrow) at the 1-4 Hz frequency range in all these patients for both the burst and suppression phases



### 2.3.3 Source analysis

Dynamic imaging of coherent sources (DICS) (Gross et al., 2001) was used to reveal the underlying neuronal network of burst suppression EEG. DICS works in the frequency domain for EEG and magnetoencephalographic (MEG) data. It is a source analysis method using a spatial filter algorithm (Drongelen et al., 1996) which is able to detect brain regions that are coherent, thus functionally related to each other. Not only cortical sources but also sources in deep brain structures such as the brainstem can be analyzed and visualized (Gross et al., 2001, Gross et al., 2002).

Two major problems need to be solved whilst working with DICS, which are the so called forward and inverse problem.

**Forward solution:** This is the computation of the scalp potentials for a set of neural current sources. It represents the individual electromagnetical and geometrical characteristics, which influence and predict the pathway of informational flow to the surface of scalp, where it is electroencephalographically measured. It is solved by estimating the lead-field matrix (LFM) with specified models for the brain. These models contain information about the individual geometry and conductivity. The LFM is multiplied with the estimated sources (the current density vector) to produce the scalp potentials. The difference between these predicted scalp potentials and the measured potentials create the basis for evaluating the real localization of the underlying sources (Michel et al., 2004).

In this study, the brain was modeled by a complex, five-concentric-sphere model (Zhang, 1995) with a single sphere for each layer corresponding to the white matter, grey matter, cerebral spinal fluid (CSF), skull and skin. In this multilayer anisotropic spheres the innermost shell is considered to be anisotropic. If the lead field is assigned due to a dipole that lies either in the center or in the surface of the sphere, it can be estimated correctly without any structural bias (Zhang, 1995).

The volume conductor model was created using standard T1 magnetic resonance images. The resulting template model was then warped onto the standard head model. The head was modeled by entering the radius and the position of the sphere with the standard electrode locations.

In the next step, in order to map the current dipoles in the human brain to the voltages on the



scalp, lead-field matrix (LFM) was calculated. The LFM was estimated using the boundary-element method (BEM) (Fuchs et al., 2002). For a more detailed description of the solution for the forward problem see Muthuraman et al. (2010b). Spherical models were successfully used before to localize brain stem sources in BAEP studies (Scherg and von Cramon, 1985), as well as in earlier studies in tremor patients (Muthuraman et al., 2012a) and in epilepsy patients (Japaridze et al., 2013, Moeller et al., 2013b, Elshoff et al., 2013).

**Inverse solution:** This is the quantitative estimation of the properties of the neural current sources underlying the EEG activity. The neural activity is modeled as a current dipole or sum of current dipoles. To calculate the power and coherence of a predefined location in the brain a linear transformation has to be applied. In this study a spatial filter was used, which discriminates signals based on their spatial origin (Drongelen et al., 1996). Coherence quantifies the linear dependency of two signals in the frequency domain and is normalized between 0 and 1.

The linear constrained minimum variance (LCMV) spatial filter was used which relates the underlying neural activity to the electromagnetic field on the surface. The main aim of the LCMV method (Drongelen et al., 1996) was to design a bank of spatial filters that attenuates activity originating from other locations and only permits signals generated from a particular location in the brain.

The DICS method employed a spatial filter algorithm to identify the spatial power maximum or coherence in the brain for a particular frequency band. It uses a regularization parameter, which determines the spatial extent of source representation. For all analyses the same regularization parameter of  $\alpha = 0.001$  was used so that the regularized DICS does not contain excessive contributions from noise. This value has been shown to yield reliable results in simulation studies and in MEG data (Kujala et al., 2008) and EEG data (Muthuraman et al., 2012a).

The brain region with the strongest power in a specific frequency band can be used as a reference region for further coherence analysis (Gross et al., 2001). The spatial filter was applied to a large number of voxels covering the entire brain to create topographic maps. Therefore the used voxel size was 5 mm. The individual maps of coherence were spatially normalized and interpolated on a standard T1 brain in SPM2.

The brain source with the strongest power in the predominant frequency range of 1–4 Hz was

estimated and defined as the reference region for further coherence analysis between brain areas. Coherence is measured between 0 and 1. The coherence of a reference region with itself is per definition always 1, consequently the reference region was projected out of the coherence matrix for evaluating further coherent brain areas. In the following process, the second strongest coherent source was considered as noise in order to locate the next correlated source. Then the same was done with the next strongest source and so on. Because there were no more disturbances from noise from the strongly correlated next source in the delta frequency band, it was possible to find even very weak sub-cortical sources, which are otherwise obscured by noise. For a description of the spatial filter see Muthuraman et al. (2008). The statistical significance of the identified coherent sources was tested by a within subject surrogate analysis. A Monte Carlo test of 100 random permutations was carried out, and the Monte Carlo p value ( $\alpha = 0.05$ ) was calculated (Maris and Oostenveld, 2007, Maris et al., 2007). This analysis was performed for each patient separately, followed by a grand average of the significant sources across all patients.

### **2.3.4 Directionality analysis**

Coherence analysis only reveals components that are common to two signals in the frequency domain. It cannot detect the direction of information flow between the two signals. In this study we applied renormalized partial directed coherence (RPDC) (Schelter et al., 2009), which is a technique performed in the frequency domain to detect the direction of informational flow from one signal to the other and vice versa. The pooled time course of all the voxel source signals identified in a source were taken for the calculation of the RPDC. This method applies a multivariate (MVAR) modeling approach which uses an autoregressive process to obtain the coefficients of the signals in the frequency band of 1–4 Hz. In order to obtain these coefficients, the correct model order needs to be chosen which is estimated by minimizing the Akaike Information Criterion (AIC) (Akaike, 1974) and gives the optimal order for the corresponding signal (Ding et al., 2000). After estimating the RPDC values, the significance level is calculated from the applied data using a bootstrapping method (Kaminski M, 2001). In this study the open source matrix laboratory (matlab) package ARFIT (Neumaier A, 2001) was used to estimate the autoregressive coefficients from the spatially filtered source signals.

## **3. Results**

### **3.1 Dynamic imaging of coherent sources (DICS)**

#### **3.1.1 Burst Phases**

All of the identified sources, the individual sources as well as the grand average over all sources, were statistically significant with a probability value of 0.0006 according to Monte Carlo random permutation across all the subjects. fig. 3A depicts the grand average over all patients and supp. fig 6, demonstrates the individual results for every single patient. DICS analysis during burst phase showed five coherent sources in every patient in the same regions, with only small differences across the patients with respect to the local maxima of the sources (supp. fig. 6). There are three sources in cortical regions and two subcortical sources, one source in the mesencephalon and one source in the diencephalon.

The source with the strongest power in the delta frequency band of 1-4 Hz was detected bilaterally in the precuneus (BA 39 and BA 7) in all 13 patients. The local maximum of this source varied across the patients (supp. fig. 6). To gain further coherent sources, this first source was defined as the reference region and projected out of the matrix as described before. The second source, which is the one with the strongest coherence with the reference region, was found bilaterally in the somatosensory cortex (BA 2 and BA 3) in all patients. The third strongest source was detected in prefrontal regions bilaterally (BA 9). The next coherent sources was identified in the thalamus (BA 23) bilaterally in eleven patients and unilaterally on the left side in two patients, whereas the last coherent source was determined in the brainstem (BA 25), or more precisely in the mid-brain tegmentum in all thirteen patients.

### **3.1.2 Suppression Phases**

Statistical significance was given for all sources, which were detected during suppression phase, according to Monte Carlo random permutation across all subjects ( $p = 0.009$ ). All in all four coherent sources were found in all patients and there were only small differences across the patients with respect to the local coherence maxima of the sources (supp. fig 6). Three of the four sources had the same localization as the sources during burst phases, although in contrast to burst phases DICS revealed no subcortical sources. The source with the strongest power in the frequency band 1-4 Hz (first source) was detected bilaterally in the precuneus (BA 39 and BA 7) in all 13 patients and was similar to the strongest source during burst phases (see fig. 4A and supp. fig. 6). The second strongest source was found bilaterally in the occipital cortex (BA 17) in eleven patients and unilaterally on the left side in two patients. The third strongest coherence value was detected bilaterally in the somatosensory cortex (third source; BA 2 and BA3). As described above, the location of these sources were analogous to the second sources during the burst phases. The last coherent source were determined bilaterally in prefrontal cortex (BA 9), similar to the third source during the burst phases.

## **3.2 Renormalized partial directed coherence (RPDC)**

### **3.2.1 Burst Phases**

During burst phases RPDC showed that the direction of informational flow was significantly stronger from the posterior regions towards the anterior regions (fig.3B). There was a significantly ( $p = 0.0060.01$ ) stronger information flow from the precuneus (source 1) towards the somatosensory cortex ( $p = 0.009$ ) (source 2) and the prefrontal cortex (source 3) ( $p = 0.004$ ). Furthermore the somatosensory cortex (source 2) showed stronger informational flow ( $p = 0.002$ ) towards the prefrontal cortex. The upward information flow was detected from the brainstem (source 5) towards the thalamus (source 4) ( $p = 0.003$ ) and from the thalamus to the precuneus ( $p = 0.007$ ) rather than vice versa. All cortical regions showed a clear trend of significant information flow from posterior regions towards anterior regions and subcortical sources towards cortical sources. The connections between sources 2 to 5 were not significant.

### **3.2.2 Suppression Phases**

RPDC did not reveal any significant differences in informational flow between the sources. This fact might indicate bidirectional and homogenous flow of information (see fig. 4B). However, all these bidirectional connections were significant in the data-driven bootstrapping method for the RPDC analyses ( $p > 0.1$ ) and TRT analyses revealed strong symmetries.

### **3.3 Comparison between burst and suppression phases**

The relative SNR was not significantly different between the burst and the suppression phases ( $p = 0.59$ ). We compared the source absolute power between the two phases and found that the burst phases were significantly ( $p = 0.009$ ) higher than those during the suppression phases (see Fig. 5). Next, we compared the total interaction strength of coherence between the sources during both phases and found that coherence values during burst phases were significantly ( $p = 0.0006$ ) higher than those during the suppression phases. In this study, the bootstrapping method followed by the TRT analyses underlined the robustness of the above-mentioned results, as any significant causal interaction identified by RDPC were identified as strong asymmetry by the TRT.

Fig. 3: The grand average of the sources during burst phases as revealed by DICS and RPDC

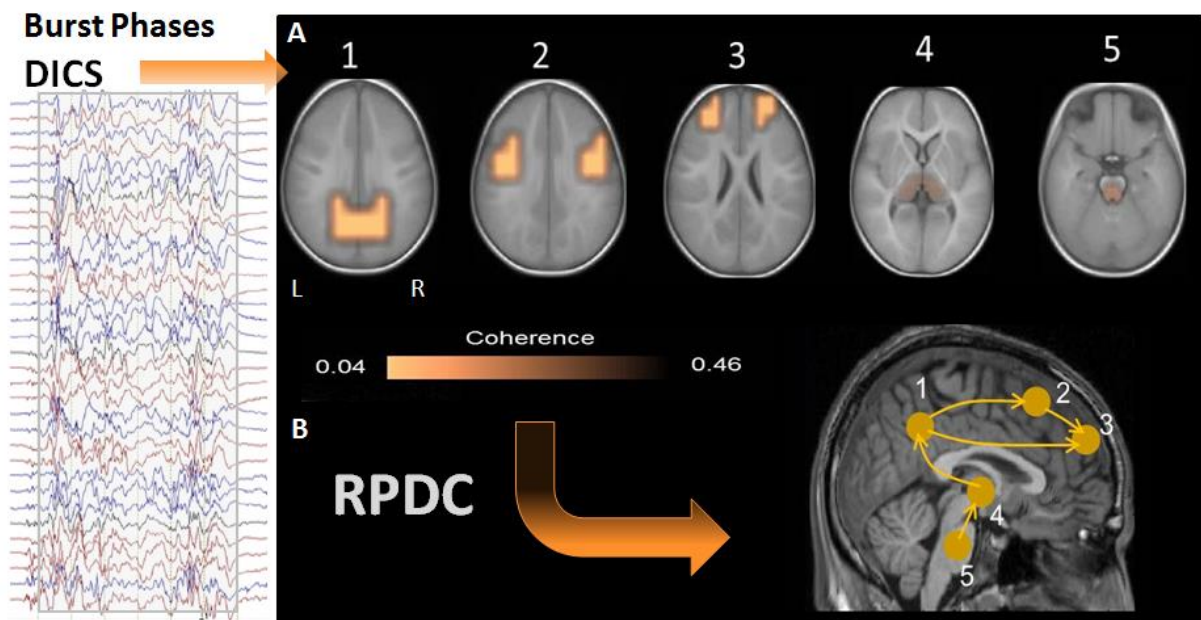


Fig.3. A. Shows the grand average of the sources described by DICS analysis during burst phase. The source of the strongest power at the frequency band 1-4 Hz was detected bilaterally in precuneus in all 13 patients. 2nd Source was bilaterally in the somatosensory cortex in all patients. 3rd Source was detected in prefrontal regions bilaterally; Subsequent sources were detected in the Thalamus (4th Source) bilaterally and the last coherent source was found in the brainstem, in all 13 patients.

B. RPDC during burst phase: showing significantly ( $p=0.003$ ) stronger information flow from precuneus (Source 1) towards somatosensory cortex ( $p=0.001$ ) (second source) and prefrontal cortex (third source) ( $p=0.004$ ) as well as from brainstem (Source 5) towards the thalamus ( $p=0.004$ ) and from thalamus to precuneus ( $p=0.004$ ) rather than wise versa. Also, the stronger RPDC was detected from the somatosensory cortex towards prefrontal cortex.

Fig. 4: The grand average of the sources during suppression phases as revealed by DICS and RPDC

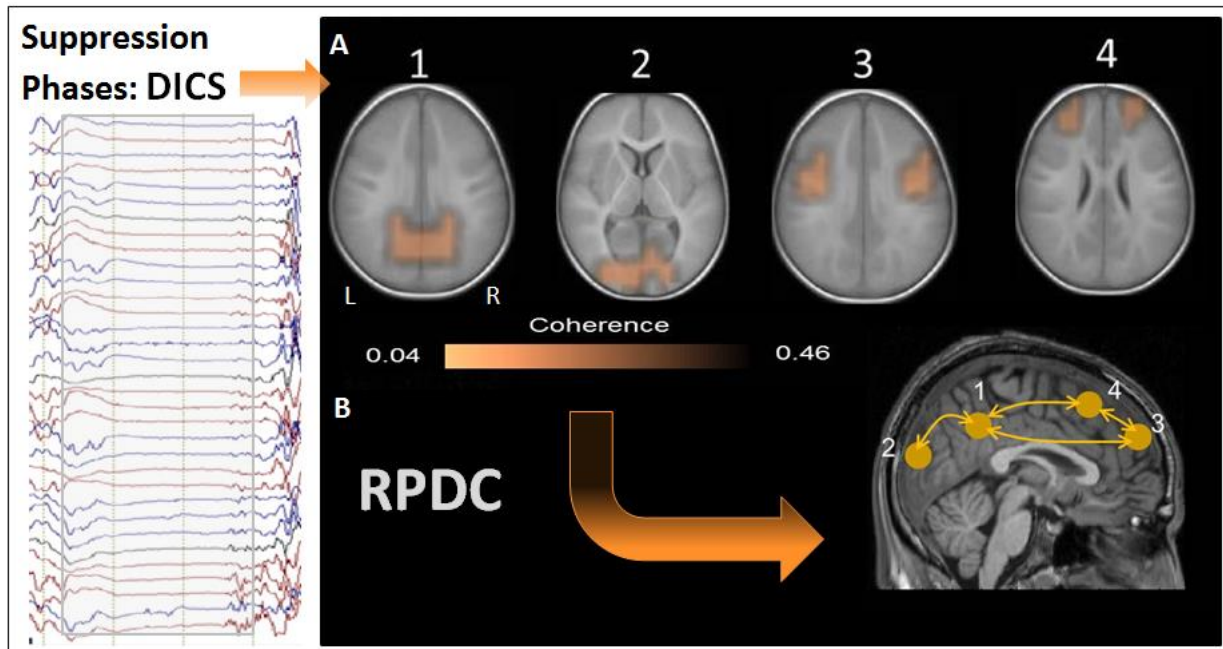
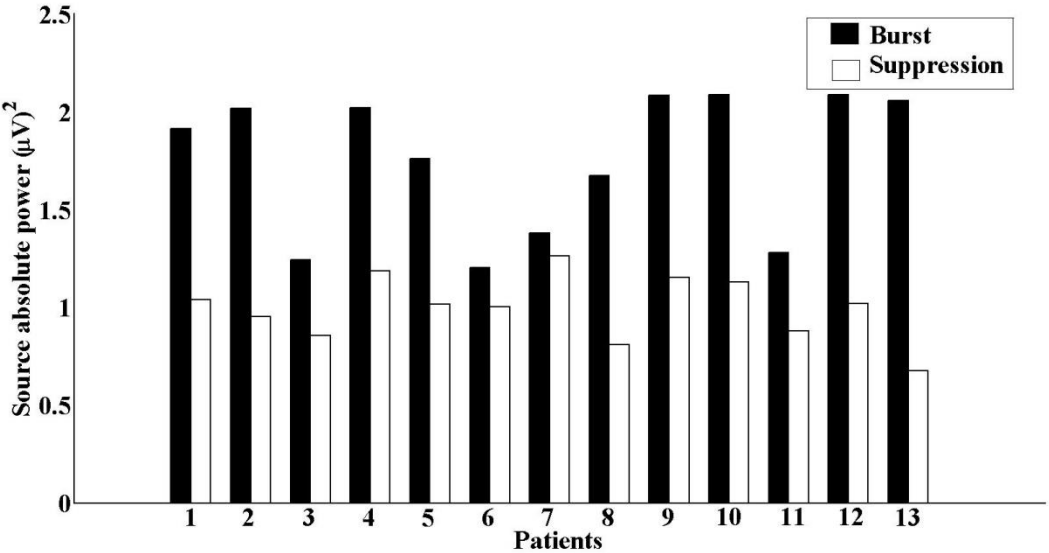


Fig.4. A. Shows DICS results for the suppression phases. The source of the strongest power at the frequency band 1-4 Hz was detected bilaterally in precuneus in all 13 patients (first source) and was very similar to the strongest sources during burst phases. 2nd strongest sources were found bilaterally in the occipital cortex in 11 patients and unilaterally on the left side in two patients. 3rd source detected in somatosensory cortex bilaterally. The last sources were detected bilaterally in prefrontal cortex, similar to the third source during the burst phases. No deep sources were discovered during the suppression phases.

B. RPDC during suppression phases showed no significant differences in information flow between sources. The connections between the sources 1 and 2 ( $p=0.49$ ), 1 and 3 ( $p=0.52$ ), 1 and 4 ( $p=0.32$ ), 2 and 3 ( $p=0.29$ ), 2 and 4 ( $p=0.42$ ), 3 and 4 ( $p=0.29$ ).

Fig.5. Strength of coherence between the sources during both phases showing higher absolute power of the sources during burst phases than during the suppression phases





## 4. Discussion

### 4.1 Functional and effective connectivity during BS

The aim of this study was to describe the dynamics of neuronal networks underlying the BS EEG pattern in neonates and infants with severe encephalopathies. During the suppression phases, source analysis showed that delta activity is characterized by coherent sources in precuneus, occipital cortex, somatosensory cortex and prefrontal cortex, whereas during burst phases, DICS analysis did not display the source in the occipital cortex and demonstrated additional sources in thalamus and brainstem. Moreover, burst phases were characterized by significantly higher source absolute power and mean coherence values between the sources and the informational flow between the identified sources was stronger as compared to suppression phases.

The significant difference in mean coherence values is not due to the difference in power between the two phases, as proven via estimation of the relative SNR on the scalp level. The same network underlying burst and suppression phases was found on both group and individual levels separately. We presume that the described network represents a stable inter-individual pattern of functional and effective connectivity, which does not depend on the etiology, but rather represents a unifying mechanism for BS.

In the context of BS it is often spoken about the central role of the thalamus and a possible thalamocortical deafferentiation (Steriade et al., 1994). The thalamus plays a central role in the presence or absence of consciousness (Schiff, 2010). It is important for the regulation of brain activation during attentive wakefulness. Kinney et al. (1994) investigated the case of a patient who was in vegetative state for ten years. They found thalamic damage, disproportionately high compared to cortical damage. This theory was strengthened by further neurophysiological study of patients in vegetative state in which Adams et al. (2000) reported bilateral death of thalamic neurons and subcortical white matter. Interestingly neuronal death of subcortical brain areas was not associated with neocortical cell death in this study, so conclusively during vegetative state a structural intact cortex is unable to function. These observations indicate that thalamic neurons play a causal role in disorders of consciousness.

According to Vogt and Laureys (2005) there are two major aspects regarding the concept of consciousness. They distinguish between the level of consciousness on the one hand, which is equatable to arousal/vigilance or wakefulness. The second aspect is the content of consciousness, the awareness of the environment and self-reflection. The thalamus receives projections from the brainstem, more precisely from the midbrain reticular formation, which provides a key coupling site between brainstem system for arousal and cortical system for cognitive processing and awareness (Vogt and Laureys, 2005). These upward projections from the brainstem “arousal systems” to the central thalamus control the activity of many cortical and thalamic neurons during the sleep-wake cycle (Schiff, 2010). The thalamus itself, among other brain areas, is connected to posterior cingulate (PCC) and retrosplenial brain areas (RSC) (Vogt and Laureys, 2005). PCC and RSC together with the precuneus (PrCC) form a critical node of the neuronal network correlates of consciousness (NNCC) (Vogt and Laureys, 2005). This is a network of regions of the forebrain, which is of special importance for the coupling between arousal and awareness and has a central position in consciousness processing. Interestingly these areas have the highest level of metabolism in human brain (Vogt and Laureys, 2005).

Precuneus additionally is an important part of the default mode network (DMN) as stated by Raichle et al (2001) by measuring of the brain oxygen extraction fraction (OEF) using positron emission tomography (PET) in healthy subjects. The DMN represents a group of brain regions, which are associated with cognitive and mental processing. The components of the DMN are activated when individuals are involved in internally focused tasks (Li et al., 2014, Buckner et al., 2008). Furthermore the default mode network is considered to be a system subserving self-reflection (Johnson et al., 2002, Moran et al., 2013). Next to the precuneus (PrCC), the posterior cingulate cortex (PCC) and the medial prefrontal cortex (MPFC) play an important role in DMN (Fransson and Marrelec, 2008).

Our study showed that precuneus was the strongest source in both burst and suppression phases, so this area seems to have one of the central positions in the context of BS. The precuneus as a central brain area for consciousness processing is affected during BS. Next to the involvement of the thalamus, this can explain the loss of cognitive impairment. It raises the question whether and why the involved cortical brain areas are more susceptible to dysfunction than others. Is it because of the high level of metabolism, which makes them more vulnerably for malfunction?

The network between precuneus and frontal brain regions comprises a multi synaptic structure

suggesting that it is part of a multiple, specialized processing system with not a single but rather a broad variety of functions (Mantini and Vanduffel, 2013). They ascertain a continuous connection between posterior association processes and anterior executive functions, which represents a high-order cognitive function (Cavanna and Trimble, 2006, Kjaer et al., 2001). Looking at it from a different perspective, other studies showed that during altered states of consciousness like general anesthesia or vegetative state there is a profound deactivation of the above mentioned network (Laureys et al., 2004, Fiset et al., 1999). In this study sources in the thalamus with statistically significant connections towards cortical sources, such as the precuneus, were only detected during burst phases. During suppression phases subcortical structures show no coherence/interaction with the cortical structures. So, thalamocortical projections seem to exist only during the burst phase and seem to be interrupted during suppression phases.

Our findings support the theory developed by Ching and colleagues (2012) that each burst phase can be seen as an attempt to recover normal neuronal dynamics. They constructed a neurophysiological-metabolical model on the cellular level for BS. They also speak for a phasic alteration in synaptic efficiency. The level of energetics in terms of adenosine triphosphate (ATP) has influence on the efficiency of synaptic transmission. According to this study, there is a circular flow of available energetics in proportion to required energetics. During bursts, the ATP-gated potassium channel, which is expressed in cortical and subcortical structures, is activated. This leads to stabilization of cellular membranes and decrease of ATP levels, which prohibits further spiking and thus produces suppression. After recovery of ATP levels and lowered conductivity of the ATP-channel, a next burst can emerge. This cycle produces rhythmicity, because available energetics (ATP) and synaptic transmission show phasic transition.

The disconnection of thalamus from cortex during BS EEG has already been suggested by Steriade and colleagues (1994) by measuring intracellular recordings of cortical and thalamic cells. Although a part of thalamic cells showed rhythmical discharges even during suppression phases, eventually all cells ceased firing as suppression phases became longer than 30 seconds. Furthermore, a functional neuroimaging study of an infant with EME, which used single photon emission computed tomography (SPECT) and (18F)-fluoro-D-deoxyglucose positron emission tomography (FDG-PET), also revealed dysfunction of thalamus and cortical regions (Hirose et al., 2010).

In a study on anesthetized cats, Amzica and colleagues (2007) showed, that during BS the cortex

is in a hyperexcitable state. Even subliminal stimuli are sufficient to raise BS. The height of extracellular calcium levels is important for the generation and maintaining of synaptic events and BS is accompanied by the phasic depletion of extracellular calcium concentration. After being restored by neuronal pumps, an increase of extracellular calcium levels enhances synaptic efficiency and triggers a bursting period, whilst a decrease of calcium levels during burst phases with high synaptic transmission leads to decline of synaptic transmission and produces suppression phases.

In an in vivo experiment on cats BS was induced by isoflurane and this study showed that cortical hyperexcitability during BS is achieved by suppressed inhibition (Ferron et al., 2009). Ferron et al. measured responsiveness of cortical neurons to stimulations of thalamic nuclei using intracellular recordings of cortical neurons. The results displayed that inhibitory processes are much more diminished during BS than excitatory progresses, which leads to imbalance (Ferron et al., 2009).

The study of Lewis and colleagues (2013) on patients with epilepsy under general anesthesia supports the hypothesis of neuronal and metabolic mechanisms of BS as discussed above. They investigated local cortical dynamics during BS with intracranial electrodes, mainly on the neo-cortical level. According to these results, BS is not a homogeneous state of brain and local cortical dynamics vary across time and space. Whilst some cortical regions showed a bursting period, others were in a different state. This perception matches our data, because there are some specific structures being involved, but not the whole cortex as the generalized EEG abnormalities would suggest.

## **4.2. Comparison of the neural networks of epileptic/non-epileptic encephalopathies and West Syndrome**

A common finding is that the predominant frequency was in the delta band. Furthermore neuronal sources were found in both cortical and subcortical regions. West syndrome patients show sources in cortical (occipital, parietal, and frontal) as well as in subcortical (lenticular nucleus, brainstem) regions (Japaridze et al., 2013). During BS EEG sources in precuneus, occipital-, somatosensory- and prefrontal cortex together with sources in thalamus and brainstem were found. Remarkably, RPDC in both studies reveals that the effective connectivity between subcortical and cortical structures seems to be important in the genesis of these neuronal disorders. Thus, in our study there is interruption of informational flow during suppression phases indicating a thalamocortical deafferentiation.

In summary it can be said, therefore, that in both studies there is a specific neuronal network that plays a role in the origin of these severe diseases. Subcortical structures and their connectivity to cortical regions have an important role in this context.

## **4.3. Methodological limitations**

A first methodological consideration is, whether it is possible to detect subcortical sources with methods, which are based on recordings of electrical activity of neurons on the scalp level. As discussed above the inverse problem complicates source analyses and may lead to blurred localization of sources in the brain, as the activity at certain electrodes do not directly predict the position of its underlying generator. There are several configurations of sources that can generate the same pattern of electrical activity on the surface. However DICS has proven that it is able to reveal brain sources in subcortical regions such as the thalamus and brainstem. Previous MEG (Gross et al., 2001, Gross et al., 2002, Sudmeyer et al., 2006a, Timmermann et al., 2003b) and EEG (Muthuraman et al., 2012a) based studies revealed subcortical sources by applying DICS to oscillatory signals (e.g. tremor), and also in healthy subjects during isometric contraction (Muthuraman et al., 2012b). Moreover DICS method has also been shown to identify successfully subcortical sources in the thalamus (Moeller et al., 2013b, Elshoff et al., 2013) and the brainstem (Japaridze et al., 2013) in the previous studies based on EEG. There have been also two validation studies with an independent method like EEG-fMRI (Moeller et al., 2013b)

and local field potentials measured simultaneously with EEG from macro electrodes in orthostatic tremor patients (Muthuraman et al., 2010b).

The second methodological limitation is the fact that we have not used realistic head modeling to account for the forward problem. Considering that current study is a retrospective study, MRIs in cases of the investigated patients were not performed according to the requirements necessary for the optimal head modeling (3D T1 and T2 Images and DTI images). In addition, some of the sequences were not fully performed, i.e. parietal or temporal parts were incompletely scanned, which is a substantial obstacle for the head modeling. It is likely that the realistic head modeling would improve the localization power of DICS.

Another limitation of our study could be the fact that we investigated suppression phases, which are phases with a very poor electrical activity and low amplitude in EEG recordings. This can be a limitation, because it poses a challenge for the source analysis algorithm. Nevertheless there was no significant difference of the relative SNR between the burst and the suppression phases ( $p = 0.59$ ). Conclusively the implication of lower electrical activity of suppression phases do not influence the quality of source localization procedure.

Another item of methodological considerations is that we assembled a heterogeneous group of patients. The infants had encephalopathies with different underlying etiologies and all of them were in a distinct stage of disease. Furthermore, they were treated with different antiepileptic medication and they were investigated with different numbers of electrodes and at different time points. However, they all show BS EEG and the same basic neuronal network as revealed by DICS. This was a consequent finding on both the individual and the group levels. We presume that the network represents a unifying mechanism of BS regardless of the etiology, severity or medical treatment. This is in line with other studies, which have shown that a certain EEG pattern, independent of etiological factors, can be represented by a common neuronal networks (Siniatchkin et al., 2010, Japaridze et al., 2013, Moeller et al., 2013b, Moeller et al., 2013a, Siniatchkin et al., 2007).

## 5. Conclusion

In this study, we investigated dynamics within the neuronal networks during BS EEG pattern in neonates and infants with severe epileptic and non-epileptic encephalopathies. Our findings are in line with the results of other studies showing a specific periodicity of neural activity during BS with special emphasis on the involvement of thalamocortical interaction. During suppression phases, there is a complete deafferentiation between subcortical and cortical structures. Especially those brain structures are affected, which are responsible and necessary for cognitive processing. However, each consecutive burst can be considered as a temporary recovery of subcortical and cortical neuronal dynamics. Despite the above mentioned limitations, our findings support the feasibility of the described methodology for the investigation of neuronal networks in infants with severe encephalopathies.

This study gives us further insights into the fundamental dynamics of BS EEG. It helps us to develop a better understanding of pathophysiological mechanisms underlying encephalopathies of very early life.

Further investigation on this topic could improve our knowledge of the etiopathogenesis of these severe diseases and may be of great importance for development of further treatment options in the future.

## 6. Summary

**Introduction:** Burst-suppression (BS) is an electroencephalography (EEG) pattern consisting of alternative periods of slow waves of high amplitude (the burst) and periods of so called flat EEG (the suppression) (Swank and Watson, 1949). It is generally associated with the reduced level of consciousness (Steriade et al., 1994).

**Aim:** The aim of this study was to reveal the neuronal network underlying both burst and suppression phases using a source analysis method: dynamic imaging of coherent sources (DICS) (Gross et al., 2001) and to describe the effective connectivity between the identified sources using renormalized partial directed coherence (RPDC) (Schelter et al., 2009).

**Material/Methods:** DICS was applied separately to the EEG segments of 13 neonates with burst and suppression EEG pattern. Power spectrum analyses were performed to identify the predominant frequencies. The brain area with the strongest power in the analyzed frequency (1-4 Hz) range was defined as the reference region. DICS was used to compute the coherence between this reference region and the entire brain. RPDC was used to describe the informational flow between the described sources.

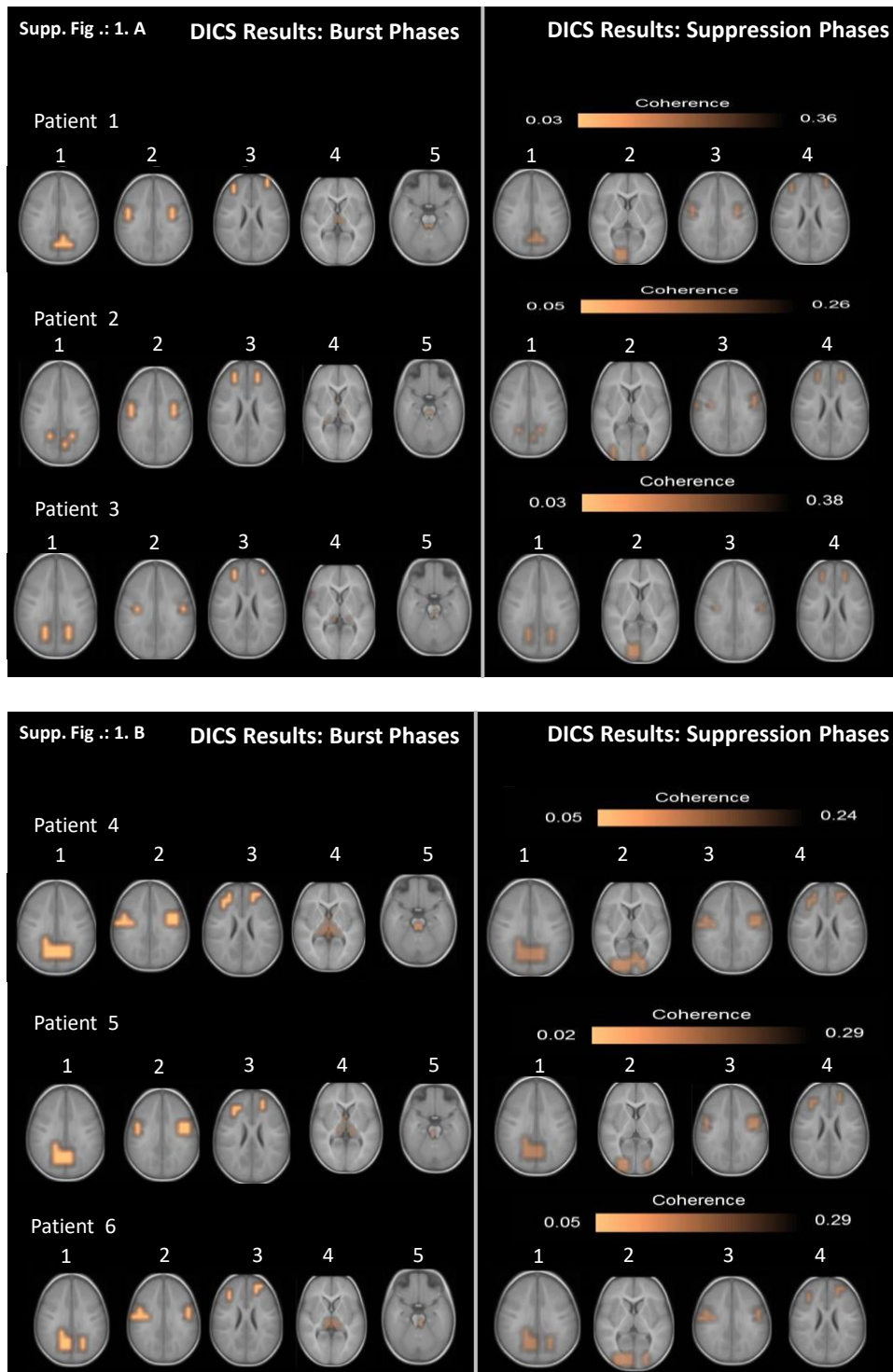
**Results/Conclusion:** Delta activity during burst phases was associated with sources in the thalamus and brainstem as well as bilateral sources in the cortical regions mainly frontal and parietal, whereas suppression phases were associated with coherent sources only in the cortical regions. Results of the RPDC analyses showed an ascending informational flow from the brainstem towards the thalamus and from the thalamus to cortical regions, which was absent during suppression phases. Especially those brain regions were affected, that are important for cognitive processing. The results of this study strengthen the assumption that there is a specific periodicity of neural activity and that thalamocortical deafferentiation is an essential feature of BS. Thus a burst can be understood as short, repetitive recovery of cortical neural dynamics. The described deafferentation may play a role in the poor neurological outcome in these encephalopathies (Beal et al., 2012).

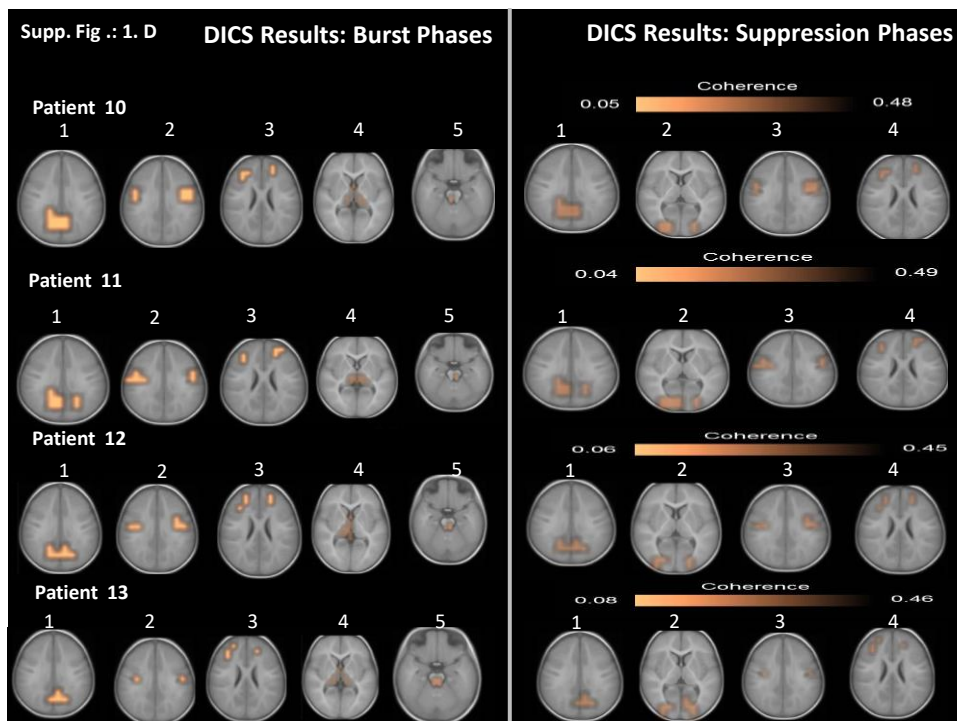
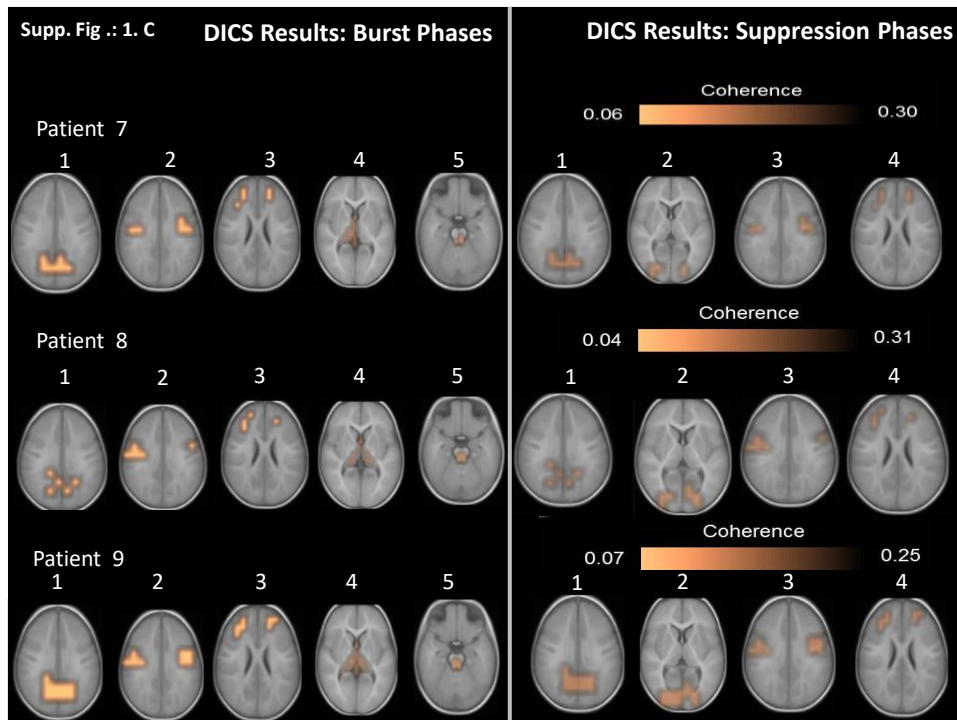


## 7. Appendix

### 7.1 Supplementary figures

Fig.6: Individual results of DICS analysis





DICS analysis results: showing all sources that were described by DICS in each patient, separately for burst and suppression phases, numbered according to the strength and sequence of the identified sources.

**Burst Phases:** the source of the strongest power (Source 1) at the frequency band 1-4 Hz was detected bilaterally in precuneus in all 13 patients. The local maximum of this source varied across the patients. In all the cases, there were four sources coherent with the first source, Sources with the strongest coherence with the reference (Source 2) source were found bilaterally in the somatosensory cortex in the patients. The next strongest sources (Source 3) were detected in prefrontal regions bilaterally; subsequent sources were found in the thalamus (Source 4) bilaterally in eleven patients and unilaterally on the left side in two patients, whereas the last coherent source was determined in the brainstem (source 5), or more precisely in the mid-brain tegmentum in all thirteen patients.

**Suppression phases:** The source of the strongest power (source 1) at the frequency band 1-4 Hz was detected

bilaterally in precuneus in all 13 patients. Second strongest source were found bilaterally in the occipital cortex (Source 2) in eleven patients and unilaterally on the left side in two patients. The next strongest coherence was detected bilaterally in the somatosensory cortex (source 3), the location of these sources were analogous to the second sources during the burst phases. The last coherent sources were determined bilaterally in prefrontal cortex (source 4).

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## **10. Ethical Publication Statement**

I confirm that this report is consistent with guidelines involved in ethical publication.

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