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Are there different mechanisms of synchronization in the course of spike-wave discharges (SWDs) burst development in WAG/Rij rats?

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Abstract. In WAG/Rij rats the pair linear correlation *r* was calculated for bipolar recordings in fronto-temporal, fronto-occipital and occipito-temporal zones of both hemispheres as well as in paleocerebellar cortex (culmen). It was shown that development of SWD bursts resulted in interhemispheric decreases of correlation between the right occipito-temporal cortical region on one side, and left fronto-temporal on the contralateral side. Towards the end of SWD, we found an increased interhemispheric correlation between left fronto-temporal and right fronto-occipital cortical zones, as well as, between both fronto-temporal zones. Paleocerebellum correlates at a weak to moderate level during different periods of SWD burst generation.

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INTRODUCTION

It has been suggested that the promotion of seizure activity is connected with decreased intrinsic "antiepileptic" mechanisms and the cessation of epileptogenesis associated with increased antiseizure defense (Godlevsky et al. 2002, Kryzhanovsky 1986, Shandra and Godlevsky 2005). Spike-wave discharges (SWD) are characteristic of seizure activity in genetic rat models of epilepsy, such as WAG/Rij and GAERS (Manning et al. 2004, Meeren et al. 2004). It is well known that cortical structures, interconnected in the cortico-thalamo-cortical network, play a leading role in the generation of SWD. The cerebellar paleocortex has been also implicated in suppression of seizure activity (Godlevsky et al. 2002, Kryzhanovsky 1986). To address the diverse roles these structures may play in the generation of seizure activity, we have recorded electrocorticograms from distinct cortical areas and determined changes in synchronous activity between pairs of structures during the beginning and cessation of SWD in WAG/Rij rats.

METHODS

Animals

Seven WAG/Rij rats (3 female and 4 male) weighing 180–270 g were used in this study. Rats were housed under standard laboratory conditions (23°C, 60% relative humidity, 12-h light/dark cycles) with food and water available *ad libitum*. All procedures involving animals and their care were conducted in accordance with the European Community Council Directive 86/609.

General surgery

Rats were anesthetized with Nembutal (Pentobarbital®, "Ceva", France, 40 mg/kg, i.p.) and recording electrodes (nichrome wires with outer diameter 120 μm) implanted bilaterally into frontal (all coordinates in mm from bregma; AP=1.5; L=1.8), temporal (AP=-5.0; L=6.0) and occipital (AP=-6.0; L=2.5) zones of cortex according to the stereotaxic atlas of Paxinos and Watson (1998). Bipolar nichrome electrodes (interelectrode distance – 2.0 mm) were implanted into culmen lobule of paleocerebellar cortex under visual control. All electrodes were fixed to the skull with quick-drying dental cement. After surgery, all animals received gentamycine (5 mg/kg, i.p., during five days) to prevent sepsis. As an analgesic Addnok (Buprenorphine®, "Rusan Pharma Ltd", India, 0.02 mg/kg s.c.) was administered immediately after surgery. One week after surgery, rats were handled daily and habituated to the experimental set-up.

ECoG recording and analysis

Bipolar ECoG was recorded with a time constant of 0.3 s from the following brain regions: (1) fronto-temporal (FT); (2) occipito-temporal (OT), and (3) fronto-occipital (FO) (Fig. 1). Corresponding leads in right hemisphere were numerated as (4), (5), and (6). Bipolar recording of paleocerebellar activity was recorded from a seventh lead.

ECoG recording, in freely moving rats, was performed at a sample rate of 256 Hz using PC-based electroencephalograph ("DX-technology", Ukraine). SWD in WAG/Rij rats has been shown to be highly dependent on the stage of vigilance (Coenen 1995, Coenen et al. 1992), therefore, rats were recorded during passive wakefulness.

Pair linear correlation between leads was calculated, using a specially designed MatLab 7.0- based program. The first second of SWD was used for calculating the *r* value, and designated as the beginning of SWD burst. The last second of SWD burst was used to calculate *r* and designated the period of cessation. Selection of SWD for calculations was based of the following requirements: (i) SWD not less than 5.0 s, characteristic of the typical duration of "absence" ECoG in

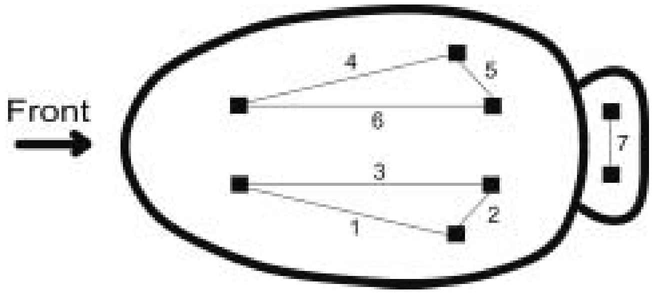

Fig. 1. Schematic location of recording electrodes. (1) fronto-temporal (FT) zone of left and (4) right hemispheres; (3) fronto- occipital (FO) cortical zone of left, and (6) right hemispheres; (2) occipito-temporal (OT) cortex of left, and (5) right hemispheres; (7) paleocerebellar cortex. All recordings were bipolar.

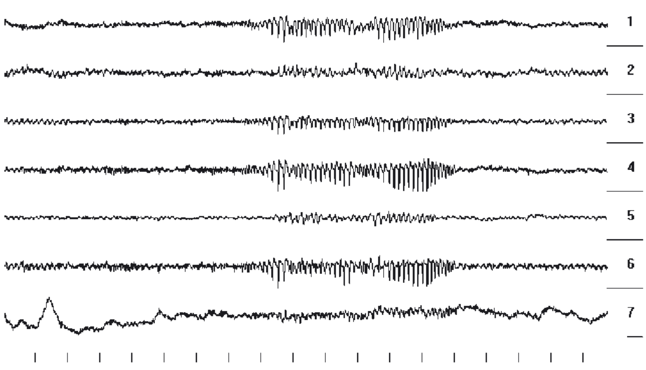

Fig. 2. Typical SWD burst generation in WAG/Rij rat. Notes: rows of EEG are marked by figures (right column, which are in correspondence with those ones marked in Fig. 1). Distance between horizontal short lines represent calibration (1.0 mV), distance between vertical short lines at the bottom of Figure is 1 second.

WAG/Rij rats (Coenen et al. 1992); (ii) well documented, presence of typical "secondary electrophysiological characteristics" associated with absence seizures, in the course of SWD bursts, determined by visual inspection.

ECoG activity was recorded for 60 min, and bursts, used for analysis in study were collected during first spontaneous period of passive wakefulness. Representative recordings from distinct cortical regions are shown in Fig. 2. All coefficients of correlation were averaged in correspondence to data derived from proper matrices of correlation, and values have been expressed as mean ± SEM. Averaged data were analyzed by a one-way ANOVA, followed by Newman–Keuls test. $P<0.05$ was considered significant.

Values of the coefficients of correlation between channels have been compared on a qualitative basis (see Table I).

RESULTS

During the development of SWD, the spikes of maximal amplitude were recorded bilaterally from FT leads and smallest spikes in OT leads (Fig. 2). In the occipital sites, spikes were hardly visible, and only a few rhythmic waves detected (Fig. 2, leads 2 and 5). The wave component became more prominent toward the end of SWD bursts, in agreement with previous studies in WAG/Rij rats (Midzianovskaia et al. 2001). The intraepisode frequency of the SWD ranged from 7 to 10

Table I

Scale for the weighting of *r*

Value of *r*	Qualitative characteristics
>0.9	Very strong
0.7–0.9	Strong
0.5–0.7	Pronounced
0.3–0.5	Moderate
<0.3	Weak

Table II

Interhemispheric coefficients of pair linear correlation between cortical leads during different phases of SWD burst development (mean ± SEM)

	Beginning of SWD burst	Cessation of SWD burst
4–1 (right FT- left FT)	0.57 ± 0.08 (#)	0.77 ± 0.03 * (#)
4–2 (right FT- left OT)	0.25 ± 0.08	0.12 ± 0.03
4–3 (right FT- left FO)	0.59 ± 0.11	0.57 ± 0.11
5–1 (right OT- left FT)	0.34 ± 0.08	0.13 ± 0.04 *
5–2 (right OT- left OT)	0.46 ± 0.10	0.60 ± 0.11
5–3 (right OT- left FO)	0.23 ± 0.09	0.39 ± 0.11
6–1 (right FO- left FT)	0.18 ± 0.07	0.62 ±0.07 ***
6–2 (right FO- left OT)	0.39 ± 0.10	0.39 ± 0.15
6–3 (right FO- left FO)	0.52 ± 0.12	0.81 ± 0.06

Notes: (#) number of observation = 7; all the rest groups are composed on the basis of 6 observations; *$P<0.05$, ***$P<0.001$; ANOVA+ Newman–Keuls test was appropriate

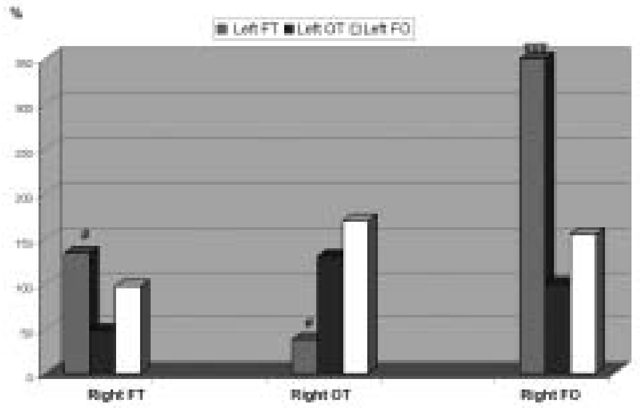

Fig. 3. Dynamics of relative values of interhemispheric coefficients of pair linear correlation calculated between cortical leads in the course of SWD bursts development. Notes: ordinate-value of *r* at the end of SWD burst pertained to *r* value at the beginning of SWD burst (100%). #P<0.05, ### P<0.001 (ANOVA+ Newman–Keuls test).

Hz consistent with findings of others (Drinkenburg et al. 1993, Meeren et al. 2004).

As shown in Table II, the correlation coefficient for interhemisphere FT leads was significantly stronger during cessation of SWD, with respect to the start ($F_{1,13}$=5.48, P<0.05). This represented a relative increase of 34.0% (Fig. 3). In contrast, a significant decrease (62.0%) in the coefficient of correlation was detected at the end of the SWD burst compared to the start for right OT and left FT zones of the cortex (Table II; $F_{1,11}$=5.51, P<0.05). The greatest difference in the value of coefficient of correlation was detected at the end of SWD burst for right FO and left FT cortices (3.5 times, Fig. 3) ($F_{1,11}$=19.76, P<0.001, Table II). It is worth noting that a pronounced but non-significant decrease (52.0%) was found between right-FT and left-OT (Fig. 3, Table II). A trend for an increase (70.0%) of *r* was found between right–OT and left-FO (Fig. 3, Table II), as well as for right- and left-FO (Fig. 3, Table II).

As shown in Table III, cessation of SWD was qualitatively associated with strengthening of the correlative relationships between cortical zones, when compared to a corresponding period at the beginning of SWD.

These data show during the development of SWD bursts the net strengthening of synchronization between fronto-temporal zones and decreased synchronization between fronto-temporal and occipito-temporal zones of cortex of both hemispheres. Our data also indicate a trend for increased synchronization between both fronto-occipital cortices.

Table III

Qualitative characteristics of *r* at different stages of SWD development (interhemispheric relationships)

r quality	Beginning of SWD burst	Cessation of SWD burst
Very strong	-	-
Strong	-	2
Pronounced	3	3
Moderate	1	1
Weak	2	-

Within cortical zones of the same hemisphere, we did not observe any significant differences in correlative strength between the first and last second of SWD bursts. However, in the left hemisphere, a trend for a reduced (45.0%) correlative strength between FT and OT zones was observed towards the end of SWD burst (Fig. 4, Table IV). Also a non-significant increase (40.8%) of *r* at the end of SWD for FT and FO of both hemispheres was detected (Fig. 4, Table IV).

Qualitative estimation of *r* values at the beginning and end of the SWD burst revealed that intrahemispheric coefficient of correlation was moderate at the beginning of bursts, while cessation of SWD burst was characterized by bi-directional deviation from that level (Table V).

There was no tendency for a change in the coefficient of correlation, which remained stable, between cerebellar paleocortex and cortical zones of both hemispheres (Table VI). This, along with the weak or moderate *r* value (Table VII) suggests that the paleocere-

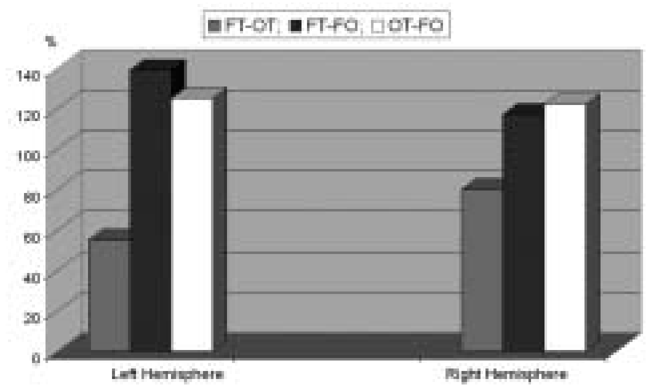

Fig. 4. Dynamics of relative values of intrahemispheric coefficients of pair linear correlation calculated between cortical leads in the course of SWD bursts development. Notes: the same as in Fig. 3.

Table IV

Intrahemispheric pair linear correlation between cortical leads during different phases of SWD bursts development (mean ± SEM)

	Beginning of SWD burst (n=6)	Cessation of SWD burst (n=6)
Left Hemisphere		
1–2 (FT- OT)	0.40 ± 0.11	0.22 ± 0.07
1–3 (FT- FO)	0.49 ± 0.09	0.69 ± 0.05
2–3 (OT- FO)	0.45 ± 0.10	0.56 ± 0.14
Right Hemisphere		
4–5 (FT- OT)	0.34 ± 0.09	0.28 ± 0.08
4–6 (FT- FO)	0.51 ± 0.12	0.59 ± 0.12
6–5 (OT- FO)	0.44 ± 0.12	0.54 ±0.14

Table V

Qualitative characteristics of r at different stages of SWD development (intrahemispheric relationships)

r quality	Beginning of SWD burst	Cessation of SWD burst
Very strong	-	-
Strong	-	-
Pronounced	1	4
Moderate	5	-
Weak	-	2

Table VI

Coefficients of pair linear correlation between cerebellum and cortical leads during different phases of SWD bursts development (mean ± SEM)

	Beginning of SWD burst (n=6)	Cessation of SWD burst (n=6)
Cerebellum-Left Hemisphere		
7–1	0.26 ± 0.09	0.29 ± 0.07
7–2	0.25 ± 0.11	0.20 ± 0.06
7–3	0.29 ± 0.07	0.34 ± 0.07
Cerebellum-Right Hemisphere		
7–4	0.28 ± 0.11	0.28 ± 0.06
7–5	0.23 ± 0.09	0.24 ± 0.08
7–6	0.19 ± 0.05	0.33 ± 0.08

Table VII

Qualitative characteristics of r for cerebellum-cortical relationships at different stages of SWD development

r quality	Beginning of SWD burst	Cessation of SWD burst
Very strong	-	-
Strong	-	-
Pronounced	-	-
Moderate	-	2
Weak	6	4

bellum (culmen zone) does not participate in the development of SWD absence like seizure bursts.

DISCUSSION

The data presented in this study have shown changes in interhemispheric synchronization between cortical zones during the course of SWD burst activity. Specifically, we identified net strengthening of correlation between fronto-temporal and fronto-occipital zones of cortex, towards the end of the SWD. A similar, but not significant increase was observed between occipito-temporal cortical zones. In contrast, we found a weaker correlation during the course of SWD development between fronto-occipital cortex and the contralateral fronto-temporal and fronto-occipital zones. These changes were more pronounced and significant for interhemispheric relationships and less evident intrahemispherically.

Taking into consideration the leading role played by the cortex in generation of SWD, it might be assumed that a dynamic "reconstruction" of the functional role played by frontal, temporal and occipital cortical zones contributes to the genesis of SWD. Thus, strengthening of interhemispheric synchronization of both frontal and occipital regions of the cortex was enhanced, while intrahemispheric relationships between these areas demonstrated the trend to their alleviating. Hence, this activity resembles a type of transversal functional block of intracortical functional links, which underlie mechanisms of SWD bursts generation.

The high level of correlation between similar cortical zones is in agreement with the known peculiarity of SWD in WAG/Rij rats, which are highly synchronous (Coenen et al. 1992). The dynamics SWD frequency, characterized by a higher frequency at the beginning of the burst than the end, is also in favor of an intracortical mechanism, responsible for the pattern of intraepisode discharge evolution (Drinkenburg et al.1993, Meeren et al. 2004).

Since interhemispheric correlative relationships are strengthened towards the end of SWD bursts, it might be assumed that the increased interhemispheric interactions contribute to the generation of an antiepileptic mechanism that suppresses SWD bursts. It has been proposed that the cerebellum may act as an "antiepileptic structure" (Godlevsky et al. 2002). Indeed, Kandel and Buzsaki (1993) described a correlation of unit activity within the cortex and cerebellum with SWD or high voltage spindles in rodents, which support the idea on cerebellum involvement in generation of components of SWD bursts. However, we did not find any change in the correlative relationships between paleocerebellar activity and cortical zones during SWD development. The observed in our investigation absence of correlative relationships between paleocerebellar activity and SWDs might be explained by methodological differences and by specificity of mechanisms of SWD generation in genetic form of absence epilepsy in WAG/Rij rats.

CONCLUSION

In the course of SWD burst development in the WAG/Rij genetic rat model of absence epilepsy a strengthening of synchronization between fronto-temporal zones and decreased synchronization between fronto-temporal and right occipito-temporal zones of cortex was observed. Hence, a transversal reduction of the interhemispheric correlation between frontal and occipital zones of cortex occurs at the moment of suppression of SWD bursts.

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