Cannabis agonist injection effect on the coupling architecture in cortex of WAG/Rij rats during absence seizures

Marina V. Sysoeva[ab], Galina D. Kuznetsova[c], Clementina M. van Rijn[d], Ilya V. Sysoev[eb]

[a]Yuri Gagarin State Technical University of Saratov, 77 Politechnicheskaya str., Saratov, Russia;
[b]Saratov Branch of Institute of Radioengineering and Electronics of RAS, 38 Zelenaya str., Saratov, Russia;
[c]Institute of Higher Nervous Activity and Neurophysiology of RAS, 5A Butlerova str., Moscow, Russia;
[d]Radboud University Nijmegen, Donders Centre for Cognition, Nijmegen, The Netherlands;
[e]Saratov State University, 83 Astrakhanskaya str., Saratov, Russia.

ABSTRACT

WAG/Rij rats are well known genetic model of absence epilepsy, which is traditionally considered as a non-convulsive generalised epilepsy of unknown aetiology. In current study the effect of (R)-(+)-WIN 55,212-2 (cannabis agonist) injection on the coupling between different parts of cortex was studied on 27 male 8 month old rats using local field potentials. Recently developed non-linear adapted Granger causality approach was used as a primary method.

It was shown that first 2 hours after the injection the coupling between most channel pairs rises in comparison with the spontaneous activity, whilst long after the injection (2-6 hours) it drops down. The coupling increase corresponds to the mentioned before treatment effect, when the number and the longitude of seizures significantly decreases. However the subsequent decrease of the coupling in the cortex is accompanied by the dramatic increase of the longitude and the number of seizures. This assumes the hypothesis that a relatively higher coupling in the cortical network can prevent the seizure propagation and generalisation.

Keywords: epilepsy, cannabinoids, Granger causality, time series analysis, local field potentials

1. INTRODUCTION

Absence epilepsy is a form of non-convulsive generalised epilepsy, which aetiology is unknown.[1] Clinically it is expressed in relatively short in time lacks of consciousness, leading to unresponsiveness and interruption of ongoing behaviour. However, absence epilepsy can be easily detected from electroencephalograms (EEGs) due to well pronounced specific type of oscillations — spike-and-wave discharges (SWDs).

Since deeper layers of somato-sensory cortex and a number of nuclei of thalamus play the main role in the SWD generation,[2] the precise and detailed investigation of absence epilepsy from scalp EEGs is not possible and intracranial electrodes are often used. Such a technique became popular owing to two well established genetic rat models of this disease: GAERS (Genetic Absence Epilepsy Rats from Strasbourg[3]) and WAG/Rij (Wistar Albino Glaxo from Rijswijk[4]).

While clinical symptoms of absence epilepsy are not violent, it often tends to change into convulsive forms if not treated. In order to make progress in treatment different kinds of medicines were studied, including drugs which directly affect the cannabinoid system by targeting CB_1 receptors.[5]

The coupling processes between different areas in cortex and thalamus nuclei were shown to be very important for absence seizure development. Various measures were used for this purpose: linear Granger causality,[6] wavelet analysis,[7] non-linear correlation function,[8] time resolved Granger causality.[9] The precursor activity before

Further author information: (Send correspondence to Ilya V. Sysoev)
Ilya V. Sysoev: E-mail: ivssci@gmail.com

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the seizure was found using continuous wavelet transform[10] and using non-linear adapted Granger causality.[11] However the effect of the drugs used for treatment on the brain areas coupling architecture was never studied.

Here, the coupling architecture between cortical areas and hippocampus was studied for spontaneous seizures and for seizures occurring after the CB_1 receptor agonist (R(+)WIN55,212-2) injection. Different seizure phases were considered. Different medicine dozes were used. Results were compared to the results of control group of animals given the oil instead of a drug.

2. ANIMALS AND DATA

27 WAG/Rij rats in edge from 6 to 8 months were studied. Rats were implanted, under complete inhalation anesthesia (isoflurane), with standard tripolar electrode sets (Plastics One MS-333/2-A, Plastic Products, Roanoke, USA). There were stainless steel insulated wire electrodes with non-insulated tip (diameter 0.2 mm). Free electrodes were located in the cortex: frontal, parietal and occipital. One electrode was located in the hippocampus. Recording electrodes were implanted unilaterally at the right hemisphere. Ground and reference electrodes were placed symmetrically over both sides of the cerebellum. Electrodes were permanently attached to the rats' skull with dental cement.

After the surgery, animals were allowed to recover during at least ten days. During this recovery period, animals received post surgery care and their weight was monitored. Upon completion of the recording sessions, rats were deeply anesthetised with overdose of sodium pentobarbital (200 mg/kg i.p.) and their brains were stained with Nissl.

Recordings were performed in freely moving rats. Each recording session lasted about eight hours during the dark period of the day-night cycle. LFP signals were fed into a multi-channel differential amplifier, filtered between 1 and 100 Hz, digitized with frequency 512 Hz (CODAS software). SWDs were detected off-line in the frontal channel using the previously specified criteria.[12]

3. METHOD

Original linear Granger causality method was proposed in application to econometrics,[13] however its modifications are now widely used also in neuroscience and climatology. Recently the non-linear extension of Granger causality with generalised polynomials used as basis functions were proposed.[14,15] The idea of the method is as following.

Given two time series ${x_n}_{n=1}^N$ and ${y_n}_{n=1}^N$ representing two underlying systems X and Y respectively, one has to answer the question: does the system Y drive the system X or not (the opposite question: does the system X drive the system Y can be solved analogously but completely separately, i. e. the method performs directed coupling analysis)? To perform this a number of steps is required:

1. The univariate prediction model (self-model) is constructed from signal ${x_n}_{n=1}^N$, e. g. in the form (1).

$$x'_{n+\tau} = f\left(x_n, x_{n-l}, \ldots, x_{n-(D_s-1)l}\right), \tag{1}$$

where $\mathbf{x}_n = \left(x_n, x_{n-l}, \ldots, x_{n-(D_s-1)l}\right)$ is a state vector reconstructed by means of the method of delays,[16] the components of which are obtained from the same observable time series by shifting it back in time by an interval of l time points $(D_s - 1)$ times; l is a time delay (or lag), and D_s is a univariate model dimension that is actually the number of components in the reconstructed state vector $\{\mathbf{x}_n\}_{n=1}^{N-(D-1)l}$; x'_n is a predicted value corresponding to a measured value x_n, τ is a length of the prediction interval (the prediction length), i. e. the time in data points between the last point used for state vector reconstruction and the predicted point. Model coefficients are estimated using the least-squares routine[17] by minimising the squared prediction error, that measures the difference between the predicted values $x'_{n+\tau}$ and the observed ones $x_{n+\tau}$.

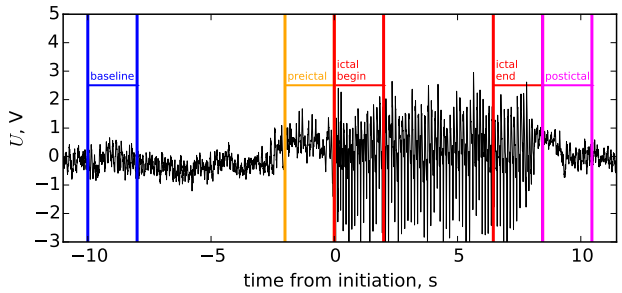


Figure 1. A typical spike-wave discharge measured in parietal cortex. Zero time corresponds to the seizure onset. Five epochs were considered: 1) baseline from 10 to 8 s before the seizure, 2) pre-ictal activity from 2 s before the seizure to the seizure onset, 3) first 2 s of seizure, 4) last 2 s of seizure, 5) post-ictal activity — first 2 s after the seizure.

2. The bivariate model (2) is constructed from both time series $\{x_n\}_{n=1}^N$ and $\{y_n\}_{n=1}^N$:

$$x''_{n+\tau} = g\left(x_n, x_{n-l}, \ldots, x_{n-(D_s-1)l}, y_n, \ldots, y_{n-(D_a-1)l}\right), \tag{2}$$

where D_a is a dimension of the state vector $\mathbf{y}_n = \left(y_n, y_{n-l}, \ldots, y_{n-(D_a-1)l}\right)$ reconstructed from the scalar time series $\{y_n\}_{n=1}^N$ in (2). So the total dimension of the bivariate model can be computed as $D_j = D_s + D_a$, and its prediction error is denoted as ε_j^2.

3. The value of the *prediction improvement* PI (3), that is considered as a main characteristic of the Granger causality method, is computed.

$$PI = 1 - \frac{\varepsilon_j^2}{\varepsilon_s^2} \tag{3}$$

If $\varepsilon_j^2 = \varepsilon_s^2$, this suggests that considering the time series $\{y_n\}_{n=1}^N$ it is not possible to improve the prediction of $\{x_n\}_{n=1}^N$, therefore one has to conclude that Y does not drive X. If $\varepsilon_s^2 > 0$ and $\varepsilon_j^2 \to 0$, providing $PI \to 1$, then the data from $\{y_n\}_{n=1}^N$ exceedingly improve prediction of $\{x_n\}_{n=1}^N$, so Y is said to drive X.

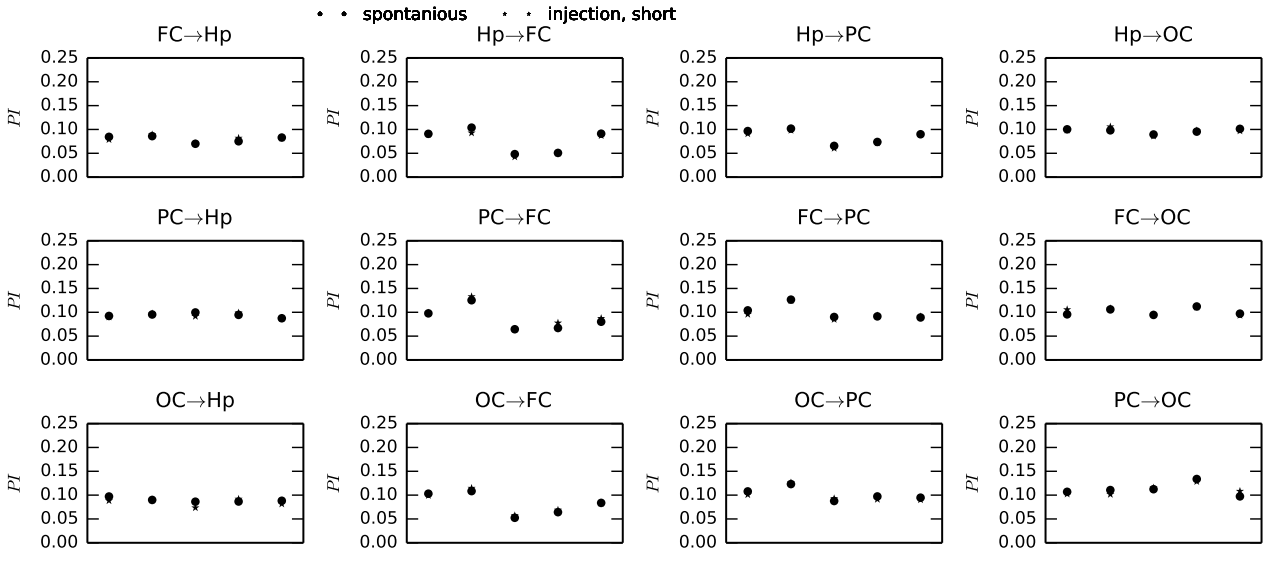
In most real cases the prediction improvement is laying somewhere in the middle between 0 and 1. This can occur due to different factors: inproper models,[18] not enough data (series are too short of sampling time is too large[19]), noises.[20] Therefore such intermediate values has to be tested for significance, since they can be interpreted either as actual coupling, or as a method artefact (see, e. g. "ears" phenomenon[18]).

In many cases the actual value of PI has no matter but its change (increase or decrease) can be interpreted as an increase or decrease of coupling strength in the considered direction. In the current paper we were interested in difference in coupling between the spontaneous seizures and seizures after the drug injection. Therefore the statistical t-test was used to establish the significant difference of the mean PI values.

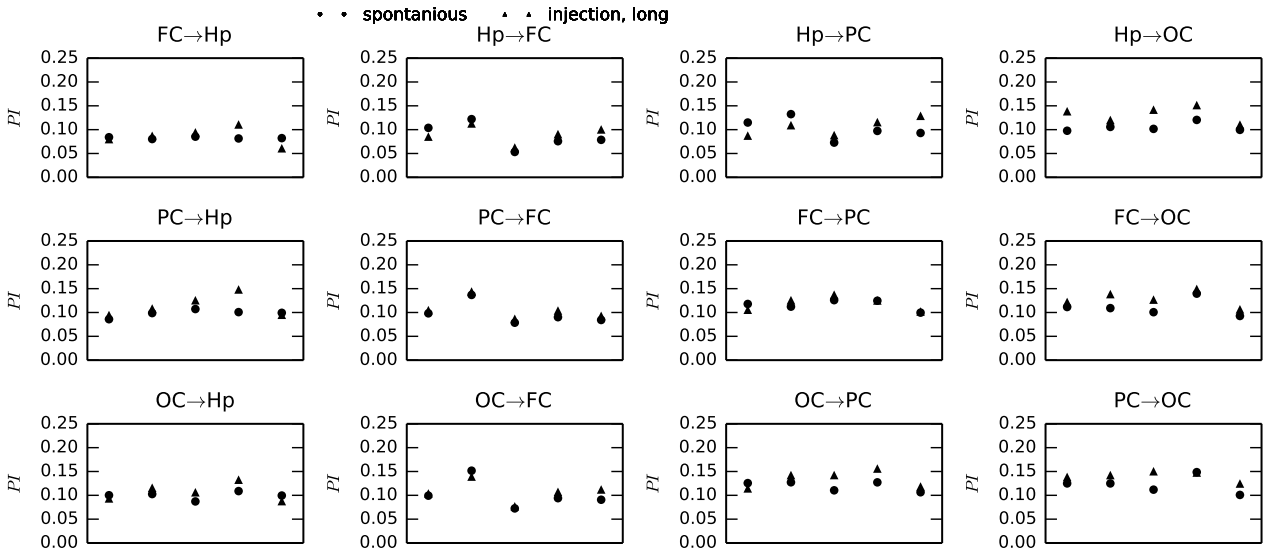
4. RESULTS

In order to be able to average over seizures of different length, all considered seizures were cut into 5 non-overlapping intervals, each being 2 s length, as it is shown on fig. 1. All appropriate seizures were divided into 6 classes based on two criteria. First, time of seizure development was used:

1. spontaneous seizures (before the WIN injection, usually about 130 minutes),
2. early affected seizures (first 120 minutes after the injection),
3. late affected seizures (from 2 hours after the injection till the recording end).



(a)



(b)

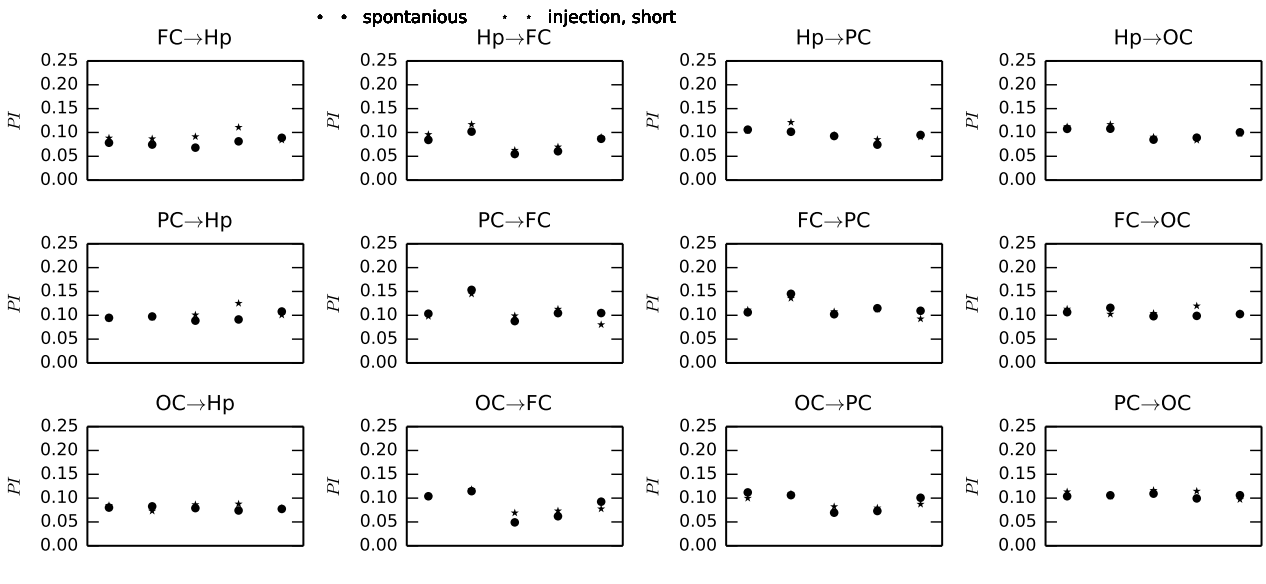
Figure 2. Mean PI values for all rats for (a) — spontaneous short seizures and short early affected seizures for oil control, (b) — spontaneous long seizures and long late affected seizures for oil control.

Second, the seizure length was considered: the seizures of length from 4 to 10 s were split from the seizures of length more than 10 s. To avoid possible problems with comparing seizures of very different length, long spontaneous seizures were compared to the long affected ones, while short spontaneous — to the short affected.

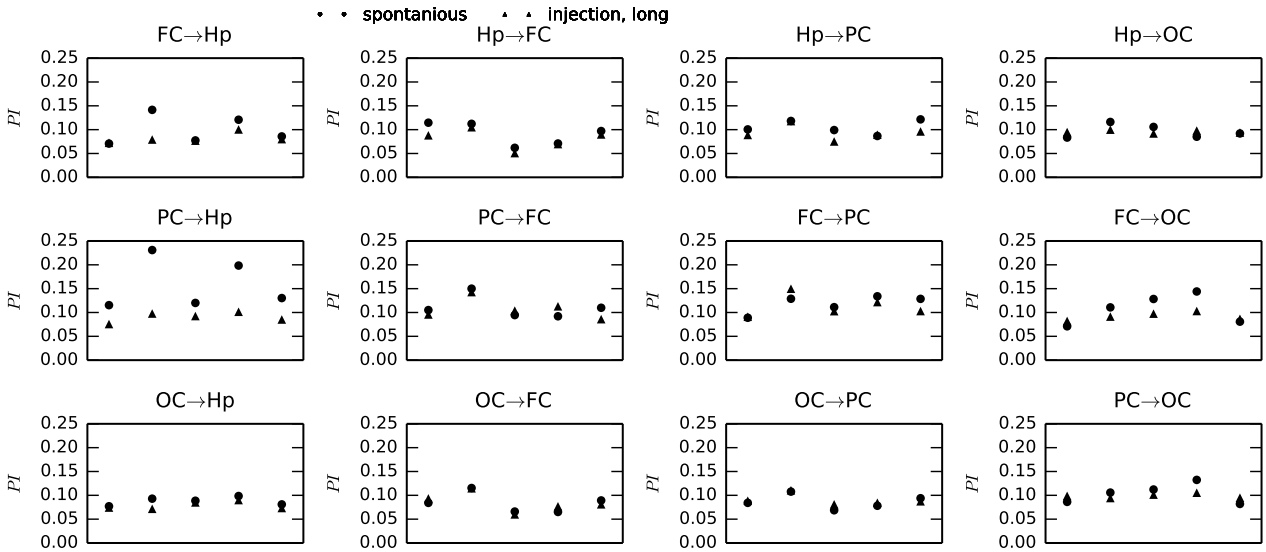
In most considered cases there was no long early affected seizures, so only short early affected seizures were compared to the spontaneous ones. In contrary, most late affected seizures were long, so only long late affected seizures were compared to the spontaneous ones.

In all cases prediction improvement was calculated and then averaged for all seizures of the same rat. Then the averaging over rats was performed, so the impact of an each rat into the resulting value was the same.

Comparison of mean PI values achieved for short seizures before and after the oil injection shows no difference (see fig. 2 a). This ensures that there is no effect due to the oil injection in first 2 hours. The same comparison



(a)



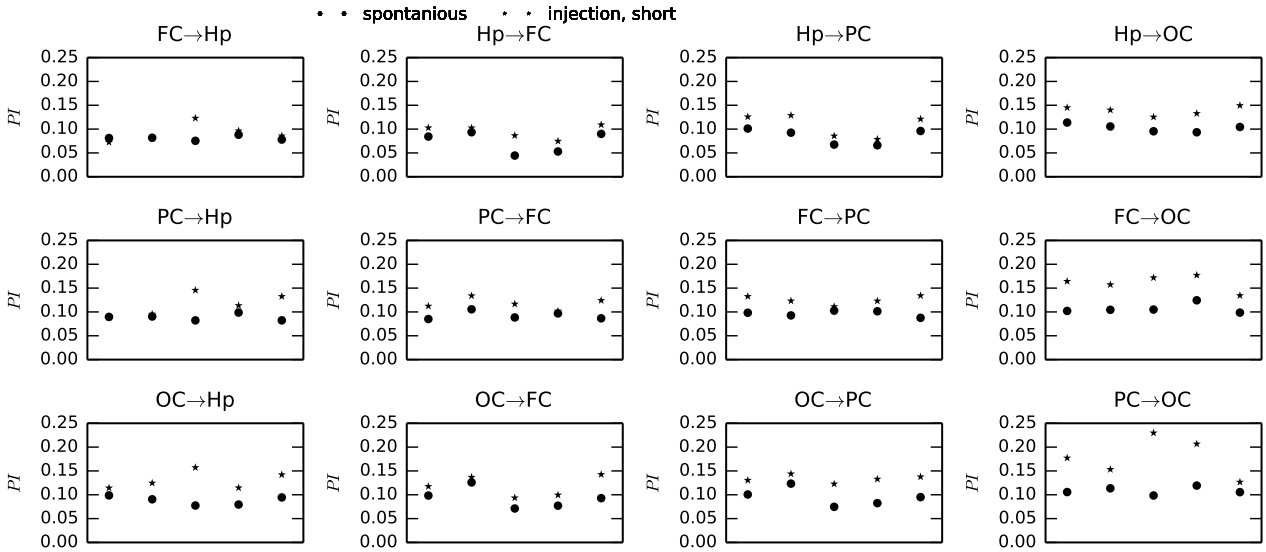
(b)

Figure 3. Mean PI values for all rats. Subplot (a) corresponds to spontaneous short seizures and short early affected seizures for dozes of R(+)WIN55,212-2 equal to 3 mg/kg; subplot (b) — to spontaneous long seizures and long late affected seizures for the same doze

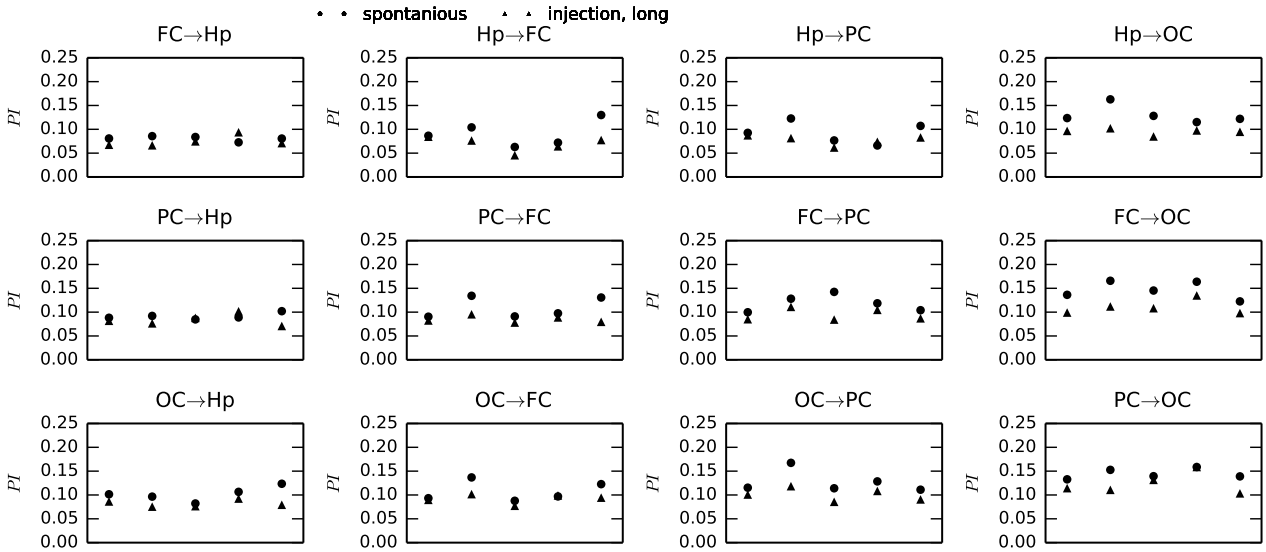
was performed for long seizures before and long after the injection (see fig. 2 b). Some difference here can be mainly explained due to very small amount of seizures of acceptable length.

For short seizures after the drug injection one can see the increase of PI for many different channel pairs. This increase is better expressed for larger dozes: 6 mg/kg (see fig. 4 a) and 12 mg/kg (see fig. 5 a) rather than for 3 mg/kg doze (see fig. 3 a). For baseline level this increase can be seen for most channel pairs except pairs their the hippocampus is drive. Also the ictal phase usually characterised with the largest value of difference between PI for spontaneous and PI for injection affected seizures.

Oppositely, for long seizures long after the injection one can see the decrease in coupling in comparison with the spontaneous seizures (see fig. 3 b, 4 b and 5 b). This decrease is not so well pronounced as the increase mentioned before. For 6 mg/kg (fig. 4 b) the pre-ictal phase is mainly affected, while for the largest doze (12

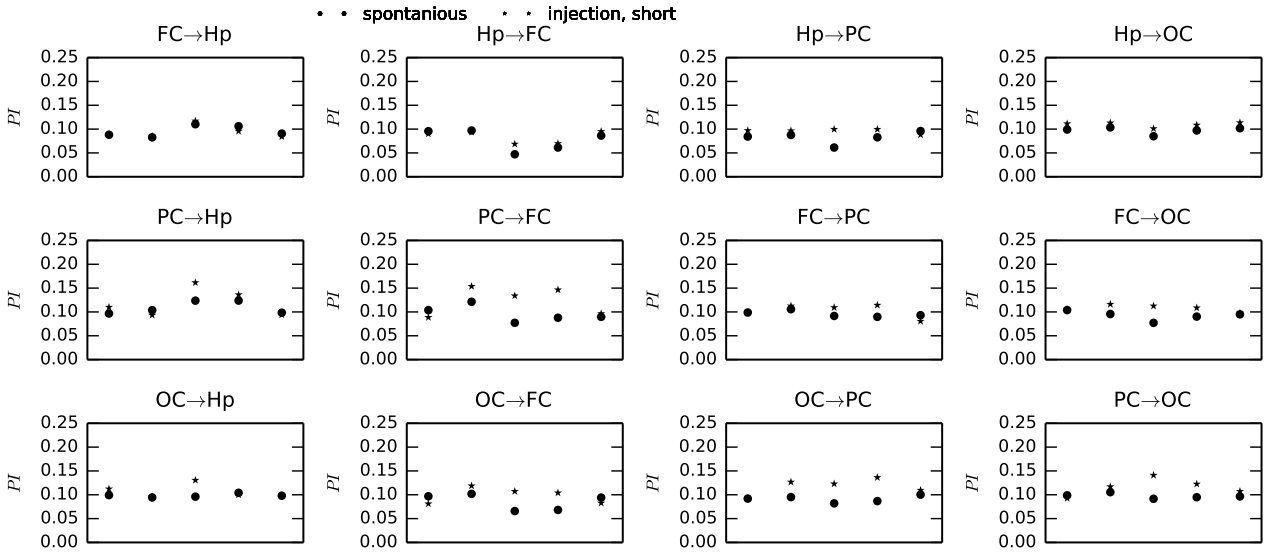


(a)

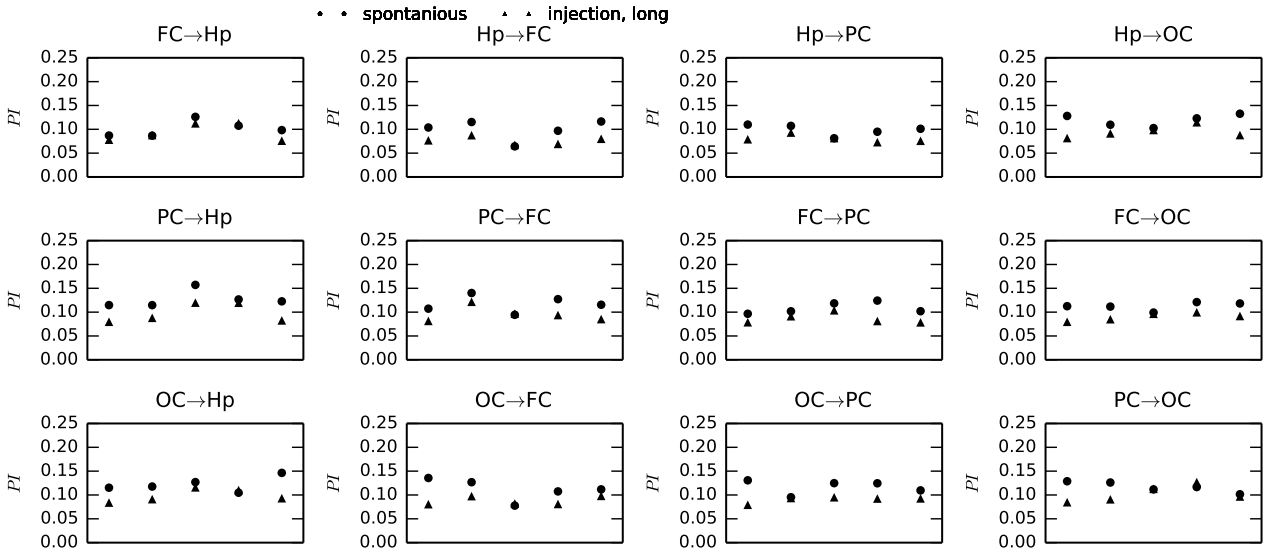


(b)

Figure 4. Mean PI values for all rats. Subplot (a) corresponds to spontaneous short seizures and short early affected seizures for dozes of R(+)WIN55,212-2 equal to 6 mg/kg; subplot (b) — to spontaneous long seizures and long late affected seizures for the same doze



(a)



(b)

Figure 5. Mean PI values for all rats. Subplot (a) corresponds to spontaneous short seizures and short early affected seizures for dozes of R(+)WIN55,212-2 equal to 12 mg/kg; subplot (b) — to spontaneous long seizures and long late affected seizures for the same doze

mg/kg, fig. 5 b) the baseline level also falls.

5. DISCUSSION

The discrepant effect of cannabis agonist (R(+)WIN55,212-2) for absence epilepsy treatment was mentioned previously.[5] For the first 2 hours after the injection one can see the decrease in number and mean longitude of seizures. We have shown that this decrease is accompanied by the rise in coupling strength between different parts of cortex and hippocampus. Oppositely, long after the injection (more than 2 hours) the number and the length of seizures rose significantly. This processes is accompanied by fall in the coupling, as it was shown here.

Based on these results we can formulate the hypothesis that a relatively high level of coupling can prevent seizures to occur (since coupling is increased for baseline and pre-ictal phase) or shorten their length if they still occur (since it is also increased for ictal phases). The relative increase of coupling in cortex and thalamus in the preictal phase is necessary for seizure generation.[11] We can propose that having higher baseline level of coupling, the thalamo-cortical system is less able to increase the coupling at the same relative value due to energetic reasons. The somato-sensory cortex keeps the leading role in the absence seizure generation,[2] therefore the effective seizure spreading can be achieved if it synchronises other cortical area and other brain structures such as hippocampus. However, these area have their own rhythms. If they become more coupled one with another they can resist to somato-sensory cortex dictate more efficiently.

This hypothesis is confirmed by the fact that when the coupling fell lower than the spontaneous level (after 2 hours after the injection), the longitude and the number of seizures greatly increased.

6. ACKNOWLEDGEMENTS

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REFERENCES

[1] Berg, A., Berkovich, S., Brodie, M., Buchhalter, J., Cross, J., van Emde Boas, W., Engel, J., French, J., Glauser, T., Mathern, G., Moshe, S., Nordli, D., Plouin, P., and Scheffer, I., "Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Comission on Classification and Terminology, 2005–2010," *Epilepsia* **51**(4), 676–85 (2010).

[2] Meeren, H., Pijn, J., van Luijtelaar, E., Coenen, A., and Lopes da Silva, F., "Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats," *J. Neurosci* **22**, 1480–1495 (2002).

[3] Vergnes, M., Marescaux, C., Depaulis, A., Micheletti, G., and Warter, J., "Spontaneous spike and wave discharges in thalamus and cortex in a rat model of genetic petit mal-like seizures," *Experimental Neurology* **96**, 127–136 (1987).

[4] van Luijtelaar, E. L. J. M. and Coenen, A. M. L., "Genetic Animal Models for Absence Epilepsy: A Review of the WAG/Rij Strain of Rats," *Behavior Genetics* **33**, 635–655 (November 2003).

[5] van Rijn, C. M., Gaetani, S., Santolini, I., Badura, A., Gabova, A., Fu, J., Watanabe, M., Cuomo, V., Gilles van Luijtelaar, Nicolett, F., and e, y. C. T. N., "WAG/Rij rats show a reduced expression of CB1 receptors in thalamic nuclei and respond to the CB1 receptor agonist, R(+)WIN55,212-2, with a reduced incidence of spike-wave discharges," *Epilepsia* **51**(8), 1511–1521 (2010).

[6] Sitnikova, E., Dikanev, T. V., Smirnov, D. A., Bezruchko, B. P., and van Luijtelaar, G., "Granger causality: cortico-thalamic interdependencies during absence seizures in WAG/Rij rats," *J Neurosci Methods* **170**(2), 245–254 (2008).

[7] Sitnikova, E., Hramov, A. E., Grubov, V. V., Ovchinnikov, A. A., and Koronovski, A. A., "On–off intermittency of thalamo-cortical oscillations in the electroencephalogram of rats with genetic predisposition to absence epilepsy," *Brain research* **1436**, 147–156 (2012).

[8] Lüttjohann, A. and van Luijtelaar, G., "The dynamics of cortico-thalamo-cortical interactions at the transition from pre-ictal to ictal LFPs in absence epilepsy," *Neurobiology of Disease* **47**, 47–60 (2012).

[9] Lüttjohann, A., Schoffelen, J. M., and van Luijtelaar, G., " Termination of ongoing spike-wave discharges investigated by cortico-thalamic network analyses," *Neurobiology of Disease* **70**, 127–137 (2014).

[10] van Luijtelaar, G., Hramov, A. E., Koronovski, A. A., and Sitnikova, E., "Spike-wave discharges in WAG/Rij rats are preceded by delta and theta precursor activity in cortex and thalamus," *Clin Neurophysiol* **122**, 687–695 (2011).

[11] Sysoeva, M. V., Sitnikova, E., Sysoev, I. V., Bezruchko, B. P., and van Luijtelaar, G., "Application of adaptive nonlinear Granger causality: Disclosing network changes before and after absence seizure onset in a genetic rat model," *J Neurosci Methods* **226**, 33–41 (2014).

[12] van Luijtelaar, E. and Coenen, A., "Two types of electrocortical paroxysms in an inbred strain of rats," *Neurosci. Lett.* **70**, 393–397 (1986).

[13] Granger, C. W. J., "Investigating Causal Relations by Econometric Models and Cross-Spectral Methods," *Econometrica* **37**(3), 424–438 (1969).

[14] Chen, Y., Rangarajan, G., Feng, J., and Ding, M., "Analyzing Multiple Nonlinear Time Series with Extended Granger Causality," *Physics Letters A* **324**(1), 26–35 (2004).

[15] Bezruchko, B. P., Ponomarenko, V. I., Prokhorov, M. D., Smirnov, D. A., and Tass, P. A., "Modeling nonlinear oscillatory systems and diagnostics of coupling between them using chaotic time series analysis: applications in neurophysiology," *Physics-Uspekhi* **51**, 304–310 (2008).

[16] Packard, N., Crutchfield, J., Farmer, J., and Shaw, R., "Geometry from a Time Series," *Phys. Rev. Lett.* **45**, 712–716 (1980).

[17] Legendre, A. M., [*Appendice sur la méthodes des moindres quarrés. Nouvelles méthodes pour la détermination des orbites des comètes*], Firmin-Didot (1805).

[18] Sysoev, I. V. and Sysoeva, M. V., "Detecting changes in coupling with Granger causality method from time series with fast transient processes," *Physica D* **309**, 9–19 (Aug. 2015).

[19] Smirnov, D. A. and Bezruchko, B. P., "Spurious causalities due to low temporal resolution: Towards detection of bidirectional coupling from time series," *Europhys. Lett.* **100**, 10005 (2012).

[20] Nalatore, H., Sasikumar, N., and Rangarajan, G., "Effect of measurement noise on Granger causality," *Physical Review E* **90**, 062127 (Dec. 2014).