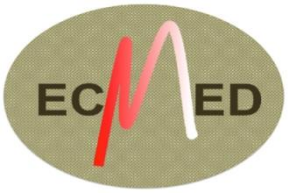


**Grant agreement no. 642881**

# ECMED

## The Extracellular Matrix in Epileptogenesis

***MSCA-ITN-2014-ETN: Marie SkłodowskaCurie Innovative Training Networks (ITN-ETN)***

# D3.5: New data on the mechanisms of epileptogenesis

Work Package: 3

Due date of deliverable: month 32

Actual submission date: 30 / 09 / 2017

Start date of project: January 1st 2015 Duration: 48 months

Lead beneficiary for this deliverable: *IIT*
Contributors: *Fabio Benfenati, Lorenzo Cingolani*

| Project co-funded by the European Commission within the H2020 Programme (2014-2020) | | |
|---|---|---|
| **Dissemination Level: CO** | | |
| **PU** | Public | |
| **CO** | Confidential, only for members of the consortium (including the Commission Services) | **X** |
| **CI** | Classified, as referred to in Commission Decision 2001/844/EC | |



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# Table of contents





*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 1. History table

| Version | Date | Released by | Comments |
|---|---|---|---|
| 1.0 | 29.09.2017 | Fabio Benfenati, Lorenzo Cingolani | |
| | | | |
| | | | |



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 2. Definition and acronyms

| **Acronyms** | Definitions |
|---|---|
| ECM | Extracellular matrix |
| KO | Knock out |
| MEA | MultiElectrode Arrays |
| PPI | Prepulse Inhibition |
| PRRT2 | Proline-Rich Transmembrane protein 2 |
| PSTH | Post-Stimulus Time Histogram |
| SNP | Single Nucleotide Polymorphism |
| | |



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 3. Introduction

Growing evidence exists for a major role of the ECM and its interactors in epilepsy. Associations have been described between ECM protein mutations and epilepsy, as well as changes in the ECM and associated proteins during the development of epilepsy and brain diseases associated with epilepsy, such as stroke, traumatic brain injury, neurodegeneration, schizophrenia or autism spectrum disorders.

In this deliverable (**D3.5**), we address the roles of integrins and PRRT2, receptors and interactors of ECM, in the mechanisms of epileptogenesis. The work here presented set new experimental models for understanding the mechanisms of epileptogenesis, and testing the validity of novel approaches to treat or prevent epilepsy.

**Integrin αVβ3**

Integrin receptors are important for many processes as cell adhesion, migration, cytoskeleton organization, cell proliferation, survival, among others (Hynes, 2002). They are are heterodimers of an α and a β subunit in which both subunits contribute to the specificity of integrin binding to their extracellular ligands (Hynes, 2002). In the brain, αV is the most abundant and broadly expressed α subunit in the neocortex, distributed across all cell layers (Pinkstaff et al., 1999). Interestingly, ablation of αv integrin in glial cells and neurons causes brain hemorrhages and thirty percent of the animals show behavioral defects, spontaneous convulsions and die by postnatal week four. The remaining animals survive several months, but show ataxia, and develop more frequent seizures by six months of age (McCarty et al., 2005). While the αV subunit forms heterodimers with six different β subunits, β3 binds exclusively to αV in the brain (Hynes, 2002). Full knockout of β3 leads to autistic-like phenotypes in mice (Carter et al., 2011a). In humans SNPs and rare mutations in the β3 integrin gene were associated with autism or seizure susceptibility (Schuch et al., 2014). It is therefore expected that αVβ3 integrin plays a role in neuronal circuit modulation.

The reason why deficiencies in αV integrin and some haplotypes of β3 increase propensity for seizures is not yet known. Our goal is to dissect ECM-integrin mediated mechanisms of epileptogenesis.

To this end, we have used mice constitutive KO for β3 integrin, in which β3 integrin is absent throughout development, and β3 integrin heterozygous mice, which express half of the amount of protein found in wild-type animals. Additionally, we have used conditional KO mice for αV integrin, in which αV integrin is ablated exclusively in excitatory neurons of the forebrain starting from the second postnatal week. Our animal



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

models were tested for Startle Response and Pre-pulse Inhibition (PPI), which is presented in this deliverable, giving us an experimental tool to explore the relationship between integrin function and reflexes responses. Moreover, we have characterized excitatory synaptic transmission between pyramidal neurons from the prefrontal cortex in these animal models to explore the physiological mechanism behind altered behavioral responses.

**PRRT2**

Mutations affecting the function or the expression of the PRoline-Rich Transmembrane protein 2 (PRRT2) underlie a group of paroxysmal disorders including epilepsy, kinesigenic dyskinesia and migraine (Ebrahimi-Fakhari et al., 2015). Predictions based on the primary structure showed similarities between PRRT2 and the dispanin family of membrane proteins (Chen et al., 2011), indicating that this protein likely worked as a synaptic adhesion molecule by interacting with proteins of the ECM and/or with extracellular domains of synaptic proteins. More recently, analysis of its exact topology has revealed that PRRT2 is a type-2 membrane protein with a C-terminal anchor, analogous to the SNARE proteins VAMP/synaptobrevin and syntaxin-1 (Rossi et al., 2016).

Acute downregulation and constitutive inactivation of the PRRT2 gene have been used to study the physiological role of PRRT2 protein as well as the pathophysiology of the paroxysmal disturbances associated with PRRT2 mutations. Acute silencing of PRRT2 *in vitro* resulted in synaptic defects including decreased synaptic density and impaired synchronous release at excitatory and inhibitory synapses, probably due to the interactions of PRRT2 with SNARE proteins and the fast $Ca^{2+}$ sensors synaptotagmins 1/2. In the developing brain knocking down PRRT2 by *in utero* electroporation also resulted in a delayed migration of newborn neurons to the cortical plate. Furthermore, the PRRT2-KO mouse summarized the paroxysmal nature of the human PRRT2-related diseases (Liu et al., 2016; Michetti et al., 2017; Valente et al., 2016).

In this work we have monitored the spiking activity of mature primary hippocampal cultures by means of MultiElectrode Arrays (MEA) to analyze the impact of chronic deletion of PRRT2 on the maintenance and stability of synaptic transmission in neuronal networks

# 4. Activities carried out and results



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

**Role of αVβ3 integrin in startle response, pre-pulse inhibition and AMPAR rectification**

Regarding the startle response, a significant statistical difference was found between control and ITGAV KO mice at 110 dB and 120 dB (Control ($ITGAV^{f/f}$; $Cre^{-/-}$), n=43; KO ($ITGAV^{f/f}$; $Cre^{+/-}$), n=32; p=0.0314 for 120 dB and p=0.0262 for 110 dB), but not between ITGB3 WT, HT and KO (WT n=28; HT n=33; KO n=31; ANOVA One-way p=0.4956). Nonetheless, it is possible to identify a trend towards increased startle responses also in this strain, as shown in **Fig. 1A**. The lack of statistical significance may be due to the high variability of the population (mean ± standard deviation for 120 dB: 59.4 ± 30.47 in WT; 69.74 ± 36.16 in HT; 64.65 ± 34.23 in KO) that was not compensated by the relatively large number of experimental animals. Additionally, Grubb's test failed to identify outliers in each group, and the standard error of the means were already small, pointing for a good estimation of the population mean (SEM: WT ±5.76; HT ±6.3; KO ±6.15). Although Startle response and PPI have been considered robust tests (Geyer and Swerdlow, 2001; Swerdlow et al., 2009), there was more variability than expected in our strains. Nonetheless, we were able to identify a significant increased response in ITGAV KO and a similar trend in ITGB3 KO.

Although ITGAV KO leads to a significant increase in startle response, both strains present similar pre-pulse inhibition (**Fig 1B**) relative to their controls (ITGB3: for 90 dB p=0.4779, for 86 dB p=0.2618, one-way ANOVA; ITGAV: for 90 dB p=0.3929, for 86 dB p=0.9006, t-test). Together, these results show that ITGAV KO mice have an increased sensibility to noise stimulus, but are able to appropriately gate this sensory-evoked motor response in the presence of a prestimulus of lower intensity.

AMPA receptors play a pivotal role in excitatory synaptic transmission, and their subunit composition affects greatly their biophysical properties. Namely, GluA2A confers impermeability to $Ca^{2+}$ (Kumar et al., 2002). In fact, more than 90% of GluA2 subunit mRNA is edited in such a way that a glutamine is replaced by an arginine, a positively-charged amino acid, which prevents the passage of highly positively charged molecules such as calcium and polyamines (e.g. spermine). Spermine, when added to the intracellular solution used for electrophysiological recordings, can therefore block AMPA receptors lacking GluA2 at positive holding membrane potentials, thus causing an inward rectification of AMPA receptor current (Pellegrini-Giampietro, 2003). Rectification of AMPA receptor currents is thus a method used to estimate the synaptic content of GluA2-containing AMPA receptors.

The rectification index of AMPAR currents was obtained by measuring AMPAR currents at holding potentials of -70 and +40mV. As shown in **Fig 1C**, pyramidal neurons from



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

P17-P21 wild type mice exhibit a strong rectification of AMPA receptor currents (current ratio (+40/-70): 0.392 ± 0.025). Pyramidal neurons from KO mice also rectified, but to a lesser extent (current ratio (+40/-70): 0.485 ± 0.019). The difference between the absolute values of the ratios is 9.3%, which represents an increase of approximately 24%. The reduction of rectification in KO pyramidal neurons points to a higher amount GluA2-containing receptors, hence to an increased level of $Ca^{2+}$-impermeable AMPA receptors.

**MEA recording from primary PRRT2 KO hippocampal cultures**

Dissociated cultures were prepared from WT and PRRT2-KO hippocampi and plated at high density onto poly-L-lysine-coated MEAs (Axion BioSystems, Atlanta, GA, USA). These MEA plates are composed of 12 wells, each containing an array of 64 nano-porous gold electrodes (30 µm electrode diameter; 200 µm center-to-center spacing). Recordings were collected between the second and the third week in vitro from a total of 95 cultures (46 from PRRT2-KO and 49 for WT, 4 independent preparations).

Spiking activity was recorded for 15/20 min at 37 °C under ambient atmospheric conditions, using Axion BioSystems hardware (Maestro amplifier and Middle-man data acquisition interface) and software (Axion Integrated Studio, version 2.1). After 1,200x amplification, raw data were digitized at 12.5 kHz/channel and stored on a hard disk for subsequent off-line analysis.

For both genotypes, basal activity ranged from isolated spikes to single-channel bursts and synchronous array-wide bursts (raster plots and inset in **Fig. 2A)** that could last from few hundreds ms up to 1 (van Pelt et al., 2004; Suresh et al., 2016). The average network bursting rate in PRRT2-KO cultures was approximately two-fold higher than that in WT cultures, with the former networks generating shorter network bursts with higher intra-burst firing rate (**Fig. 2B**). Consistent with the observation that bursts were the predominant pattern of activity in PRRT2-KO networks (bar plot in **Fig. 2A)**, the burst percentage was significantly higher than in WT networks. The degree of synchronization of bursting activity throughout each network was also expressed in terms of synchrony index (Paiva et al., 2010). The mean synchrony index was significantly higher for mutant cultures than for WT ones, confirming that mature PRRT2-KO networks express a more pronounced synchronization in their bursting behavior (**Fig. 2B**).

To test whether the observed hyperactivity of PRRT2-KO networks was due to an impairment inhibitory transmission, mature cultures were acutely exposed to bicuculline (BIC, 30 µM) to block $GABA_A$ receptor-mediated transmission. In both WT and PRRT2-KO cultures, BIC sharply increased bursting activity, while random spiking was almost



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

completely abolished (raster plots in **Fig. 2C**). To directly evaluate the impact of BIC treatment across genotype, the values of each activity parameter were normalized to the corresponding value before the addition of BIC (BIC/base ratio; right panels in **Fig. 2C**). WT and PRRT2-KO networks displayed qualitatively similar changes in firing rate, bursting rate and burst duration, that were accompanied by significant increases in network synchronization and fraction of spikes within bursts (**Fig. 2C**). However, these effects were more pronounced in mutant cultures, resulting in significantly higher BIC/base ratios for firing rate, bursting rate, burst percentage and synchrony index in PRRT2-KO networks than in WT.

Because all PRRT2-associated diseases share paroxysmal manifestations that involve network instability, we sought to estimate a possible role for PRRT2 in the maintenance of stable neuronal circuits. We thus challenged WT and PRRT2-KO cultures with trains of localized extracellular stimulation, delivered at 0.2 Hz (Chiappalone et al., 2009). For each channel of the dataset, the firing frequency resulting from the 50 stimuli in the train was cumulated over time and used to calculate post-stimulus time histograms (PSTHs). While the response pattern was homogeneous within single networks, marked changes were observed between WT and PRRT2-KO cultures. Temporal analysis of the evoked responses in WT cultures revealed the presence of a short latency window in which the maximum firing probability was reached, followed by a later firing window in which the firing probability progressively decreased (**Fig. 3C**). The spiking activity profile of PRRT2-KO cultures displayed a significantly higher firing probability peak in the early phase with respect to WT networks that sharply decreased in the later phase to become significantly lower than WT networks (**Fig. 3C,D**).

Data are plotted as means ± standard error of the mean (SEM) for WT (black) and PRRT2-KO (red bars) cultures. Statistical analysis was carried out in Prism 5 (GraphPad Software, Inc.) Unpaired Mann-Whitney's/Student's *t*-test was used to compare PRRT2-KO and WT cultures under the same experimental conditions (*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$). Data in Fig. 1A ware analyzed using a Fisher's statistics. Within-genotype effects of BIC treatment were analyzed by either Wilcoxon or paired Student's *t*-test (°$p < 0.05$; °°$p < 0.01$; °°°$p < 0.001$).



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 5. Figures

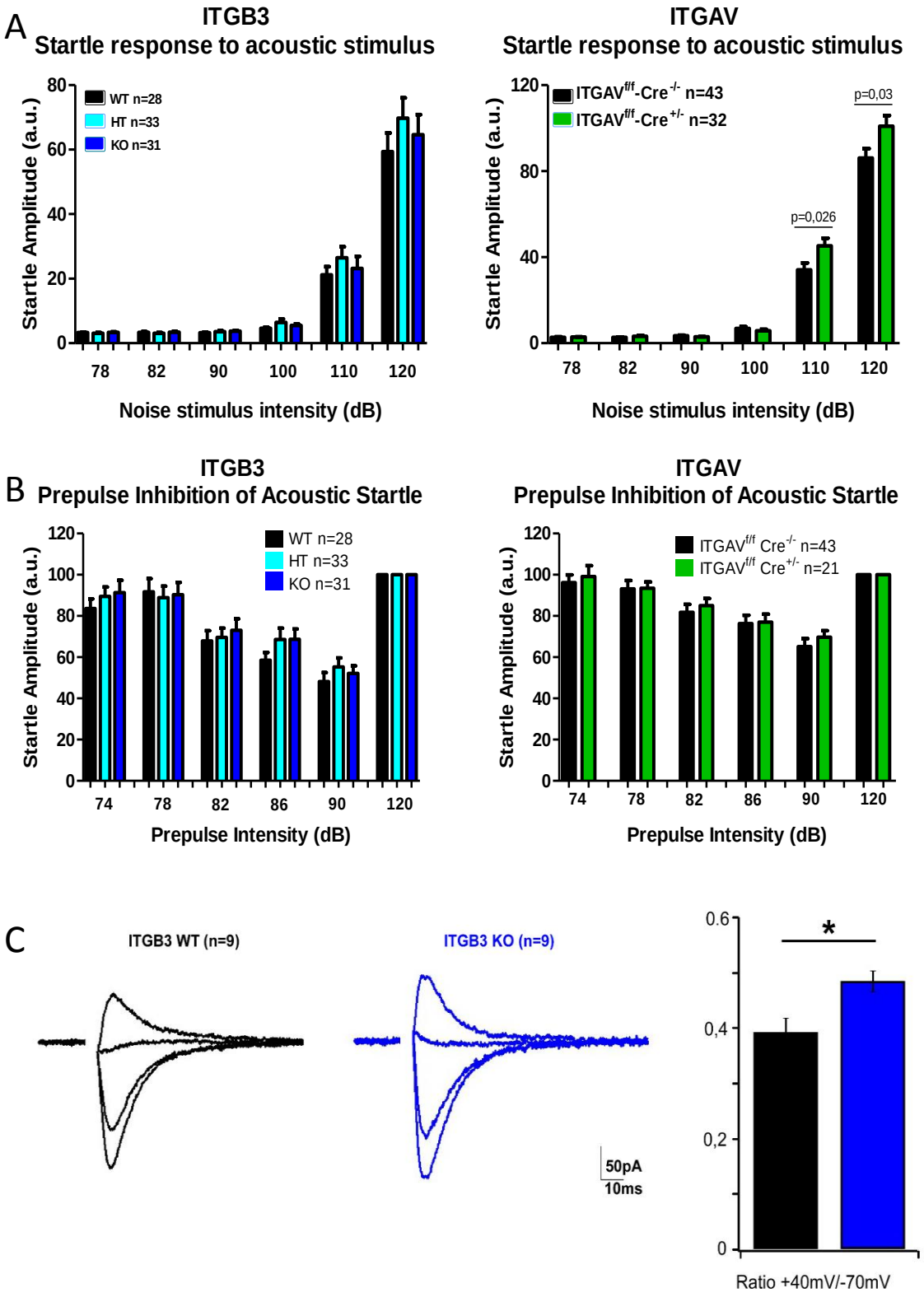


**Figure 1. A**. Startle response for an acoustic stimulus. Each value of intensity was displayed pseudo-randomly six times and the startle response was averaged. ITGAV KO mice show increased startle reflex in comparison to control (p=0,0262 for 110 dB and



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

p=0,0314 for 120 dB). ITGB3 KO mice show no differences as compared to wild type mice (p=0.5168 for 110 dB and p=0.4956 for 120 dB). Mean ± SEM. **B**. Sensorimotor gating of acoustic stimuli. Each pair of stimuli, consisting of a 120 dB stimulation preceded by a pre-pulse of different intensity, was displayed randomly six times and the startle response was averaged. Both strains showed a strong inhibition of startle when the strong stimulus was preceded by a pre-pulse of 82, 86 or 90 dB, but none of the strains showed an effect due to the genotype. Mean ± SEM. **C**. AMPA receptor currents, recorded in P17-P21 mice, rectify significantly less in ITGB3 KO than in WT neurons. AMPA receptor currents were elicited at different holding membrane potentials (-70, -40, 0 and +40mV) in the presence of D-APV (100 µM). The ratio of the amplitude of AMPAR current recorded at +40mV and -70mV was taken as inward rectification index. *Left:* representative traces recorded at holding potentials of -70, -40, 0 and +40 mV. The stimulation artifact was blanked. *Right:* bar graph of the rectification index (black WT n=9; blue KO n=9, *p=0,03).



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

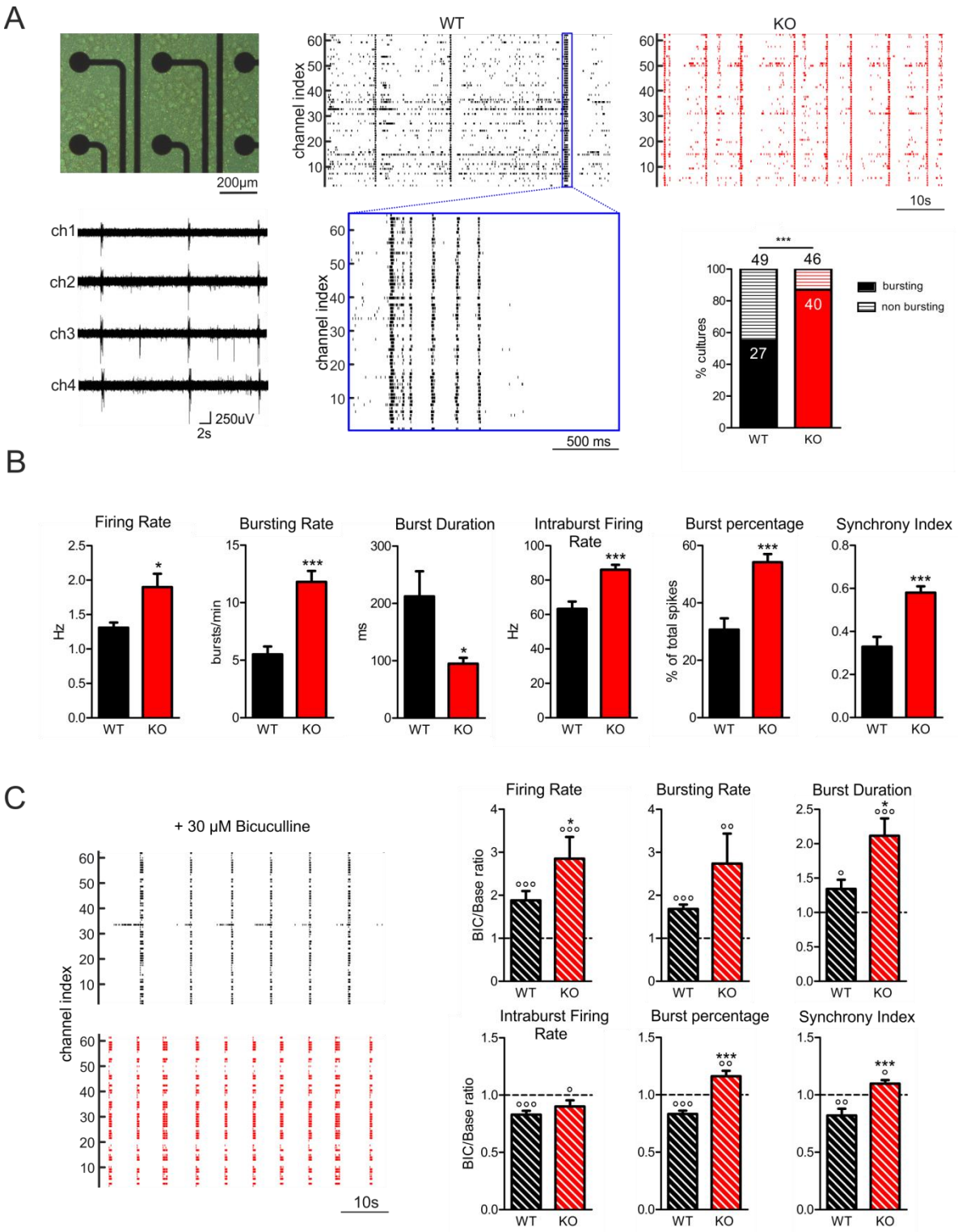


**Figure 2. A**. A light microscope view of a WT network cultured over a MEA at 14 DIV (top left), and spiking activity recorded from 4 electrodes of a well (bottom left). On the right, representative raster plots of the activity recorded from WT and PRRT2-KO cultures over 60 s. The magnification shows spontaneous population bursting generated by a mature WT culture. The bar plot shows the fraction of networks generating population bursts, expressed as a percentage of total cultures of each genotype. **B**. Firing rate, network bursting rate, network burst duration, intra-burst firing rate, burst percentage and synchrony index measured for each experimental group during the third



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

week *in vitro*. **C.** Effect of BIC treatment on the same activity parameters, expressed as ratios between individual values of each network parameter under BIC and the corresponding values under basal conditions (BIC/base ratio).

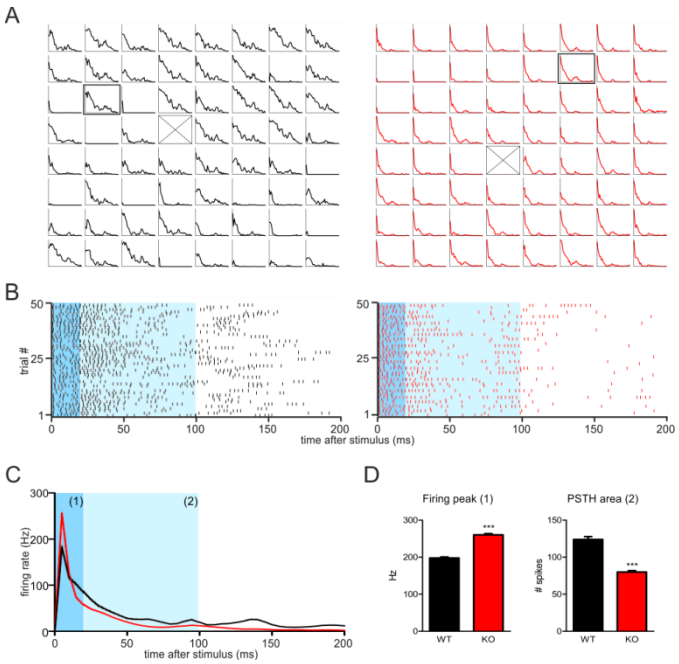


**Figure 3. A**. 8x8 PSTH maps from selected WT (left) and PRRT2-KO (right panel) cultures. Crossed boxes indicate the site of stimulus delivery. **B**. Raster plots of evoked spiking activity recorded from two representative WT and PRRT2-KO channels (boxed in Fig. 2A) over 200 ms after stimulation. **C**. Population mean PSTH of evoked activity averaged over 50 consecutive stimulations for all the active WT (black trace) and PRRT2-KO (red trace) channels. **D**. Bar plot of mean (± SEM) firing peaks and PSTH areas (i.e., overall number of spikes evoked by a single stimulation session) for all the active channels of the dataset.



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 6 Conclusions

ITGAV conditional KO mice had increased startle responses and ITGB3 KO showed a reduction in the rectification of AMPA receptor currents, suggesting an increase in GluA2 content. Future experiments will address more directly the propensity of ITGB3 and ITGAV KO mice for seizures *in vivo*. These tests will be done in collaboration with our partner DZNE (Magdeburg, Germany). At the physiological level, the rectification of AMPA receptor currents will be measured in ITGAV conditional KO mice in order to understand the synaptic roles of αVβ3 integrin and how to manipulate this integrin for anti-epileptic purposes.

PRRT2-KO hippocampal networks grown on MEAs display significant signs of hyperexcitability, resulting in enhanced network synchronization and short-term responsiveness to external stimuli.

The enhancement of the differences between the two experimental groups under $GABA_A$ receptor blockade is consistent with the strengthened inhibitory tone for mutant cells pointed out by our group in patch-clamp recordings. Hyperactivity in mutant networks could then result from an increased synaptic facilitation of excitatory transmission, which is known to deeply affect activity dynamics in reverberant networks (Abbott and Regehr, 2004). Indeed, different population bursting dynamics, and not single-channel bursting dynamics, were observed in the two groups.

Emphasizing the role of PRRT2 in the stability of neuronal networks, mutant cultures sustained evoked spiking at higher frequencies in response to electrical stimulation. Their responses were also more temporally focused, probably due to a faster recruitment of the inhibitory mechanisms.

Future experiments on acute brain slices will better elucidate the role of this synaptic modulator in brain circuits potentially involved in the generation of PRRT2-related paroxysms.



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

# 7 Bibliographical references

Abbott, L.F., and Regehr, W.G. (2004). Synaptic computation. Nature *431*, 796–803.

Carter, M.D., Shah, C.R., Muller, C.L., Crawley, J.N., Carneiro, A.M.D., and Veenstra-VanderWeele, J. (2011a). Absence of preference for social novelty and increased grooming in integrin β3 knockout mice: Initial studies and future directions. Autism Res. *4*, 57–67.

Carter, M.D., Shah, C.R., Muller, C.L., Crawley, J.N., Carneiro, A.M.D., and Veenstra-VanderWeele, J. (2011b). Absence of preference for social novelty and increased grooming in integrin ??3 knockout mice: Initial studies and future directions. Autism Res. *4*, 57–67.

Chen, W.-J., Lin, Y., Xiong, Z.-Q., Wei, W., Ni, W., Tan, G.-H., Guo, S.-L., He, J., Chen, Y.-F., Zhang, Q.-J., et al. (2011). Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. Nat. Genet. *43*, 1252–1255.

Chiappalone, M., Casagrande, S., Tedesco, M., Valtorta, F., Baldelli, P., Martinoia, S., and Benfenati, F. (2009). Opposite changes in glutamatergic and GABAergic transmission underlie the diffuse hyperexcitability of synapsin i-deficient cortical networks. Cereb. Cortex *19*, 1422–1439.

Ebrahimi-Fakhari, D., Saffari, A., Westenberger, A., and Klein, C. (2015). The evolving spectrum of PRRT2-associated paroxysmal diseases. Brain *138*, 3476–3495.

Geyer, M. A, and Swerdlow, N.R. (2001). Measurement of startle response, prepulse inhibition, and habituation. Curr. Protoc. Neurosci. *Chapter 8*, Unit 8.7.

Hynes, R.O. (2002). Integrins: Bidirectional, allosteric signaling machines. Cell *110*, 673–687.

van Pelt, J., Wolters, P.S., Corner. M.A., Rutten. W.L., and Ramakers, G.J.. (2004). Long-Term Characterization of Firing Dynamics of Spontaneous Bursts in Cultured Neural Networks. *51*, 2051–2062.

Kumar, S.S., Bacci, A., Kharazia, V., and Huguenard, J.R. (2002). A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. J Neurosci *22*, 3005–3015.

Liu, Y.-T., Nian, F.-S., Chou, W.-J., Tai, C.-Y., Kwan, S.-Y., Chen, C., Kuo, P.-W., Lin, P.-H., Chen, C.-Y., Huang, C.-W., et al. (2016). PRRT2 mutations lead to neuronal



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

dysfunction and neurodevelopmental defects. Oncotarget *7*, 39184–39196.

McCarty, J.H., Lacy-Hulbert, A., Charest, A., Bronson, R.T., Crowley, D., Housman, D., Savill, J., Roes, J., and Hynes, R.O. (2005). Selective ablation of alphav integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. Development *132*, 165–176.

Michetti, C., Castroflorio, E., Marchionni, I., Forte, N., Sterlini, B., Binda, F., Fruscione, F., Baldelli, P., Valtorta, F., Zara, F., et al. (2017). The PRRT2 knockout mouse recapitulates the neurological diseases associated with PRRT2 mutations. Neurobiol. Dis. *99*, 66–83.

Paiva, A.R.C., Park, I., and Príncipe, J.C. (2010). A comparison of binless spike train measures. Neural Comput. Appl. *19*, 405–419.

Pellegrini-Giampietro, D.E. (2003). An activity-dependent spermine-mediated mechanism that modulates glutamate transmission. Trends Neurosci. *26*, 9–11.

Pinkstaff, J.K., Detterich, J., Lynch, G., and Gall, C. (1999). Integrin subunit gene expression is regionally differentiated in adult brain. J Neurosci *19*, 1541–1556.

Pozo, K., Cingolani, L.A., Bassani, S., Laurent, F., Passafaro, M., and Goda, Y. (2012). β3 integrin interacts directly with GluA2 AMPA receptor subunit and regulates AMPA receptor expression in hippocampal neurons. Proc. Natl. Acad. Sci. U. S. A. *109*, 1323–1328.

Rossi, P., Sterlini, B., Castroflorio, E., Marte, A., Onofri, F., Valtorta, F., Maragliano, L., Corradi, A., and Benfenati, F. (2016). A novel topology of proline-rich transmembrane protein 2 (PRRT2): Hints for an intracellular function at the synapse. J. Biol. Chem. *291*, 6111–6123.

Schuch, J.B., Muller, D., Endres, R.G., Bosa, C.A., Longo, D., Schuler-Faccini, L., Ranzan, J., Becker, M.M., dos Santos Riesgo, R., and Roman, T. (2014). The role of β3 integrin gene variants in autism spectrum disorders - diagnosis and symptomatology. Gene *553*, 24–30.

Suresh, J., Radojicic, M., Pesce, L.L., Bhansali, A., Wang, J., Tryba, A.K., Marks, J.D., and van Drongelen, W. (2016). Network burst activity in hippocampal neuronal cultures: the role of synaptic and intrinsic currents. J. Neurophysiol. *115*, 3073–3089.

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., and Braff, D.L. (2009). NIH Public Access.



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*

Valente, P., Castroflorio, E., Rossi, P., Fadda, M., Sterlini, B., Cervigni, R.I., Prestigio, C., Giovedì, S., Onofri, F., Mura, E., et al. (2016). PRRT2 Is a Key Component of the $Ca^{2+}$-Dependent Neurotransmitter Release Machinery. Cell Rep. *15*, 117–131.



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No **642881**"*