Final report

FLAG-ERA:
How rigid and plastic circuits contribute to hippocampal function
2019.01.01.-2021.12.31.

The current research project – as part of a consortium endeavor – set out to investigate the relevance of developmental origin as a predictor of structural and functional diversity of pyramidal neurons in the hippocampal CA1 area. Our working hypothesis was that CA1 pyramidal neurons that are generated at different time points during development have different cellular, intrinsic and connectivity properties (in vitro) and specific coding properties as place cells (in vivo). Parts of this hypothesis were to be tested by our collaborator laboratories in France (Rosa Cossart and Jerome Epsztein labs). The aim of our laboratory in the research project was to **elucidate whether developmentally distinct populations of CA1 pyramidal neurons express differential intrinsic properties and dendritic processing of synaptic information**, using state-of-the-art *in vitro* methods to compare excitability, dendritic integration of synaptic inputs and plasticity thereof, in CA1 pyramidal neurons born at different time points, as identified by genetic fate-mapping.

I. Set-up of the transgenic mouse line for fate-mapping

To mark glutamatergic neurons generated during different times of embryogenesis, we use the technique of inducible genetic fate-mapping, based on the transient expression of the proneural bHLH transcription factor Neurogenin2 in pyramidal glutamatergic neurons as they are becoming postmitotic. At the start of the grant, the first task was to set up the new transgenic mouse line in our Institute. We have imported frozen sperm of NgN2CreER(+/-)/Ai14(+/-) transgenic mice from the Cossart laboratory. After the initial in vitro fertilization and breeding, we crossed double-heterozygous male offspring mice with Ai14(+/+) females to obtain a line with homozygous TdTomato marker NgN2CreER(+/-)/Ai14(+/+). The new colony has been successfully established by the end of the first year.

Fate-mapping by TdTomato-expression of hippocampal pyramidal neurons can be achieved in this line by tamoxifen (TMX) administration during the E12-E17 period, using oral gavage of pregnant female CD1 mice crossed with NgN2CreER(+/-)/Ai14(+/+) male mice. According to our experience with the protocol, approximately 40-50% of the TMX-treated pregnancies ended without viable pups (due to miscarriage, still-birth or maternal cannibalism). Preparing brain slices from ~2month-old viable offsprings, we confirmed highly specific labelling of pyramidal cells in TMX-treated NgN2CreER(+/-)/Ai14(+/+) mice (**Figure 1A**). In contrast, no labelling was seen in control experiments including: 1) NgN2CreER(+)/Ai14(+) mice without TMX treatment, 2) NgN2CreER(+)/Ai14(-) mice with TMX treatment, and 3) NgN2CreER(-)/Ai14(+) mice with TMX treatment.

In our initial workplan we aimed to compare the properties of two developmental groups: early (E12-13) and late (E15-16) born CA1 pyramidal neurons. However, during the course of the grant, our consortium partner (Cossart lab) has found that, in the ventral hippocampus, there is a development-related variability in CA1 pyramidal cell properties on a finer temporal scale. Specifically, among ventral CA1 pyramidal cells, those born at E14.5 had higher input membrane resistance, higher somatic excitability and longer primary apical dendritic trunk than the earlier- and later-born counterparts, and several other differences in synaptic input connectivity and c-fos activation were found that were specific to one of the three developmental age group. These results prompted us to **modify the workplan of our experiments**, and instead of studying only two time groups as originally planned, **we compared the properties of three categories: pioneer (E12-13), early-born (E14-15) and late-born (E16-17) neurons**.

II. Topographic distribution of fate-mapped CA1 pyramidal neurons in the dorsal hippocampus

Consistent with the results by the Cossart lab and with the classical inside-out pattern of cell migration during development, our experiments showed that pioneer CA1 pyramidal cells are spread out relatively widely along the radial axis of the pyramidal cell layer. Late born cells were more numerous, less dispersed than pioneer and early cells, and were more confined to the superficial layers of str. pyramidale (Figure 1).

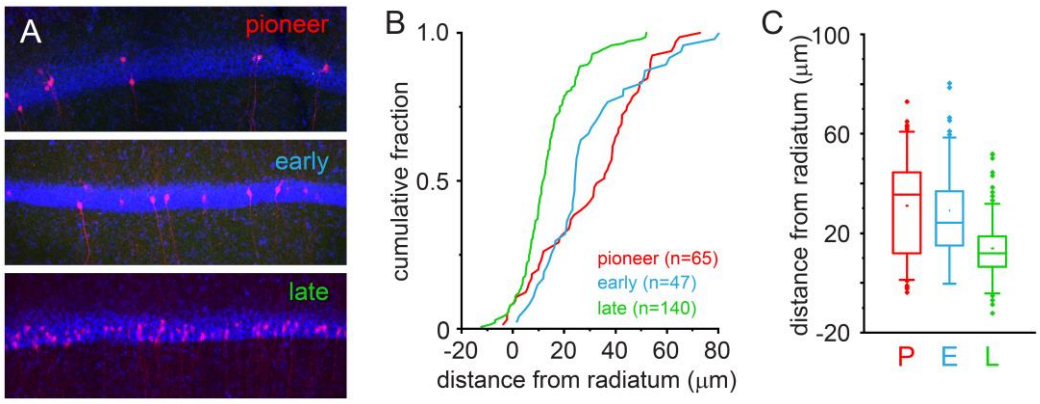

Figure 1. *A, confocal stacks from the CA1 region. Pioneer, early and late born CA1 pyramidal cells are labelled by TdTomato (shown in magenta). Blue: DAPI staining. B, radial distribution of labelled neurons in the three fate-mapped groups. C, median, interquartile ranges, 5-95% percentiles and outliers indicated for the three groups.*

III. Basic intrinsic electrophysiological properties of fate-mapped CA1 pyramidal neurons

Using acute slices from ~2-month-old offspring, we performed somatic current clamp recordings combined with two-photon (2P) microscopy after targeted patching of TdTomato-expressing CA1 pyramidal neurons (**Figure 2A**) in all three birthdate groups.

Using depolarizing and hyperpolarizing current injection step series, we determined membrane properties (resting membrane potential, input resistance, sag ratio) and action potential (AP) properties. These basic properties were similar in the three groups, only AP amplitude was slightly smaller in late-born cells (**Figure 2B-D**).

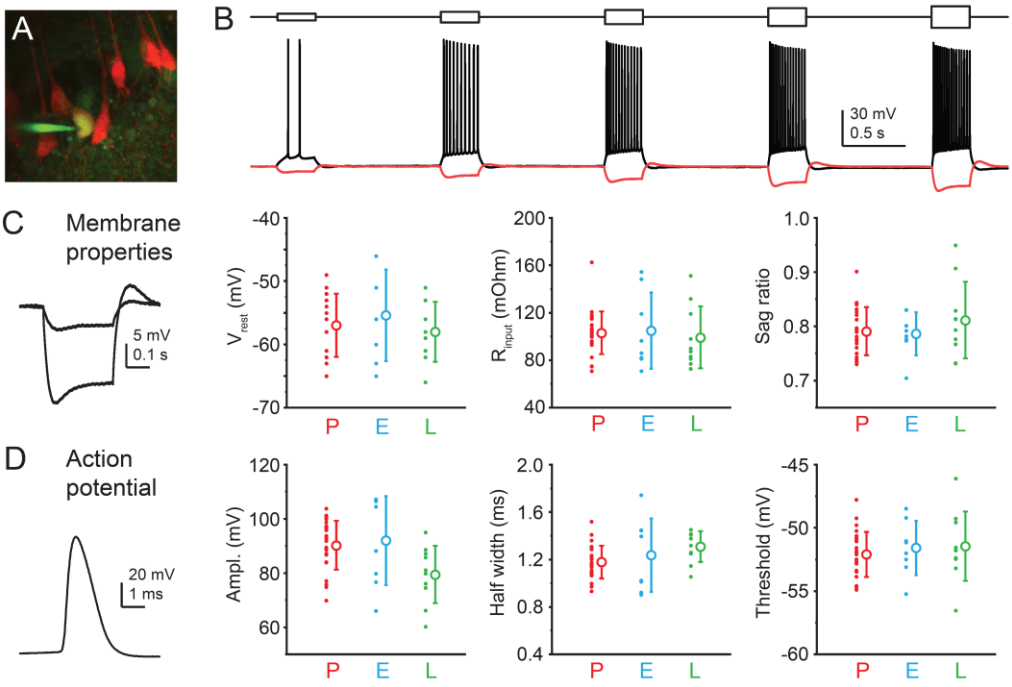

Figure 2. *A, 2P image showing targeted patching of a TdTomato-labelled red CA1 pyramidal neuron. The cell was loaded with a green Ca^{2+} sensor. B, Protocol for testing somatic electrophysiological properties. C-D, Basic membrane properties (C) and action potential parameters (D) in the three groups.*

IV. Dendritic properties of fate-mapped CA1 pyramidal neurons

1) Using the green fluorescent Ca^{2+} indicator OGB-1, we measured dendritic Ca^{2+} signals along the main apical trunk dendrite systematically at increasing distances from the soma (**Figure 3**). APs were evoked at various numbers and frequencies (1 or 5 APs at 20-100 Hz). The AP-evoked Ca^{2+} signals decayed in all groups with distance from the soma. There seems to be a trend for stronger backpropagation of APs at higher frequencies to distal apical dendrites in early-born

CA1 pyramidal cells, which would be consistent with higher dendritic excitability in this developmental group. We will increase the number of experiments here to be able to draw a statistically supported conclusion.

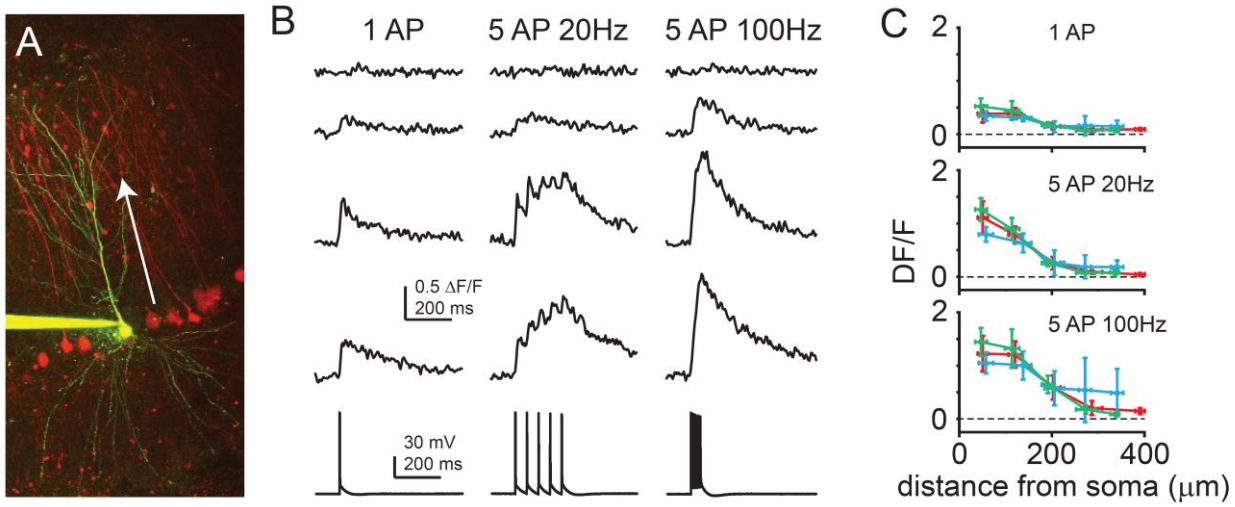

Figure 3. *A, 2P image of a patched TdTomato-labelled red CA1 pyramidal neuron. The cell was loaded with a green Ca^{2+} sensor. B, backpropagating AP-evoked Ca^{2+} signals by different AP patterns in an example cell, measured in the apical trunk dendrite at increasing distances from the soma (as indicated in A). C, Summary of distance-dependent bAP-evoked Ca^{2+} signals in the three groups. Red: pioneer (10 cells), blue: early-born (7 cells), green: late-born (8 cells).*

2) To measure dendritic Ca^{2+}-spike associated burst propensity, we applied series of 100-ms-long step current injections (300 and 600 pA), while measuring Ca^{2+} signals in distal apical dendrites. Small spikelets (possibly caused by failed axonal APs) compromised these recordings, and complex spike bursting could not be unambiguously verified or excluded in several cells.

3) To determine the strength of dendritic Na^+ spikes in thin perisomatic dendrites (a measure potentially indicating plasticity of dendritic excitability, Losonczy, Makara, Magee 2008, Makara et al 2009) we used 2P glutamate uncaging to synchronously stimulate small groups (5-10) of spines on basal dendrites. Characteristic Na^+ spikes could be evoked in dendrites of all three cell groups (**Figure 4**). Due to the high variance of Na^+ spike strength (as measured at the soma by the first temporal derivative component, dV/dt) depending on branch position and location distance (expected based on our previous work), we need to obtain a large dataset to draw a clear conclusion.

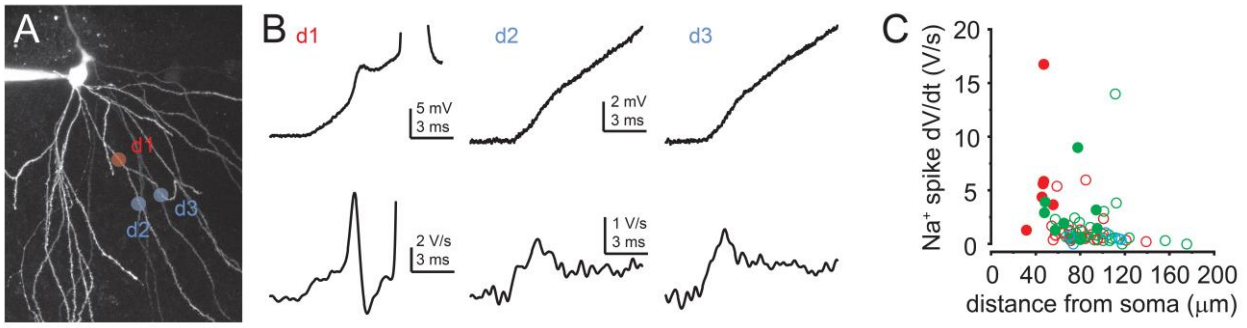

Figure 4. *A, 2P image of a patched TdTomato-labelled CA1 pyramidal neuron. B, 2P glutamate uncaging evoked basal dendritic Na^+ spikes in an example cell. Top: voltage; bottom: first temporal derivative (dV/dt; a measure of Na^+ spike propagation strength). C) Summary of Na^+ spike strength data collected so far in the three cell groups. Filled syambols: parent dendrites; open symbols: terminal branches.*

4) To help reconstruction of dendritic arbors and to assess the relative radial position of the patched cell in the pyramidal cell layer, we loaded a subset of neurons with biocytin and established a staining protocol for DAPI/biocytin/Alexa594 labeling. Recontruction and analysis of neurons is underway.

5) Since our recent work indicated that the dendritic properties of native CA3 pyramidal neurons is much more variable than those of CA1 pyramidal cells (Raus Balind et al., 2019 Nature Communications; Magó, Kis et al., 2021 eLife), we began to extend similar investigations into the CA3 area, to test whether the observed heterogeneity can be related to birth date.

6) TdTomato labeling by birth date can be easily combined with AAV-mediated expression of GCaMP in juvenile mice for in vivo two-photon Ca^{2+} imaging. We have optimized techniques for head fixation and implantation of a hippocampal imaging cannula window. Our preliminary results indicate that we have adequate optical access to image pyramidal cell somata and dendrites in the CA1 and distal CA3 area. We also developed a head-fixed behavior paradigm for studying dendritic function while animals perform a virtual reality navigation task in different environments. This technique will allow us to begin longitudinal in vivo investigation of dendritic and somatic activity and tuning properties of fate-mapped CA1 and CA3 pyramidal neurons in behaving animals.

Difficulties and modifications of the workplan

Due to facing difficulties in recruiting an experienced postdoctoral fellow, the project was assigned to a well-trained student (now PhD student), Noémi Kis, who has taken over the experimental work of the project full time. Since she already had experience with performing

electrophysiological and two-photon imaging experiments, relatively little training time was needed. Noémi was appointed for the position in November 2019, when the first transgenic fate-mapped mice became available for experiments. This change in plan resulted in underspending in the salary budget category than originally expected.

The Covid -19 pandemic presented an unexpected global threat that affected the execution of our program since 2020. Because of the virus situation we often had to pause experiments or implement working restrictions in the lab to reduce the risk of infection among lab members, slowing the experiments and compromising the workflow. Due to the pandemic several thematic international conferences (such as the Dendrite Gordon Research Conference, EMBO Dendrites meeting) were cancelled. Therefore unfortunately we were not able to present our results at relevant dendrite conferences, and the planned travel expenses had not realized.

In addition, a serious infection in the animal facility of the Institute required a major sanitization break and restarting the breeding of the colony in the summer of 2021, leading to delays in experimentation.

As indicated above, based on ventral hippocampal results obtained by consortium partners, we decided to modify the original workplan and included an additional developmental age group in the project. This required more experiments to be performed for Aim 1-2 than originally expected, which we prioritized over proceeding in Aim 3.

Overall, our results indicate that the so far investigated intrinsic and dendritic properties of CA1 pyramidal neurons born at different time points during development are not overly different, although some of the experiments require further increase in sample size, and variations of properties that we have not yet investigated cannot be ruled out. We hypothesize that variations depending on birth date may be more robust in the CA3 area, which we began to investigate. In the next year we hope to begin the preparation for a potential publication of the results.