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RESEARCH THESIS PROJECT

WHO GIVES IN FIRST? REGION-SPECIFIC VULNERABILITY TO EPILEPTOGENESIS AND THE ROLE OF THE ENDOGENOUS CORTICAL NETWORK ACTIVITY

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2020

Who gives in first? Region-specific vulnerability to epileptogenesis and the role of the endogenous cortical network activity

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Summary

Distinct brain regions display different seizure thresholds, as evident by epidemiological studies, but previous *in vitro* studies that have attempted to investigate seizure thresholds in different brain regions have yielded conflicting results. Furthermore, studying the connection between slow wave sleep and epilepsy investigation might help elucidate underlying mechanisms of epileptogenesis. Here we used an *in vitro* model of epileptiform activity to examine the seizure threshold of two different neocortical regions (M1, S1BF) and a hippocampal region (CA3). We found that the neocortex exhibits a reduced epileptiform activity threshold compared to the hippocampus, which is strongly associated with the presence of Up state activity. We also examined how the properties of the physiological endogenous cortical network activity change as it transitions to a hypersynchronous paroxysmal state. Our data reveal that under epileptogenic conditions, Up state activity is accelerated, until Up states are eliminated and replaced by epileptiform discharges.

Highlights

- Under epileptogenic conditions, cortical Up states become more frequent and shorter in duration until they disappear and are replaced with epileptiform activity
- Up state activity in the neocortex is associated with reduced threshold for epileptiform activity compared to the hippocampus and the silent neocortex

Keywords

CA3, epileptiform, hippocampus, low Mg^{2+}, M1, neocortex, S1BF, Up state

Abbreviations

(N)REM – (non) rapid eye movement
ACSF – artificial cerebrospinal fluid
CA – cornu ammonis
EEG – electroencephalogram
FED – first epileptiform discharge
GABA – γ-aminobutyric acid
IED – interictal epileptiform discharge
LFP – local field potential
M1 – primary motor cortex
MEA – mature epileptiform activity
mEPSP – miniature excitatory post-synaptic potential
NMDA – N-methyl-D-aspartate
SLE – seizure-like event
S1BF – primary somatosensory cortex barrel field
SWD – spike-wave discharge
SWR – sharp-wave ripple

Introduction

Epilepsy is a spectrum of disorders characterized by recurrent, unprovoked epileptic seizures. (ILAE, 1993). It is one of the most common chronic neurological disorders worldwide, affecting ~1% of the population. About 30% of patients develop pharmacoresistant epilepsy, and 15-25% experience adverse effects from antiepileptic medication (Chen et al., 2017). Epilepsy preferentially affects the young brain, with approximately 75% of all epilepsies appearing during childhood, with the higher incidence during the first year of life. This pinpoints to an increased susceptibility of the developing brain to seizure activity, that could be attributed to the higher number of glutamatergic synapses during the synaptogenesis peak, together with the fact that the neurotransmitter GABA is excitatory in early life (Sánchez Fernández and Loddenkemper, 2014; Stafstrom and Carmant, 2015).

Temporal lobe epilepsy, followed by frontal lobe epilepsy, are the two most common forms of focal epilepsy, thus highlighting the temporal and frontal areas as the most common sources of epileptic activity and as especially vulnerable to epileptogenic insults (Semah et al., 1998). However, *in vitro* studies using several different models have reported that seizure-like events (SLEs) are initiated more reliably in cortical regions than in hippocampal regions (Avoli et al., 2002; Bear and Lothman, 1993; Codadu et al., 2019; Lücke et al., 1995; Shi et al., 2014; Tancredi et al., 1990). The above studies have revealed a significant contribution of the entorhinal cortex to seizure-like activity recorded in the hippocampus, suggesting that the hippocampus might not be as prone to seizure generation as initially thought. This raises the question of whether other, more distant cortical brain regions also display higher vulnerability that the hippocampus itself.

An important aspect of epileptic conditions, that could prove useful in elucidating the underlying mechanisms, is their relation to sleep. Both Interictal Epileptiform Discharges (IEDs) and seizures are activated preferentially during NREM sleep and inhibited during REM

sleep, with IEDs most prevalent in slow wave sleep, and seizures in lighter stages of NREM sleep (St. Louis, 2011). During NREM sleep, the predominant oscillations that can be recorded with scalp EEG or intracranial local field potentials (LFPs) are the slow oscillation (<1 Hz), the delta oscillation (1-4 Hz), sleep spindles (waxing-and-waning 10–16 Hz oscillations), and sharp wave–ripples (SWRs, 100–250 Hz). These sleep related oscillations result from thalamic, neocortical or hippocampal synchronous activity. In particular, neocortical slow oscillations reflect slow alternations of neuronal depolarized (active, "Up") and hyperpolarized (inactive, "Down") states (Steriade et al., 1993) and are considered a predominantly cortical phenomenon, with the thalamus having a role in regulating their properties (Sanchez-Vives and McCormick, 2000). They represent the most highly synchronized activity in the healthy brain, and they are hypothesized to play a role in memory consolidation, synaptic plasticity, cellular homeostasis and downregulation of synaptic strengths (Neske, 2016). Along with the coincidence of certain seizure types with slow-wave sleep, experimental paradigms have demonstrated that seizure activity develops from a gradual acceleration of the slow wave rhythm, reflecting a transition from a synchronous to a hypersynchronous, paroxysmal state of neural activity (Timofeev and Steriade, 2004). Thus, it has been hypothesized that some seizure types could actually be dysregulated Up states (Žiburkus et al., 2013).

A profound problem with epilepsy research is that ictal and interictal activity are not stereotypical, and EEG manifestations of seizures differ depending on the seizure type and/or localization (Fisher et al., 2014). Also, due to the low spatial resolution of the EEG, the exact localization of the epileptic foci is very difficult without surgical intervention. So far, there have been scarce data reporting the epidemiology of epilepsy with respect to the brain region affected, and most studies rely on the symptomatology and EEG picture in order to diagnose the epilepsy disorder and pinpoint to the region(s) affected (Manford et al., 1992). Animal studies are better suited for this kind of research, and brain slice preparations in particular are ideal for isolating and studying different brain regions under identical experimental conditions. Many *ex vivo* studies have characterized epileptiform activity and studied the mechanisms of epileptogenesis in animal brain slices, with most of them focusing on temporal lobe regions, mainly the hippocampus and entorhinal cortex and sometimes containing other parahippocampal regions, such as the subiculum and the piriform cortex (Codadu et al., 2019; Hamidi et al., 2014; Librizzi and De Curtis, 2003; Shi et al., 2014). These regions are usually studied in combined slices, in which all of the regions are contained in one slice and are not anatomically separated. On top of that, the epileptiform activity induction protocols as well as animal species and age are very diverse in the literature. Since the epileptiform signal differs with brain area (Fisher et al., 2014), subject age (Wong and Yamada, 2001) and induction protocol (Antonio et al., 2016; Avoli and Jefferys, 2016), it becomes clear that there is a need for a study that examines various brain regions at different ages under identical conditions, in order to correctly determine regional vulnerability to epileptogenesis.

Here we used an *ex vivo* model of acute mouse brain slices that spontaneously express synchronized network activity in the form of Up/Down states, similar to the slow oscillation activity observed *in vivo* during NREM sleep and certain types of anesthesia (Sanchez-Vives and McCormick, 2000). For the induction of epileptiform activity we implemented the widely used low Mg^{2+} model, which works well in both cortical and hippocampal regions, thus allowing us to study the transition from the physiological endogenous cortical network

activity to paroxysmal synchronization with high temporal resolution due to its slow-induction property (Antonio et al., 2016). Since clinical studies have revealed a clear developmental effect on epilepsy incidence, we conducted experiments on both young and adult mice, and recorded local field potentials from three different brain regions: primary motor cortex (M1), primary somatosensory cortex barrel field (S1BF) and hippocampal Cornu Ammonis 3 (CA3). Our results reveal both regional and age-dependent differences in onset and pattern of epileptiform activity, thus highlighting the importance of such studies.

STAR★Methods

Key Resources Table

REAGENT or RESOURCE	SOURCE
Experimental Models: Organisms/Strains	
Mouse: C57BL/6	Jackson Laboratory
Software and Algorithms	
Axograph X (version 1.3.5)	https://axograph.com
LFP Analyzer	Tsakanikas et. al, 2017 doi: 10.1038/s41598-017-03269-9
SPSS (version 25)	https://www.ibm.com/analytics/spss-statistics-software
Prism	GraphPad https://www.graphpad.com

Experimental Model and Subject Details

Animals C57Bl/6J mice were bred in the animal facility of the Center for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens. The facility is registered as a breeding and experimental facility according to the Presidential Decree of the Greek Democracy 160/91, which harmonizes the Greek national legislation with the European Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The present study was approved by the Regional Veterinary Service, in accordance to the National legal framework for the protection of animals used for scientific purposes (reference number 3372/05-07-2018). Mice were weaned at 21 days postnatally (P21, considering P0 as the day of birth), housed in groups of 2–12, in 267 mm × 483 mm × 203 mm cages supplied with bedding material and kept at a 12–12 dark-light schedule. Food was provided *ad libitum*. For the experiments, male pups (P16-20) and adults (3-6 months old) were used.

Slice preparation Coronal brain slices (400 μm) were prepared from (a) primary somatosensory cortex of the whiskers [i.e., barrel cortex, S1BF; Anterior-Posterior from Bregma (A/P): 0.38 to -1.94 mm, Medial-Lateral (M/L): 2.50 to 4.00 mm], (b) primary motor cortex (M1; A/P: 1.70 to 0.98 mm, M/L: 1.00 to 2.75 mm) and (c) CA3 (CA3; A/P: -1.34 to -2.06 mm, M/L: 0.50 to 2.50 mm) of the right hemisphere. The mice were sacrificed with cervical dislocation, and the brain was rapidly extracted and placed in oxygenated (95% O2–5% CO2) ice-cold dissection buffer containing, in mM: KCl 2.14; $NaH_2PO_4.H_2O$ 1.47; $NaHCO_3$ 27; $MgSO_4$ 2.2; D-Glucose 10; Sucrose 200; and $CaCl_2.2H_2O$ 2; osmolarity (mean ± SD): 298 ± 5 mOsm, pH: 7.4±0.2. Brain slices were cut using a vibratome (VT 1000S, Leica) and placed in

a holding chamber with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 126; KCl 3.53; NaH_2PO_4.H2O 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; D-Glucose 10 and $CaCl_2.2H_2O$ 2 [osmolarity (mean ± SD): 317 ± 4 mOsm, pH: 7.4±0.2], where they were left to recover at room temperature (RT: 24–26°C) for at least 1 h before transferred to the recording chamber.

Ex vivo **electrophysiology** Following recovery, slices were transferred to a modified submerged type chamber (Luigs & Neumann) coated with a layer of transparent silicone onto which up to four slices could be pinned, in order to maximize yield and compare different experimental groups under identical conditions. The slices were gravity-perfused at high flow rates (10–15 ml/min) for optimal oxygenation of the cortical tissue (Hájos et al., 2009). All recordings were performed at room temperature (RT: 24–27°C).

Control recordings were performed in "*in vivo*-like" ACSF (composition same as above, but with 1mM $CaCl_2$). This ionic buffer is thought to better mimic cerebrospinal fluid *in vivo* (Harris et al., 1984; Jones and Keep, 1988) and cortical slices develop spontaneous activity in the form of alternating Up and Down states (Hájos et al., 2009; Rigas et al., 2015; Sanchez-Vives and McCormick, 2000; Sigalas et al., 2015). Recordings were performed after 1 h of incubation in 1 mM [$CaCl_2$] ACSF.

Epileptiform activity was induced with the low Mg^{2+} model, in which the composition of the ACSF was the same as the "*in vivo*-like" ACSF, but no Mg^{2+} ions were added. The timepoint of exchange of the "*in vivo*-like" ACSF with the low Mg^{2+} ACSF was marked as "Mg^{2+} Switch". The slices were perfused for up to 90 minutes to ensure that for pattern of epileptiform activity has become stabilized.

Network activity was assessed by local field potential (LFP) recordings (sampled at 5 or 15 kHz, low-pass filtered at 200 Hz) which were obtained from cortical layers II/III and from CA3 *stratum pyramidale/stratum radiatum* using low impedance (~0.5 MΩ) glass pipettes filled with ACSF. Signals were acquired and amplified (MultiClamp 700B; Molecular Devices), digitized (InstruTech; ITC-18) and viewed on-line with appropriate software (AxoGraph X, version 1.3.5).

Data analysis LFP traces were exported to MATLAB format and analyzed with custom-made MATLAB software (LFP Analyzer) (Tsakanikas et al., 2017). The LFP signal was visually inspected and all events' onset and offset were marked. Each recording session was separated in a control segment (under "*in vivo*-like" ACSF) and three 30 min low Mg^{2+} segments (Early, Intermediate and Late Mg^{2+} Period respectively, adding up to a total of 90 min under low Mg^{2+}). For each of these segments we calculated the average *occurrence* (events/min) and *duration* (seconds) of Up states. For epileptiform activity, we measured the latency (a) to the *first* epileptiform discharge (FED), and (b) to the onset of *mature* epileptiform activity (MEA), for each recording separately (expressed in min after the Mg^{2+} switch). FED was defined as the first instance of epileptiform events, whether or not they had acquired a stable morphology and whether or not they co-existed with Up state activity. MEA was defined as recurring large amplitude epileptiform events, consistent with literature descriptions, that didn't co-exist with Up state activity.

Statistical Analysis Statistical analyses were performed using SPSS (version 25) software. Sample size was defined based on the number of slices. Data were tested for outliers

(assessed by boxplot), normality (Shapiro- Wilk's test of normality), homogeneity of variances (Levene's test of homogeneity of variances), homogeneity of covariances (Box's test of equality of covariance matrices) and sphericity (Mauchly's test of sphericity) where necessary. Appropriate transformation of the dependent variable was performed where necessary. Data that represented extreme outliers (defined as any data points that lie more than 3.0 times the interquartile range below the first quartile or above the third quartile…) were discarded. In cases of departure from sphericity, the *p values* reported are corrected using the Greenhouse-Geisser estimate of sphericity. For multiple comparisons, we performed ANOVA followed by *post hoc* test. For the measurements regarding changes in the dependent variables "*Up state occurrence"* and "*Up state duration"*, we performed a two-way mixed ANOVA with two between-subjects factors (variables "brain region" and "age") and one within-subjects factor ("variable Mg^{2+} Period with 4 levels": (1) Control Period, (2), Early Mg^{2+} Period, (3) Intermediate Mg^{2+} Period and (4) Late Mg^{2+} Period). For measurements regarding the dependent variables related to the latency to the development of epileptiform activity, we performed a two-way ANOVA with two between-subjects factors ("brain region" and "age"). In cases where there was no significant two-way interaction, only the main effects are reported, while in cases of significant two-way interaction the simple main effects are investigated using *post hoc* pairwise comparisons. Statistical significance was set at $p < 0.05$. All data are represented as mean ± SD, unless otherwise stated.

Results

Cortical slices perfused with "*in vivo*-like" ACSF exhibit spontaneous Up and Down states

Both cortical regions (S1BF and M1) exhibited spontaneous activity in the form of short sustained LFP bursts alternated by longer epochs of quiescence (Fig. 1). As we and others have previously shown, these network events correspond to widely synchronous Up and Down states, respectively (Mann et al., 2009; Rigas et al., 2015; Sanchez-Vives and McCormick, 2000). In our experiments, Up state activity in S1BF could be induced more reliably than in M1, in both young and adult mice. S1BF expressed Up state activity in 100% and 61.1% of young and adult mice slices, respectively; while in the case of M1 the respective numbers drop to 61.5% in young and 30.8% in adult mice. This is consistent with previously published work on the development of this type of endogenous network activity (Rigas et al., 2015).

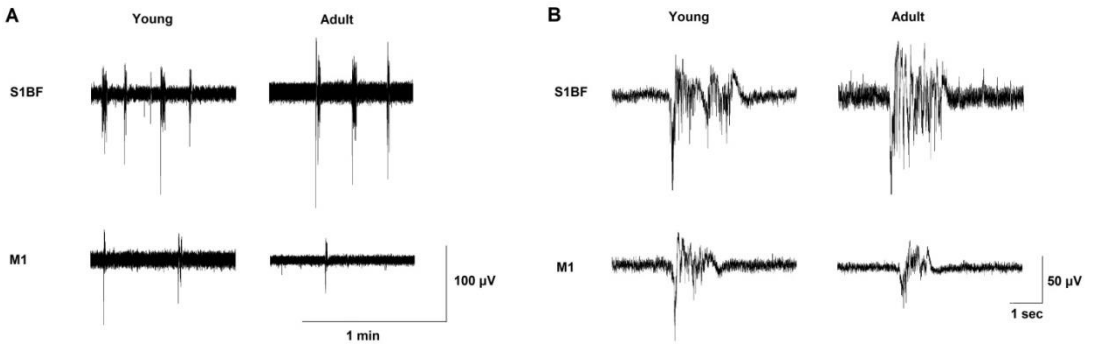

Figure 1. Cortical slices perfused with "*in vivo*-like" ACSF exhibit spontaneous Up and Down states. **A,B.** LFP trances of Up state activity at (A) lower and (B) higher temporal resolution, obtained from young (left) and adult (right) mice, in both cortical regions: S1BF (top traces) and M1 (bottom traces).

In S1BF, Up states of young mice (n = 8 slices) had an occurrence of 1.85 ± 0.49 events/min and a duration of 1.93 ± 0.58 sec, while adult mice Up states (n = 8 slices) had an occurrence of 1.74 ± 0.62 events/min and a duration of 1.51 ± 0.42 sec. In M1, Up states of young mice had an occurrence of 0.52 ± 0.28 events/min and a duration of 1.38 ± 0.39 sec, while adult mice Up states (n = 8 slices) had an occurrence of 0.73 ± 0.32 events/min and a duration of 1.46 ± 0.21 sec.

Up states undergo a gradual transformation under low Mg^{2+} ACSF

After switching to low Mg^{2+} ACSF, Up states exhibited gradual changes in both occurrence and duration, until they were eventually eliminated and replaced by epileptiform events (Fig. 2). To provide a quantitative description of these changes, the recording session was split into 3 equal 30min periods (defined as Early, Intermediate and Late) and Up state properties were measured separately for each period. This analysis revealed a qualitatively similar pattern for both cortical areas and both ages, despite isolated/individual statistical differences (described in more detail below). Specifically, Up states became more frequent during the Early Period, then subsequently decreased to below control levels during the Intermediate period and were eventually eliminated in the Late Period. Up state duration on the other hand, showed a gradual decrease, that started from the Early and continued to the Intermediate Period until Up states disappeared in the Late Period (Table 1).

Table 1. Qualitative description of changes in Up state parameters after switching to low Mg^{2+} ACSF. Arrows indicate direction of change (upward: increase, downward: decrease) and asterisks indicate statistical significance (vs control). Two-way mixed ANOVA,**p<0.01; ***p<0.001. The grey arrow indicates trend for increase which does not reach statistical significance.

Up state occurrence								
		Early	**Intermediate**	**Late**		**Early**	**Intermediate**	**Late**
S1BF	Young	↑***	↓**	↓***	M1	↑	↓***	↓***
	Adult	↑***	↓**	↓***				
Up state duration								
		Early	**Intermediate**	**Late**		**Early**	**Intermediate**	**Late**
S1BF		↓***	↓***	↓***	M1	↓***	↓***	↓***

The next two sections describe in detail the statistical comparison of Up state properties in these 3 periods and the control, for the two ages and the two cortical regions. Two-way mixed ANOVA was used for each cortical region separately, as described in the Methods section.

Network activity in S1BF

Up state occurrence: Statistical analysis revealed a significant two-way interaction between age and Mg^{2+} Period ($F_{(3,42)} = 5.56$, $p = 0.003$, partial $\eta^2 = 0.284$) (Fig. 2B, left). There was a statistically significant effect of Mg^{2+} period on Up state occurrence in both young ($F_{(3,21)} = 74.48$, $p < 0.001$, partial $\eta^2 = 0.914$) and adult ($F_{(3,21)} = 185,66$, $p < 0.001$, partial $\eta^2 = 0.964$) mice. In young mice, Up state occurrence showed a 142% increase during the Early Mg^{2+} Period compared to Control ($p = 0.002$), and a decrease in all other comparisons (Control vs. Intermediate, $p = 0.006$, Control vs. Late, $p < 0.001$, Early vs. Intermediate, $p < 0.001$) and Early vs. Late, $p < 0.001$). The pattern was similar for adult mice, with an even larger increase

(310%) between Control and Early Mg^{2+} Period ($p < 0.001$), and a decrease in all other comparisons (Control vs. Intermediate, $p = 0.002$, Control vs. Late, $p < 0.001$, Early vs. Intermediate, $p < 0.001$ and Early vs. Late, $p < 0.001$). There was also a significant simple main effect of age during the Early Period, with adults exhibiting higher occurrence ($F_{(1,14)} = 11.80$, $p = 0.004$, partial $\eta^2 = 0.457$).

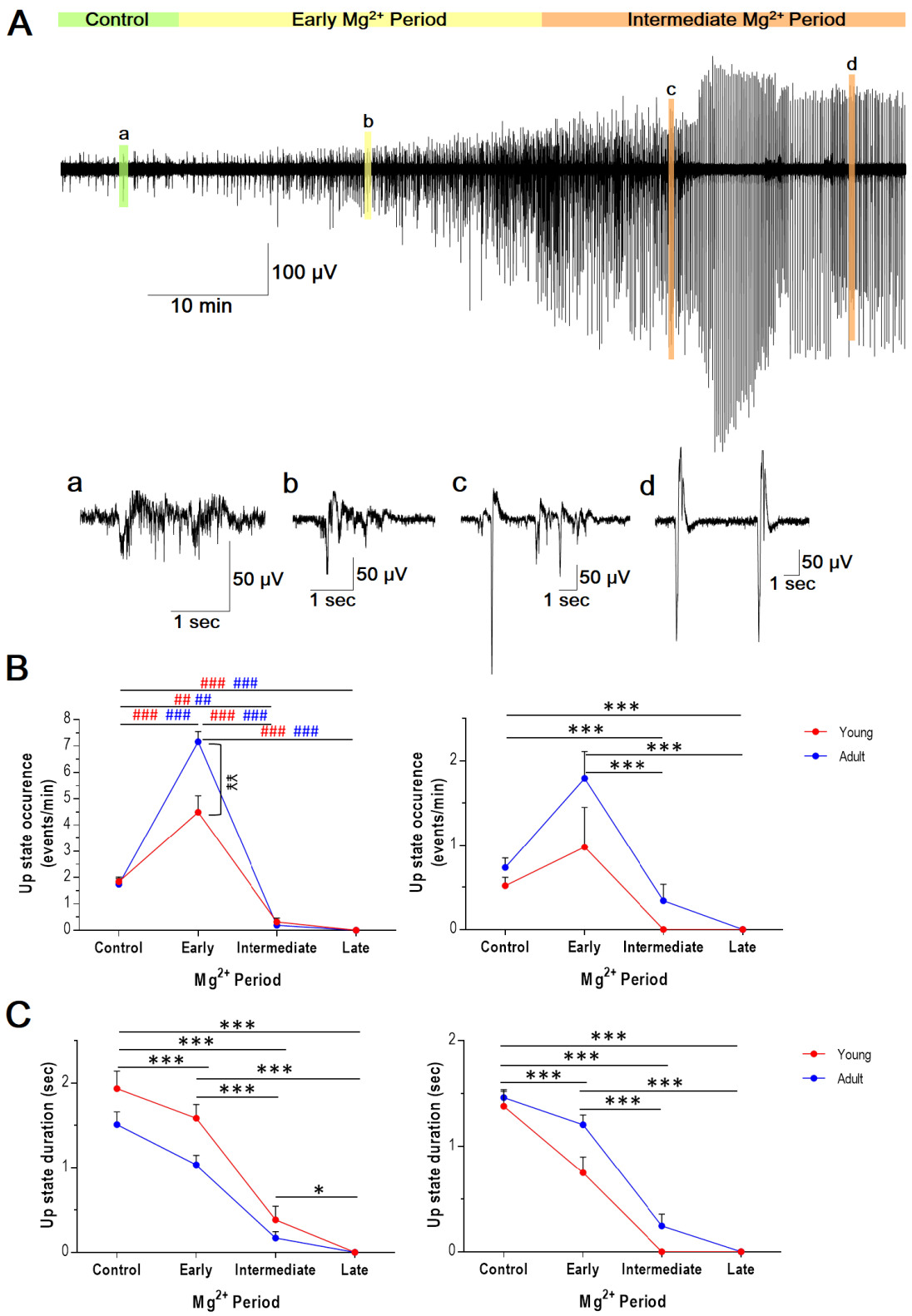

Figure 2. Up states undergo a gradual transformation under low Mg^{2+} ACSF. **A.** Example LFP trace of a young mouse S1BF slice recording showing the Control, Early and Intermediate Mg^{2+} Periods. Underneath, characteristic events of each Mg^{2+} Period are shown in higher temporal resolution. a) A control Up state event, b) an Up state

during the Early Mg2+ Period, c) a premature SWD followed by an Up state, and d) mature epileptiform activity in the form of SWDs. **B.** Effect of low Mg^{2+} on Up state occurrence in S1BF (left) and M1 (right). In S1BF, an initial occurrence increase is observed in the Early Mg^{2+} Period that is much larger in adults. This increase is followed by a subsequent decrease and disappearance of Up states. In M1, no change of occurrence in the Early Mg^{2+} Period is observed. Following, Up states become less frequent and disappear. **C.** Effect of low Mg^{2+} on Up state duration in S1BF (left) and M1 (right). Same overall pattern of transition. Initial mild decrease in duration is followed by subsequent larger decrease and disappearance of Up states. Graphs show mean ± SEM. Red and blue # indicate differences between Mg^{2+} Periods for young and adult mice respectively. ¥ indicates age differences in specific Mg^{2+} Periods (in cases where two-way interaction was observed. Asterisk indicates differences between Mg^{2+} Periods in cases where no two-way interaction was observed (age groups were collapsed). Age differences with collapsed Mg^{2+} Periods (in cases of no two-way interaction) are not represented (see text). Two-way mixed ANOVA, *p<0.05, **p<0.01, ***p<0.001, ##p<0.01, ###p<0.001, ¥¥p<0.01.

Up state duration: Contrary to Up state occurrence, statistical analysis did not reveal a significant two-way interaction between age and Mg^{2+} Period for Up state duration ($F_{(1.81,25.38)}$ = 2.26, p = 0.129, partial η^2 = 0.139) (Fig. 2C, left). The main effect of Mg^{2+} Period showed a reduction in mean Up state duration at the different Mg^{2+} Periods ($F_{(1.81,25.38)}$ = 104.97, p < 0.001, partial η^2 = 0.882), which was statistically significant for all comparisons: Control vs. Early (23.84%, p < 0.001), Control vs. Intermediate (p < 0.001), Control vs. Late (p < 0.001), Early vs. Intermediate (p < 0.001), Early vs. Late (p < 0.001) and Intermediate vs. Late (p = 0.046). The main effect of age showed that young mice have significantly longer Up states ($F_{(1,14)}$ = 6.07, p = 0.027, partial η^2 = 0.302), as previously shown (Rigas et al., 2015).

To sum up, Up states in S1BF exhibit a dramatic increase in occurrence during the period immediately following the Mg^{2+} Switch in both ages, although to a larger degree in adults. This increase is followed by a large (92.87% and 97.35% in young and adults respectively) reduction in occurrence (to below normal levels) and the eventual complete disappearance of Up state activity. Up state duration is affected similarly in both ages, gradually decreasing until Up states disappear. **These results indicate that the mechanisms underlying the Down-to-Up state transition (or the Up state generation) are, at least initially, more strongly affected in adults, while the mechanisms regulating the termination of Up states – and, consequently, their duration – are gradually failing in a similar fashion in both ages.**

Network activity in M1

Up state occurrence: In contrast to S1BF, statistical analysis did not reveal a significant two-way interaction between age and Mg^{2+} Period in M1 cortex ($F_{(1.95,27.27)}$ = 1.81, p = 0.184, partial η^2 = 0.114) (Fig. 2B, right). There was a significant main effect of Mg^{2+} Period ($F_{(1.95,27.27)}$ = 37.76, p < 0.001, partial η^2 = 0.730) that became apparent only from the Intermediate Period onwards. Specifically, Up state occurrence was significantly reduced in Control vs. Intermediate (91.55%, p < 0.001), Control vs. Late (p < 0.001), Early vs. Intermediate (p < 0.001) and Early vs. Late Period (p < 0.001). In addition, there was a main effect of age revealing a significantly higher mean occurrence in adult compared to young mice ($F_{(1,14)}$ = 9.79, p = 0.007, partial η^2 = 0.412).

Up state duration: Similarly to Up state occurrence, there was no two-way interaction between age and Mg^{2+} Period for Up state duration ($F_{(2.07,28.95)}$ = 3.17, p = 0.055, partial η^2 = 0.185) (Fig. 2C, right). There was a main effect of Mg^{2+} Period on Up state duration ($F_{(2.07,28.95)}$ = 144.82, p < 0.001, partial η^2 = 0.912), that was almost identical to the pattern observed in S1BF: a gradual reduction during successive Mg^{2+} Periods until the eventual elimination of Up state activity. Specifically, Up state duration was significantly reduced in Control vs. Early

(30.99%, $p < 0.001$), Control vs. Intermediate ($p < 0.001$), Control vs. Late ($p < 0.001$), Early vs. Intermediate ($p < 0.001$) and Early vs. Late Period ($p < 0.001$). The main effect of age showed that adults had statistically significantly longer mean Up state duration ($F_{(1,14)} = 5.12$, $p = 0.040$, partial $\eta^2 = 0.268$), as previously shown (Rigas et al., 2015).

To sum up, Up state occurrence in M1 is seemingly unaffected in the period immediately following the Mg^{2+} Switch in both ages, and subsequently decreases until Up states disappear; whereas duration exhibits a gradual decrease until Up states are eliminated. **These results indicate that the mechanisms underlying the Down-to-Up state transition (or the Up state generation) are initially unaffected, while the mechanisms regulating the termination of Up states – and, consequently, their duration – are gradually failing in a similar fashion in both ages.**

Cortical and hippocampal slices developed distinct patterns of epileptiform activity following perfusion with low Mg^{2+} ACSF

After sufficient bathing of the slices in low Mg^{2+} ACSF, all brain regions gradually developed stable paroxysmal activity. Among cortical slices, those that spontaneously expressed endogenous network activity in the form of Up states also expressed premature spike-and-wave activity in-between Up states during the transition period (Fig. 2Ac). Eventually, Up states in the cortex disappeared and mature epileptiform activity became established (Fig. 2Ad). CA3 did not display any premature epileptiform activity. Nevertheless, the different regions and/or ages progress towards this stable pattern with different speeds and distinct patterns, as described in the following section.

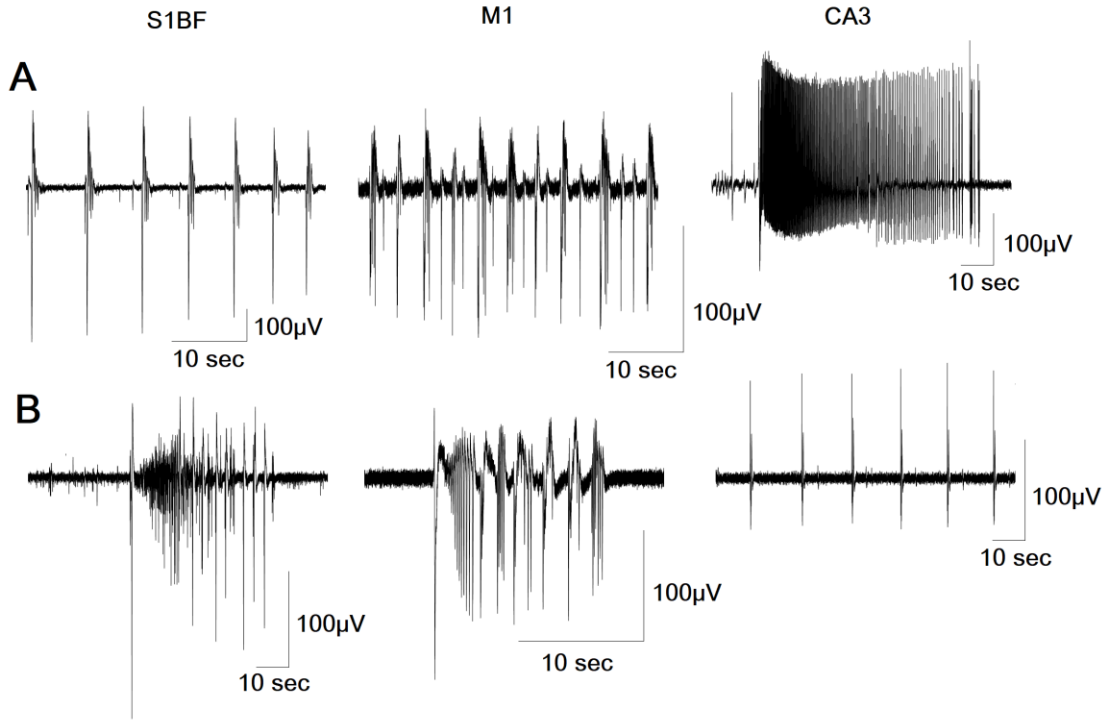

Figure 3. Characteristic LFP traces depicting the various epileptiform patterns developed in different regions and ages after perfusion with low Mg^{2+} ACSF. **A.** Epileptiform activity in young mouse slices. S1BF (left), and M1

(middle) developed short SWDs with or without afterdischarges. CA3 (right) developed long SLEs with tonic-clonic-like characteristics. **B.** Epileptiform activity in adult mouse slices. S1BF (left) developed long SLEs with tonic-clonic-like characteristics, while M1 (middle) developed shorter events with afterdischarges. CA3 (right) developed short epileptiform events.

In young mouse slices, both cortical regions (Fig. 3A, left and middle panels) developed frequent (~12 events/min) and fairly short (~2 sec) epileptiform events that resembled SWDs, either without afterdischarges in the case of S1BF (occurrence 8.99 ± 2.62 events/min, duration 1.92 ± 0.45 sec), or with afterdischarges in the case of M1 (occurrence 14.13 ± 1.69 events/min, duration 1.47 ± 0.15 sec). These afterdischarges are also called "~10Hz oscillations" by some (Castro-alamancos et al., 2007). In contrast, CA3 developed much less frequent (0.15 ± 0.03 events/min) and dramatically longer (103.39 ± 46.98 sec) SLEs with tonic-clonic-like characteristics (Fig. 3A, right).

In adult mouse slices, epileptiform activity was more variable. Here, S1BF developed long SLEs with tonic-clonic-like characteristics (Fig. 3B, left) with an occurrence of 0.34 ± 0.04 events/min and duration of 53.40 ± 9.08 sec, while M1 developed shorter epileptiform events (Fig. 3B, middle) with occurrence 1.54 ± 0.40 events/min and duration 13.90 ± 3.64 sec. CA3 developed two different patterns of activity: The majority of slices (60%) expressed short (1.05 ± 0.25 sec) epileptiform events with an occurrence of 3.91 ± 0.79 events/min (Fig. 3B, right panel), while the remaining 40% expressed long (37.94 ± 23.71 sec) SLEs with an occurrence of 0.29 ± 0.23 events/min (data not shown).

These results indicate that the type and pattern of epileptiform activity that develops after an epileptic insult depends not only on the paricular brain region examined, but also on the specific age tested.

The latency to the development of epileptiform activity is region and age-specific

In order to quantify and compare the latency with which each region and age develop epileptiform activity, we measured 2 parameters: (a) the onset of first epileptiform events (defined as the first instance of epileptiform events, whether or not they had aquired a stable morphology and whether or not they co-existed with Up state activity); and (b) the onset of mature epileptiform activity (defined as recurring large amplitude epileptiform events that didn't co-exist with Up state activity)

Overall findings: We observed that the latency to develop epileptiform activity was region-dependent, as both FED and MEA appeared sooner in neocortex compared to hippocampus; and this difference was significant at both ages. The latency to develop mature epileptiform activity was also age-dependent, as adult mice took longer to develop MEA compared to young mice, although this difference was not significant for the FED (Fig. 4A,B).

<u>Latency to the first epileptiform discharge</u>

Both young and adult cortex exhibited premature epileptiform events that resembled SWDs in-between Up states, before epileptiform activity matured and aquired its stable pattern. Thus, the FED appeared much faster in cortical slices compared to CA3. Mean latency to the FED was 22.68 ± 8.69 min for young S1BF (n = 8 slices), 26.45 ± 3.5 min for young M1 (n = 8 slices), and 55.67 ± 14.32 min for young CA3 (n = 8 slices). For adults, it was 21.42 ± 7.49 min (n = 10 slices), 30.15 ± 9.66 min (n = 8 slices), and 58.75 ± 11.82 min (n = 10 slices),

respectively. Statistical analysis did not reveal a significant two-way interaction between age and region for the latency to the FED ($F_{(2,46)}$ = 0.33, p = 0.724, partial η^2 = 0.014). There was no main effect of age in the latency to the first epileptiform discharge ($F_{(1,46)}$ = 0.45, p = 0.506, partial η^2 = 0.010). In contrast, there was a significant main effect of brain region, revealing significant difference in the latency to the FED between the three regions ($F_{(2,46)}$ = 63.92, p < 0.001, partial η^2 = 0.738). With the age level collapsed, M1, S1BF and CA3 latency to the FED was 28.30 ± 7.28 min, 21.98 ± 7.82 min, and 57.38 ± 12.68 min respectively. **These results indicate that – irrespective of age – CA3 is much slower to develop epileptiform discharges compared to either M1 or S1BF, while the two cortical regions do not differ significantly from each other (see below and Fig. 4A,Ba).**

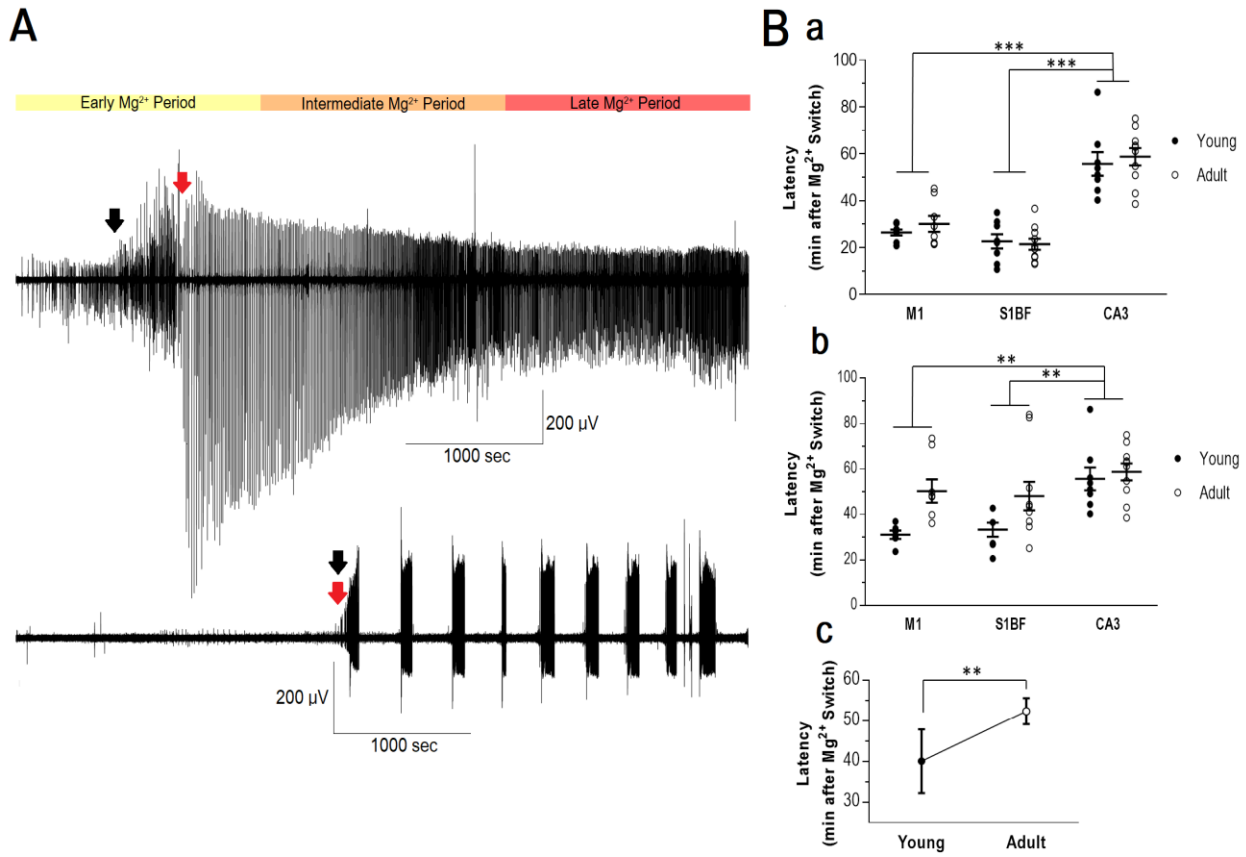

Figure 4. The latency to the development of epileptiform activity shows regional and age specificity. **A.** Example LFP traces from young S1BF (top) and young CA3 (bottom), depicting the development of epileptiform activity. Red arrows indicate the onset of mature epileptiform activity, while black arrows indicate the onset of the first epileptiform discharge (in CA3 the two coincide). **B.** The first epileptiform discharge appears fist in the cortex with no age difference (a), while mature epileptiform activity appears first in the cortex (b) and faster in young mice (c). Graphs show mean ± SEM. Two-way ANOVA, **p<0.01, ***p<0.001.

Latency to the development of mature epileptiform activity

Young S1BF (n = 8 slices), M1 (n = 6 slices) and CA3 (n = 8 slices) developed MEA with a mean latency of 33.35 ± 8.90 min, 31.15 ± 4.50 min, and 55.67 ± 14.32 min respectively. Adult S1BF (n = 10 slices), M1 (n = 8 slices) and CA3 (n = 10 slices) displayed a mean latency of , 48.15 ± 19.80 min, 50.33 ± 14.50 min and 58.75 ± 11.82 min respectively. Statistical analysis did not reveal a significant two-way interaction between age and region ($F_{(2,44)}$ = 1.85, p = 0,169, partial η^2 = 0.078), indicating that all three brain regions behave similarly at both

ages, regarding the latency to the MEA. The main effect of age, however, revealed that **adult mice display a significantly longer latency to MEA than young mice** ($F_{(1,44)}$ = 11.50, p = 0,001, partial η^2 = 0.207) (Fig. 4Bc). The main effect of region also revealed a statistically significant difference in the latency between the three regions ($F_{(2,46)}$ = 9.59, p < 0.001, partial η^2 = 0.304), with **the two cortical regions again displaying lower latency than CA3** (Fig. 4Bb).

Taken together, these results show that the neocortex develops the first signs of epileptiform activity – in the form of SWDs in-between Up state activity – about twice as fast as CA3 displays any epileptiform activity. These "premature" SWDs then gradually develop into MEA, again faster (~ 30%) than any indication of epileptiform activity in CA3.

The presence of Up states correlates with earlier onset of epileptiform activity

In the case of S1BF, Up state activity could be readily induced in both young and adult mice, and slices that did not express Up states in the control period were very rare, as mentioned before. M1, however, displayed a more stochastic expression of Up state activity at both ages, with 61.5% of young and 30.8% of adult mouse slices expressing spontaneous Up states (called active slices hereafter). We observed that slices that didn't exhibit Up state activity in the control condition (called silent slices hereafter) displayed a much longer latency for the development of mature epileptiform activity (Fig. 5B, left).

In young mice, active M1 slices (n = 6) had a mean latency of 31.15 ± 4.50 min (as mentioned before), while silent slices (n = 5) which had a 75% longer latency (54.49 ± 5.78 min). The same was true for adult mice, with active M1 slices (n = 8) showing a mean latency of 50.33 ± 14.50 min, compared to silent slices (n = 7) with a mean latency of 59.68 ± 16.05 min.

Statistical analysis did not reveal a significant two-way interaction between age and Up state presence for the latency to MEA onset ($F_{(1,22)}$ = 2.09, p = 0.163, partial η^2 = 0.087). The main effect of age revealed that adult mice display a significantly longer mean latency compared to young mice ($F_{(1,22)}$ = 6.33, p = 0,020, partial η^2 = 0.223) (Fig. 5B, right). The main effect of Up state presence revealed that **active slices develop epileptiform activity significantly faster than silent slices** ($F_{(1,22)}$ = 11.38, p = 0.003, partial η^2 = 0.341).

Given this finding of slower onset of epileptiform activity in silent cortical slices, we also investigated whether the latency of the M1 silent slices differs from the (slower) latency of CA3 slices. Statistical analysis did not reveal a significant two-way interaction between age and region for the latency to MEA onset $F_{(1,26)}$ = 0.05, p = 0.831, partial η^2 = 0.002). The main effects of age ($F_{(1,26)}$ = 0.72, p = 0.405, partial η^2 = 0.027) and region ($F_{(1,26)}$ = 0.00, p = 0.979, partial η^2 = 0.00) were also not statistically significant (data not shown).

These results confirms our previous finding that, adult M1 cortex is more resistant to the development of epileptiform activity compared to young M1 cortex, and also reveal that the presence of Up state activity in M1 correlates with a shorter latency to the development of epileptiform activity, regardless of age. Furthermore, when we compare the silent M1 slices with hippocampal slices, both regional and age differences that were present in active M1 slices disappear. This finding suggests that **the presence of Up state activity – at least in M1 slices – accelerates the appearance of epileptiform activity.**

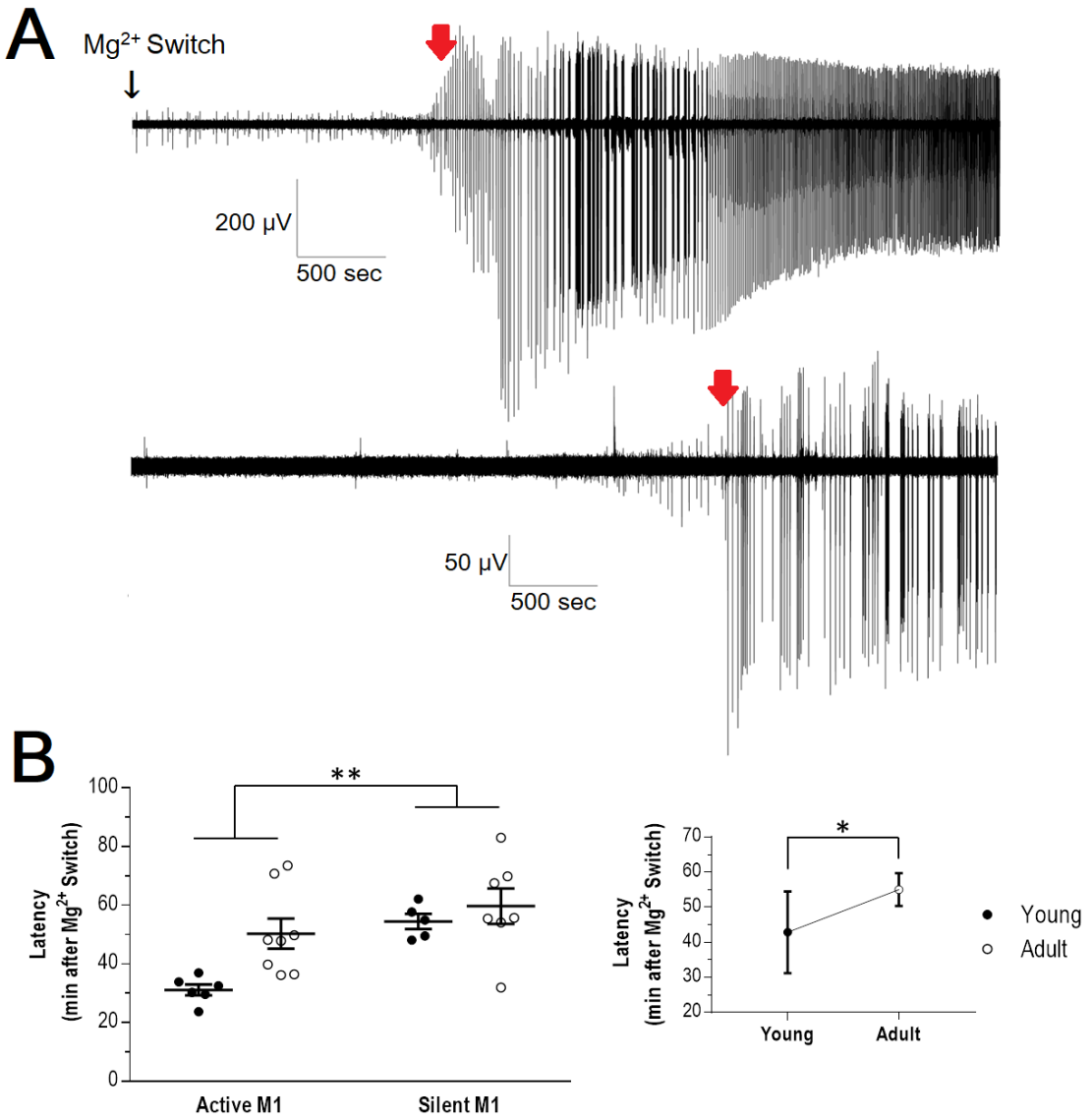

Figure 5. The presence of Up state activity in the control correlates with earlier onset of epileptiform activity in M1 slices. **A.** Representative traces after the Mg^{2+} Switch, from a young M1 slice with Up state activity (top), and without (bottom). Red arrows indicate the onset of mature epileptiform activity. **B.** Mature epileptiform activity appears earlier in active slices (left, with the age level collapsed). Also, mature epileptiform activity appears earlier in slices from young mice compared to adults (right, with the level of Up state presence collapsed). Graphs show mean ± SEM. Two-way ANOVA, *p<0.05, **p<0.01.

Discussion

Previous studies have yielded conflicting results when investigating susceptibility to seizures in distinct brain regions, which might be partly due to the variability of models and/or animals' ages used. Thus, a unifying study that examines different brain regions at different developmental stages under identical conditions is paramount. Here we implemented a widely used ex vivo model of epileptiform activity to examine the seizure threshold of two different cortical regions (M1, S1BF) and a hippocampal region (CA3). Our findings show that S1 and M1 cortex exhibit an increased vulnerability for epileptiform activity compared to CA3, independent of the age of the animal; however, this difference disappears if silent cortical slices are compared to the hippocampus, suggesting that susceptibility to develop paroxysmal activity is state-dependent. We also show that the properties of the

physiological endogenous cortical network activity undergo a gradual transformation as the network transitions to an epileptiform state, with an initial transient acceleration of the Up state rhythm, followed by a sharp reduction of Up states and their eventual elimination and replacement with epileptiform discharges.

Transformation of Up state activity under epileptogenic conditions

The effect of low Mg^{2+} on Up state occurrence and duration can be used to pinpoint specific mechanisms underlying the transition from a physiological to a pathological network synchronization in cortical tissue. Our results show that initially, the Up state rhythm is accelerated with Up states becoming more frequent and shorter in duration.

Epileptiform activity in the low Mg^{2+} model is thought to develop as the Mg^{2+} block of the NMDA receptor is gradually released due to the lack of Mg^{2+} ions in the perfusing ACSF (Antonio et al., 2016). Up states are thought to initiate in cortical layer V – and then spread to lower and upper layers (Beltramo et al., 2013; Neske, 2016; Sanchez-Vives and McCormick, 2000; Steriade and Amzica, 1996; Wester and Contreras, 2012) –, either via temporal summation of spontaneous mEPSPs in layer V pyramidal neurons (Bazhenov et al., 2002; Chauvette et al., 2010; Timofeev, 2000), or via persistent firing of layer V pyramidal neurons that initiate Up states when refractory mechanisms related to the previous Up state have diminished (Le Bon-Jego, 2007; Crunelli et al., 2012; Hasenstaub et al., 2007; Neske et al., 2015; Sakata and Harris, 2009; Sanchez-Vives and McCormick, 2000). After the termination of an Up state, sufficient network synaptic activity must be built up for the generation of the next Up state. Furthermore, in order for Up states to properly initiate and persist, a fine balance between excitation and inhibition is required (Shu et al., 2003). An enhancement of synaptic activity through increased NMDA receptor conductance (due to the release of the NMDA receptor block) could explain the fact that in low Mg^{2+} conditions Up states initially become more frequent. When the balance is tipped towards excitation, as in our case, inhibition might not be able to keep up for Up states to persist normally, resulting in a decreased Up state duration. Also, inhibitory mechanisms have been shown to be crucial for the proper sustenance of Up state duration, and when fast GABAA receptor-dependent inhibition is blocked, Up state duration is decreased (Mann et al., 2009).

Thus, it could be hypothesized that in our preparation the network is being tipped towards excitation, which leads to more frequent Up state activity, and subsequently, the inhibition mechanisms responsible for Up state persistence and proper termination start to fail, which leads to reduced Up state duration. Longer perfusion of the slices in low Mg^{2+} ACSF leads to further disturbance of this balance, resulting in complete elimination of normal Up state activity. Instead, hypersynchronous recurrent epileptiform events appear. The regional heterogeneity that was observed between M1 and S1BF cortices could be explained by differences in intrinsic properties of the local networks, such as the lack of layer IV in M1 (Donoghue and Wise, 1982) or different interlaminar inhibitory connections (Kätzel et al., 2011).

Elevated epileptiform activity threshold of the hippocampus compared to the active cortex

In our results we demonstrate that active cortical slices (i.e slices that exhibited spontaneous Up state activity in the control condition) display a lower threshold for the development of

epileptiform activity than the hippocampus CA3. The active cortex exhibited premature SWDs in-between Up states, which appeared long before the CA3 displayed paroxysmal activity, at both ages. These early SWDs developed into mature epileptiform activity (MEA) that again preceded any signs of paroxysmal activity in CA3, but, contrary to premature SWDs, this process took longer in adults. We also demonstrate that epileptiform activity develops and matures faster in active cortical slices compared to silent slices, and that the silent slice latency does not differ from the hippocampus latency.

Animal studies to date have yielded conflicting result when comparing seizure thresholds of different brain regions, displaying both elevated and reduced seizure threshold of the neocortex compared to the hippocampus (Abdelmalik et al., 2005; Codadu et al., 2019). This could be attributed to the different induction models and/or animal ages used. Here we examined distinct brain regions from different developmental stages under identical conditions, and we demonstrate a potential relationship between slow-wave-like activity in the neocortex and reduced seizure threshold. Our results are relevant since previous studies have also shown that slow-wave sleep and epilepsy are interconnected in many aspects (Bazil and Walczak, 1997; St. Louis, 2011; Malow et al., 1998, 2000; Sammaritano et al., 1991). The highly synchronized persistent activity during the Up state depends on a precise excitation/inhibition (E/I) balance (Shu et al., 2003), and the fact that seizures are thought to arise as a result of a disruption in the physiological E/I balance, has led to the hypothesis that Up state dysregulation could lead to seizures (Žiburkus et al., 2013). The mechanism by which the presence of Up states accelerates the onset of epileptiform activity in our preparation was beyond the scope of the present study, but a correlation is clearly evident, and requires further research. Of note, some studies have reported an increased susceptibility of the neocortex compared to the hippocampus even in experimental conditions in which the slices were not able to produce Up and Down states. However, these studies have mainly focused on the temporal neocortex, which is highly vulnerable to epileptogenesis and the potentially crucial role it plays in temporal lobe epileptogenesis is getting more and more recognised (Avoli et al., 2002; Bear and Lothman, 1993; Codadu et al., 2019; Lücke et al., 1995; Shi et al., 2014; Tancredi et al., 1990). Our results cleary show that active cortical slices have much lower threshold to epileptiform activity compared to hippocampus, and in this way confirm these aforementioned studies; but they also show that this increased vulnerabilty is state-dependent, at least in the primary motor cortex. Further work aiming to explore the mechanisms underlying this differentiation should have important implications for the development of effective anti-epileptic therapies.

To conclude, our results demonstrate a clear pattern of transition of physiological to pathological brain activity, as well as a correlation between cortical Up state activity and reduced seizure threshold. An experimental approach that focuses on determining the proportions of excitatory and inhibitory synaptic conductances during control conditions and also during different stages of transition to epileptiform synchronization, including during epileptiform discharges would help elucidate some of the mechanisms that govern the sudden transitions from physiology to pathology that characterize epileptic disorders.

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References

Abdelmalik, P.A., Burnham, W.M.I., and Carlen, P.L. (2005). Increased seizure susceptibility of the hippocampus compared with the neocortex of the immature mouse brain in vitro. Epilepsia.

Antonio, L.L., Anderson, M.L., Angamo, E.A., Gabriel, S., Klaft, Z.J., Liotta, A., Salar, S., Sandow, N., and Heinemann, U. (2016). In vitro seizure like events and changes in ionic concentration. J. Neurosci. Methods.

Avoli, M., and Jefferys, J.G.R. (2016). Models of drug-induced epileptiform synchronization in vitro. J. Neurosci. Methods.

Avoli, M., D'Antuono, M., Louvel, J., Köhling, R., Biagini, G., Pumain, R., D'Arcangelo, G., and Tancredi, V. (2002). Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. Prog. Neurobiol.

Bazhenov, M., Timofeev, I., Steriade, M., and Sejnowski, T.J. (2002). Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. J. Neurosci.

Bazil, C.W., and Walczak, T.S. (1997). Effects of sleep and sleep stage on epileptic and nonepileptic seizures. Epilepsia.

Bear, J., and Lothman, E.W. (1993). An in vitro study of focal epileptogenesis in combined hippocampal-parahippocampal slices. Epilepsy Res.

Beltramo, R., D'Urso, G., Dal Maschio, M., Farisello, P., Bovetti, S., Clovis, Y., Lassi, G., Tucci, V., De Pietri Tonelli, D., and Fellin, T. (2013). Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat. Neurosci.

Le Bon-Jego, M. (2007). Persistently active, pacemaker-like neurons in neocortex. Front. Neurosci.

Castro-alamancos, M.A., Rigas, P., and Tawara-hirata, Y. (2007). Resonance (~10 Hz) of excitatory networks in motor cortex: Effects of voltage-dependent ion channel blockers. J. Physiol.

Chauvette, S., Volgushev, M., and Timofeev, I. (2010). Origin of active states in local neocortical networks during slow sleep oscillation. Cereb. Cortex.

Chen, B., Choi, H., Hirsch, L.J., Katz, A., Legge, A., Buchsbaum, R., and Detyniecki, K. (2017). Psychiatric and behavioral side effects of antiepileptic drugs in adults with epilepsy. Epilepsy Behav. *76*, 24–31.

Codadu, N.K., Parrish, R.R., and Trevelyan, A.J. (2019). Region-specific differences and areal interactions underlying transitions in epileptiform activity. J. Physiol.

Crunelli, V., Lorincz, M.L., Errington, A.C., and Hughes, S.W. (2012). Activity of cortical and thalamic neurons during the slow (<1 Hz) rhythm in the mouse in vivo. Pflugers Arch. Eur. J. Physiol.

Donoghue, J.P., and Wise, S.P. (1982). The motor cortex of the rat: Cytoarchitecture and microstimulation mapping. J. Comp. Neurol.

Fisher, R.S., Scharfman, H.E., and DeCurtis, M. (2014). How can we identify ictal and interictal abnormal activity? Adv. Exp. Med. Biol.

Hájos, N., Ellender, T.J., Zemankovics, R., Mann, E.O., Exley, R., Cragg, S.J., Freund, T.F., and Paulsen, O. (2009). Maintaining network activity in submerged hippocampal slices: Importance of oxygen supply. Eur. J. Neurosci.

Hamidi, S., Lévesque, M., and Avoli, M. (2014). Epileptiform synchronization and high-frequency oscillations in brain slices comprising piriform and entorhinal cortices. Neuroscience.

Harris, R.J., Wieloch, T., Symon, L., and Siesjo, B.K. (1984). Cerebral extracellular calcium activity in severe hypoglycemia: Relation to extracellular potassium and energy state. J. Cereb. Blood Flow Metab.

Hasenstaub, A., Sachdev, R.N.S., and McCormick, D.A. (2007). State changes rapidly modulate cortical neuronal responsiveness. J. Neurosci.

Jones, H., and Keep, R. (1988). Brain Fluid Calcium Concentration and Response To. J Physiol.

Kätzel, D., Zemelman, B. V., Buetfering, C., Wölfel, M., and Miesenböck, G. (2011). The columnar and laminar organization of inhibitory connections to neocortical excitatory cells. Nat. Neurosci.

Librizzi, L., and De Curtis, M. (2003). Epileptiform ictal discharges are prevented by periodic interictal spiking in the olfactory cortex. Ann. Neurol.

St. Louis, E.K. (2011). Sleep and epilepsy: Strange bedfellows no more. Minerva Pneumol.

Lücke, A., Nagao, T., Köhling, R., and Avoli, M. (1995). Synchronous potentials and elevations in [K+]o in the adult rat entorhinal cortex maintained in vitro. Neurosci. Lett.

Malow, B.A., Lin, X., Kushwaha, R., and Aldrich, M.S. (1998). Interictal spiking increases with sleep depth in temporal lobe epilepsy. Epilepsia.

Malow, B.A., Bowes, R.J., and Ross, D. (2000). Relationship of Temporal Lobe Seizures to Sleep and Arousal: A Combined Scalp-Intracranial Electrode Study. Sleep.

Manford, M., Hart, Y.M., Sander, J.W.A.S., and Shorvon, S.D. (1992). The National General Practice Study of Epilepsy: The Syndromic Classification of the International League Against Epilepsy Applied to Epilepsy in a General Population. Arch. Neurol.

Mann, E.O., Kohl, M.M., and Paulsen, O. (2009). Distinct roles of GABAA and GABAB receptors in balancing and terminating persistent cortical activity. J. Neurosci.

Neske, G.T. (2016). The slow oscillation in cortical and thalamic networks: Mechanisms and functions. Front. Neural Circuits.

Neske, G.T., Patrick, S.L., and Connors, B.W. (2015). Contributions of diverse excitatory and inhibitory neurons to recurrent network activity in cerebral cortex. J. Neurosci.

Rigas, P., Adamos, D.A., Sigalas, C., Tsakanikas, P., Laskaris, N.A., and Skaliora, I. (2015). Spontaneous Up states in vitro: A single-metric index of the functional maturation and regional differentiation of the cerebral cortex. Front. Neural Circuits.

Sakata, S., and Harris, K.D. (2009). Laminar Structure of Spontaneous and Sensory-Evoked Population Activity in Auditory Cortex. Neuron.

Sammaritano, M., Luigi Gigli, G., and Gotman, J. (1991). Interictal spiking during wakefulness and sleep and the localization of foci in temporal lobe epilepsy. Neurology.

Sanchez-Vives, M. V., and McCormick, D.A. (2000). Cellular and network mechanisms of rhytmic recurrent activity in neocortex. Nat. Neurosci.

Sánchez Fernández, I., and Loddenkemper, T. (2014). Subunit Composition of Neurotransmitter Receptors in the Immature and in the Epileptic Brain. Biomed Res. Int.

Semah, F., Picot, M.C., Adam, C., Broglin, D., Arzimanoglou, A., Bazin, B., Cavalcanti, D., and Baulac, M. (1998). Is the underlying cause of epilepsy a major prognostic factor for recurrence? Neurology.

Shi, Y.J., Gong, X.W., Gong, H.Q., Liang, P.J., Zhang, P.M., and Lu, Q.C. (2014). Effect of the entorhinal cortex on ictal discharges in low-Mg 2+-induced epileptic hippocampal slice models. Neural Plast.

Shu, Y., Hasenstaub, A., and McCormick, D.A. (2003). Turning on and off recurrent balanced cortical activity. Nature.

Sigalas, C., Rigas, P., Tsakanikas, P., and Skaliora, I. (2015). High-affinity nicotinic receptors modulate spontaneous cortical up states in vitro. J. Neurosci.

Stafstrom, C.E., and Carmant, L. (2015). Seizures and epilepsy: An overview for neuroscientists. Cold Spring Harb. Perspect. Biol.

Steriade, M., and Amzica, F. (1996). Intracortical and corticothalamic coherency of fast spontaneous oscillations. Proc. Natl. Acad. Sci. U. S. A.

Steriade, M., Nunez, A., and Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: Depolarizing and hyperpolarizing components. J. Neurosci.

Tancredi, V., Hwa, G.G.C., Zona, C., Brancati, A., and Avoli, M. (1990). Low magnesium epileptogenesis in the rat hippocampal slice: electrophysiological and pharmacological features. Brain Res.

Timofeev, I. (2000). Origin of Slow Cortical Oscillations in Deafferented Cortical Slabs. Cereb. Cortex.

Timofeev, I., and Steriade, M. (2004). Neocortical seizures: Initiation, development and cessation. Neuroscience.

Tsakanikas, P., Sigalas, C., Rigas, P., and Skaliora, I. (2017). High-Throughput Analysis of in-vitro LFP Electrophysiological Signals: A validated workflow/software package. Sci. Rep.

Wester, J.C., and Contreras, D. (2012). Columnar interactions determine horizontal propagation of recurrent network activity in neocortex. J. Neurosci.

Wong, M., and Yamada, K.A. (2001). Developmental characteristics of epileptiform activity in immature rat neocortex: A comparison of four in vitro seizure models. Dev. Brain Res.

Žiburkus, J., Cressman, J.R., and Schiff, S.J. (2013). Seizures as imbalanced up states: Excitatory and inhibitory conductances during seizure-like events. J. Neurophysiol.