Complex Cell Surface Distribution of Voltage- and Ligand-Gated Ion Channels on Cortical Pyramidal Cells

Ph.D. Thesis

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INTRODUCTION

Principal neurons are the most numerous cells of the brain, and their primary function is to receive and process information and finally to transmit the information to other neurons at long distance away. The activity of neurons is controlled by the orchestrated function of ion channels. While voltage-gated ion channels are responsible for the action potential generation and propagation, thus conduct electrical signals along the plasma membrane at long distance away, the activation of ligand-gated ion channels (i.e. synaptic receptors) produces small localized changes in the membrane potential.

Among the voltage-gated ion channels, potassium channels have received special attention due to their molecular and functional heterogeneity. Each of these potassium channel types has a unique subcellular distribution pattern along the plasma membrane of neurons, suggesting distinct roles in controlling the excitability of nerve cells. Potassium channels are categorized into four main channel families, the largest being the voltage-gated potassium channel (Kv) superfamily. The Kv4 channels, which form tetramers, generate the transient A-type potassium current (I_A), which has a major role in a large variety of dendritic processes that control the excitability of nerve cells. Electrophysiological experiments demonstrated a six-fold increase in the density of I_A as a function of distance from the soma to distal apical dendrites of hippocampal CA1 pyramidal cells (PCs). Although light microscopic immunoreactions localized the Kv4.2 subunits to the CA1 region, the underlying mechanism of the increased current density in the distal dendrites of CA1 PCs, as well as the precise subcellular distribution and densities, remained elusive.

The inwardly rectifying potassium channels (Kir) represent a minor group of the potassium channel superfamily; however, their electrophysiological properties make them important contributors in decreasing the excitability of neurons. Kir channels are tetramers, composed of two transmembrane segments, and form macromolecular complexes with G protein coupled receptors. Previous light microscopic immunoreactions for the Kir3.1, Kir3.2 and Kir3.3 subunits revealed an uneven distribution of the immunosignals in the hippocampal CA1 region. Moreover, cell-attached patch-clamp recordings demonstrated increased spontaneous Kir3 channel activity in the distal apical dendrites of CA1 PCs compared to somata. Although the Kir3 subunits have been localised with electron microscopic immunogold methods to dendritic shafts, spines and axon terminals, their relative densities in different axo-somato-dendritic compartments at various distances from the soma are still unknown.

The activity of principal cells is shaped by the compartment specific distribution of GABAergic synapses provided by local interneurons (INs). Fast inhibition is mediated through the activation of synaptic $GABA_A$ receptors ($GABA_ARs$) which are heteropentameric ligand-gated ion channels, composed of two α, two β and one γ2 subunit. The γ2 subunit in some cells can be replaced by the δ, ε, γ1, or γ3 subunits. Receptors incorporating the γ2 subunit cluster at GABAergic postsynaptic densities and mediate fast inhibitory synaptic currents. The γ2 subunit is unique among the other subunits, as deletion of the γ2, but not α or β subunits, leads to early postnatal lethality. Moreover, some studies indicated the role of γ2 subunits in clustering $GABA_ARs$ at the synapse, because genetic deletion of the γ2 subunit resulted in decreased clustering of both $GABA_ARs$ and gephyrin. Surprisingly, a previous study from our laboratory detected $GABA_AR$-mediated miniature inhibitory postsynaptic currents (mIPSCs) in cultured $\gamma2^{-/-}$ neurons, demonstrating that $GABA_ARs$ could still generate synaptic-like currents without the γ2 subunit. However, it should be noted that the presence of synaptic-like currents does not necessarily mean that the underlying $GABA_ARs$ are concentrated within the GABAergic postsynaptic specializations.

AIMS

In the first part of the dissertation, my general aims were to investigate the cell surface distribution of two different potassium channel in rat CA1 PCs by using the highly selective high-resolution sodium dodecylsulphate-digested freeze-fracture replica labelling (SDS-FRL) method.

In the second part of the dissertation I used the Cre-dependent virus-mediated γ2 subunit deletion strategy in combination with light microscopic immunofluorescent and electron microscopic SDS-FRL techniques in mouse somatosensory cortex, to challenge the longstanding view on the role of γ2 subunits in clustering $GABA_ARs$ at inhibitory postsynaptic specializations. This work was done in collaboration with my colleague Dr. Mark D. Eyre, who performed all whole-cell patch-clamp recordings that I am not going to present in my thesis, while I carried out all light microscopic immunofluorescent and electron microscopic immunogold labelling of distinct $GABA_AR$ subunits.

My specific aims for the first part are:

1. What is the precise subcellular distribution pattern of two potassium channel subunit (Kv4.2, Kir3.2) on distinct axo-somato-dendritic compartments in CA1 PCs?

2. Does the Kv4.2 channel density follow the six-fold increase in I_A density along the proximo-distal axis of PCs?
3. Is the increased Kir3 channel activity in the distal dendrites of CA1 PCs mirrored by an increased channel density?

My specific aims for the second part are:

1. Is the γ2 subunit necessary for clustering $GABA_AR$s at inhibitory postsynaptic specializations in the sensorimotor cortex of $GABA_AR\gamma2^{77I}$lox mice?
2. What is the precise subcellular location of $GABA_AR$s underlying the mIPSCs in cortical layer 2/3 cells lacking the γ2 subunit?
3. What is the subunit composition and densities of synaptic $GABA_AR$s in neurons lacking the γ2 subunit?

MATERIALS AND METHODS

Virus injection

Male and female $GABA_AR\gamma2^{77I}$lox mice between 22 and 40 days postnatal (P) were anaesthetized and 0.6 µl adeno associated virus expressing a Cre-GFP fusion protein with a nuclear localization signal motif under a human synapsin promoter was stereotaxically injected into the somatosensory cortex at 0.1 µl min^{-1} flow rate, and then animals were allowed to recover either 2 weeks or 6 weeks post injection.

Tissue preparation

Adult male Wistar rats (P 25–52; n = 17), male wild-type (n = 3) and $Kv4.2^{-/-}$ mice (P 68–217; kindly provided by Prof. Daniel Johnston; n = 3) as well as male and female $GABA_AR\gamma2^{F77I}$lox mice (P 36–80; n = 22) were deeply anesthetized then transcardially perfused. For light microscopic immunofluorescent reactions animals were perfused with a fixative containing either 2% or 4% paraformaldehyde (PFA) and 15v/v% picric acid (PA) made up in 0.1 M phosphate buffer (PB) for 15–20 minutes, or with 2% PFA in 0.1 M Na-acetate for 15 minutes. Some animals were perfused with ice cold oxygenated artificial cerebrospinal fluid (ACSF) for 4 minutes, then the brains were removed and postfixed in 4% PFA and 15v/v % PA in 0.1 M BP for 50 minutes. Afterwards 60 or 70 µm coronal forebrain sections were cut with a vibratome. For SDS-FRL animals were perfused with a fixative containing 2% PFA and 15v/v% PA in 0.1 M PB for 15–16 minutes. Coronal sections of 80 µm thickness were cut, and then small tissue blocks from the dorsal hippocampus and from

injected somatosensory cortex were trimmed. Tissue blocks from the injected cortical area were cut out based on the endogenous GFP signal in a way that a small non-injected area surrounded the injection zone. Sections were then cryoprotected overnight in 30 % glycerol.

For electrophysiological recordings of tonic currents injected mice (n = 12) at two weeks post injection were deeply anaesthetized, following decapitation, the brain was removed and placed into ice-cold ACSF. Coronal slices from the cerebral cortex were cut at 250 μm thickness and stored in ACSF and bubbled continuously with 95 % O_2 and 5 % CO_2, resulting in a pH of 7.4. After a 30 minute recovery period at 33 °C, slices were further incubated at room temperature until they were transferred to the recording chamber.

Fluorescent immunohistochemistry

Following several washings in 0.1 M PB and Tris-buffered saline (TBS), free-floating sections were blocked in 10 % normal goat serum (NGS), followed by an overnight incubation in a mixture of primary antibody solution made up in TBS containing 2 % NGS and 0.1% Triton X-100. Next, sections were incubated in a mixture of secondary antibody solutions made up in TBS containing 2 % NGS with or without 0.1 % Triton X-100 for 2 hours. Images from the CA1 region and from injected or non-injected cortices were acquired using a confocal laser scanning microscope (FV1000; Olympus, Tokyo, Japan).

SDS-FRL

Small tissue blocks from the CA1 region and injected cortex were frozen in a high-pressure freezing machine and fractured at -135 ºC in a freeze-fracture machine. The fractured tissue surfaces were coated with thin layers of carbon (5 nm), platinum (2 nm) and carbon (20 nm). The replicas were digested in a solution containing 2.5 % SDS and 20 % sucrose in TBS (pH = 8.3) at 80 ºC overnight. Following several washes in TBS containing 0.05% bovine serum albumin (BSA), replicas were blocked in TBS containing 0.1% BSA–5% BSA for 1 hour, then incubated overnight in blocking solution containing the primary antibodies. Replicas were then incubated for 2 hours in TBS containing either 1 % or 5 % BSA and the gold coupled secondary antibodies. In double-labelling reactions, a mixture of the two primary antibodies was applied, followed by a mixture of the two secondary antibodies. For $GABA_AR$ labelling in cortex, the antibodies were applied sequentially. Specimens were analysed with a transmission electron microscope (JEM-1011, JEOL Ltd., Tokyo, Japan).

Quantitative analysis of immunogold particles labelling for the Kv4.2 and Kir3.2 subunits and for different $GABA_AR$ subunits

Quantitative analysis of immunogold labelling for the Kv4.2 and the Kir3.2 subunits was performed on CA1 PC somata, axon initial segments (AISs), 11 different dendritic compartments and axon terminals from strata radiatum (SR) and lacunosum-moleculare (SLM) of the CA1 area (n = 5 rats for each subunit). The following layer categorizations were used to group the subcellular compartments: 0-120 μm: proximal SR; 120-240 μm: middle SR; 240-360 μm: distal SR and above 360 μm: SLM. Spines were identified either based on their ultrastructure or from the presence of a PSD on isolated spine heads identified by labelling the PSD-95. Axon terminals were identified either based on the presence of gold particles labelling the SNAP-25; or based on the presence of an active zone facing a post synaptic density on the opposing exoplasmic-face (E-face) of a spine or dendrite. To unequivocally identify the AISs the molecular marker pan-Neurofascin was used. The $GABA_AR$ β3 subunit was used to identify GABAergic synapses on PC somata and dendrites. Antibodies against the Kv4.2 and Kir3.2 subunits recognized intracellular epitopes on their target proteins and consequently were visualized by gold particles on the protoplasmic-face (P-face). Nonspecific background labelling was measured on E-face structures surrounding the measured P-faces and was subtracted from the mean gold particle densities of different subcellular compartments.

For quantifying the immunogold particles labelling different $GABA_AR$ subunits, the 'mirror replica' technique was used. One face of the replica was labelled for Neuroligin-2 (NL-2) alone, or in a subset of experiments together with the guinea-pig anti-$GABA_AR$ β3 antibody. The complementary face of the replica was labelled for either the rabbit anti-$GABA_AR$ γ2, or the guinea pig anti-$GABA_AR$ γ2 and rabbit anti-$GABA_AR$ α1. On one face of the replica somata of layer 2/3 cells were randomly chosen at low magnification and images were taken at 15000X–25000X magnification for all NL-2-containing synapses and for the surrounding extrasynaptic regions. Then, the complementary face of the replica was scanned to locate the same soma based on nearby morphological landmarks, and the mirror half of the same synapses were imaged. The synaptic area in each image was delineated based on intramembrane particle (IMP) clusters and the NL-2 labelling on the P-face. The E-face image was then superimposed on the P-face image in Photoshop CS3, and the synaptic area was projected into the E-face image. Gold particles inside this polygon shaped synaptic area and up to 30 nm away from its edges were counted for NL-2, γ2, α1 and β3 subunits on both sides of a replica. Extrasynaptic gold particles were counted on the same imaged somatic surfaces. The nonspecific background labelling was calculated on either the E-face or the P-face

depending on the location of the epitope of a given antibody and subsequently subtracted from the mean gold particle densities.

Gold particle counting and area measurements were performed with iTEM software. Gold particle densities are presented as mean ± standard deviation (SD). Statistical comparisons were performed with STATISTICA software and significance was taken as $p < 0.05$.

Electrophysiological recordings of tonic $GABA_AR$-mediated currents

Electrophysiological recordings of tonic $GABA_AR$-mediated currents were conducted by my colleague, Dr. Mark D. Eyre. Somatic whole-cell voltage-clamp recordings (-70 mV) were were carried out using a mixed K-gluconate- and KCl-based intracellular solution. Kynurenic acid was used to inhibit ionotropic glutamate receptors. After initial stabilization and a baseline recording period of 6 minutes, 1 μM THIP was washed in for 18 minutes, and then followed by 20 μM SR95531. Cells were identified based on their morphology and their location in cortical layer 2/3 using IR-DIC optics. Additionally, a sequence of hyper- and depolarizing current injections was used to determine firing parameters. To define the change in holding current of each cell, 100 ms-long segments of holding current recordings, resampled every second, were binned into 1 minute intervals, and differences between the minute immediately preceding drug application and the second minute post-drug-wash in were calculated. Spontaneous IPSCs were recorded from INs and further analysed off-line with EVAN 1.5. After recordings, slices were fixed in 0.1 M PB containing 2% PFA and 15 v/v % PA for 24 hours prior to *post-hoc* visualization of the biocytin-filled cells by immunolabelling for mouse anti-Cre and rabbit-anti GFP primary antibodies.

RESULTS

Unique somato-dendritic distribution of the Kv4.2 subunits on hippocampal CA1 PCs

Light microscopic immunofluorescent reactions for the Kv4.2 subunit revealed a rather uniform labelling pattern throughout the stratum oriens (SO) and SR of the CA1 area of rat dorsal hippocampus with a slightly reduced fluorescent intensity in the SLM, which is in line with previous reports. In order to validate the specificity of the immunofluorescent reactions I repeated the labelling in wild-type and $Kv4.2^{-/-}$ mice. The labelling pattern in control mice was similar to that seen in rat, but the immunofluorescent signal was completely absent in the $Kv4.2^{-/-}$ mice, demonstrating that the immunolabelling at the light microscopic level is due to specific antibody-antigen interactions.

Electron microscopic analysis of the replicas revealed many gold particles labelling the Kv4.2 subunit on the P-face, which were apparently randomly distributed on the plasma membranes of somata and main apical dendrites of CA1 PCs. Quantitative analysis revealed a moderate distance-dependent increase in gold particle density along the proximo-distal axis of the main apical dendrites within the SR with a slight decrease in the dendrites of the SLM. The average relative increase from the proximal to distal dendrites within the SR was 69 ± 50 %. Similarly, gold particle density in oblique dendrites and dendritic spines within the SR and SLM showed an almost identical increasing-decreasing pattern to that found for the main apical trunks. In each subregion of the SR, the average densities in oblique dendrites and spines were only 26 ± 16 % higher than those found in the main apical trunks, demonstrating the lack of large quantitative differences in the densities of Kv4.2 subunit among these distinct dendritic compartments. Despite the increasing-decreasing tendency in the density of gold particles along the dendritic regions, statistical comparisons of the background subtracted densities revealed no significant difference among these dendritic compartments ($p = 0.08$, One-way ANOVA).

To validate the specificity of the immunogold labelling on SDS-FRL, the reactions were repeated in control and Kv4.2$^{-/-}$ mice. In control mice, the strength of the immunoreaction was similar to that obtained in rats. In contrast, in Kv4.2$^{-/-}$ mice the mean gold particle density on P-face structures was not significantly different from that obtained in the E-face structures around them ($p = 0.94$, One-way ANOVA).

Surprisingly, I observed that gold particles for the Kv4.2 subunit were not only confined to somato-dendritic plasma membranes, but were present in presumed presynaptic axon terminals at low (2.4 ± 0.6 gold particles/μm^2) but significant density compared to the background labelling (0.5 ± 0.4 gold/μm^2; $p < 0,01$ unpaired Student's *t*-test). The presence of significant immunolabelling in axon terminals is intriguing, because the Kv4.2 subunit is believed to be a somato-dendritic ion channel. To confirm the specificity of immunogold labelling on axon terminals, I repeated these experiments in control and Kv4.2$^{-/-}$ mice. The gold particle density for the Kv4.2 subunit in axon terminals was very similar in control and Kv4.2$^{-/-}$ mice (2.6 ± 1.6 gold/μm^2 and 2.2 ± 0.4 gold/μm^2; n = 3 mice; $p = 0.69$, unpaired Student's *t*-test). Taken together, these results reveal that the immunogold labelling in the somato-dendritic compartments is due to specific antibody-Kv4.2 subunit interactions; however, the same antibody under identical experimental conditions provides a weak, nonspecific labelling in axon terminals.

Finally, I investigated whether the Kv4.2 subunit is concentrated in glutamatergic or GABAergic synapses as suggested to occur in certain brain areas. I performed double-labelling with the excitatory synapse marker PSD-95 or the GABAergic synapse marker $GABA_AR\beta3$ subunit, respectively, but I could not find enrichment of Kv4.2 subunits in neither GABAergic nor glutamatergic synapses.

Distance-dependent increase in the density of Kir3.2 subunits in CA1 PC apical dendrites

In the CA1 area, the Kir3.1, Kir3.2 and Kir3.3 subunits showed a qualitatively similar fluorescent labelling pattern. The SO, SP and the proximal part of the SR was weakly labelled and the labelling intensity increased gradually towards the distal SR and SLM. Out of the three subunits, the overall intensity was highest for the Kir3.2 and weakest for the Kir3.3 subunit. These light microscopic observations are in line with those of previous reports. Because the Kir3.2 subunit is an essential component of the heteromeric Kir3 channels, I performed quantitative SDS-FRL of this subunit.

High-resolution electron microscopic analysis revealed only very few gold particles for the Kir3.2 subunit on the P-face membranes of somata and proximal apical dendrites, while more gold particles were observed on apical dendrites in distal SR and SLM. In SR, spiny oblique dendrites and dendritic spines were also moderately labelled. Following quantitative analysis of the Kir3.2 subunit, I found that the densities of gold particles in proximal, middle, distal apical dendrites and dendritic tufts in SLM showed an apparently linear increase as a function of distance from the soma. The densities of gold particles in oblique dendrites and dendritic spines showed a similar distance-dependent increase. Statistical comparisons of the background subtracted densities revealed significantly higher gold particle density for all subcellular compartments in the distal SR and SLM compared to PC somata (One-way ANOVA with Dunnett's *post hoc* test, $p < 0.05$).

To determine the density of gold particles in the AISs, I carried out double-labelling experiments with the AIS marker pan-Neurofascin and the Kir3.2 subunit. The density of gold particles in the AISs was not significantly different from background ($p < 0.001$; One-way ANOVA with Dunnett's *post-hoc* test: $p = 0.95$; $n = 3$ rats). Finally, I investigated the density of the Kir3.2 subunit in presynaptic nerve terminals in SR and SLM of the CA1 area. The densities of gold particles labelling for the Kir3.2 subunit on the axon terminals were not significantly different ($p < 0.001$; One-way ANOVA with Dunnett's *post-hoc* test: $p > 0.05$; $n = 3$ rats) from the background labelling.

Clustering of $GABA_ARs$ without the γ2 subunit in cortical perisomatic synapses

Electrophysiological experiments conducted by my colleague Dr. Mark D. Eyre revealed the presence of inhibitory synaptic currents in cortical layer 2/3 PC perisomatic synapses in the absence of the $GABA_AR$ γ2 subunit. Although this suggests that synaptic-like currents can be generated without the γ2 subunit, I have to emphasize that these currents cannot be taken as evidence that the underlying receptors are indeed concentrated within the postsynaptic specializations of GABAergic synapses. To gain insight into the localization, subunit composition and densities of $GABA_ARs$ underlying the remaining inhibitory postsynaptic currents, I performed light microscopic and electron microscopic SDS-FRL studies.

Light microscopic examination of the injection zone in the cortex of $GABA_AR\gamma2^{77I}lox$ mice revealed strong immunofluorescent labelling against the Cre-recombinase, which overlapped with a strong reduction in the immunoreactivity for the $GABA_AR$ γ2 subunit. In contrast, immunoreactivity for both $GABA_AR$ α1 and β3 subunits appeared unchanged within the injection zone at low magnifications. When examined at high magnification, immunofluorescent labelling for both the α1 and β3 subunits was punctate and of similar intensities for both Cre^+ and Cre^- layer 2/3 putative PCs. The lack of change in punctate immunofluorescent labelling for α1 and β3 subunits despite the absence of the γ2 subunit in Cre^+ cells suggest the possible synaptic clustering of $GABA_ARs$. Therefore it is conceivable that the remaining α and β subunits form αβ subunit-only heteropentameric receptor channels. However, application of low concentrations of Zn^{2+} (pharmacological experiments performed by Mark Eyre) failed to block mIPSCs in Cre^+ cells, indicating that αβ subunit-only receptors are unlikely to be responsible for the remaining mIPSCs following the γ2 subunit deletion. This raises the possibility that a different $GABA_AR$ subunit took the place of the γ2 subunit in the pentameric channel. Potentially the δ, ε, γ1 and γ3 subunits could associate with the remaining αβ subunits.

To test whether the γ2 subunit was replaced with the δ subunit, I performed fluorescent immunolabelling for the δ subunit in the virus-injected cortex. Surprisingly, I found increased immunosignal for the δ subunit within the Cre immunoreactive injection zone. At high magnification it was evident that a subset of cortical INs immunopositive for Cre showed strong immunoreactivity for the δ subunit in their plasma membrane. Indeed, Cre^+, PV-containing INs (PV INs) showed strong labelling for the δ subunit in their somato-dendritic membrane. In contrast, PV INs outside the injection area showed a rather cytoplasmic staining for the δ subunit. These results indicate that in the absence of the γ2

subunit, Cre^+, PV-containing, putative fast-spiking interneurons (FSINs) upregulate the δ subunit in their plasma membrane. Unfortunately, specific antibodies for the ε, γ1 and γ3 subunits were not available; therefore I could not test for the potential presence of these subunits in Cre^+ cells.

To gain insight into the subunit composition of $GABA_A$Rs in Cre^+ δ subunit-expressing putative FSINs, I performed immunofluorescent labelling for the α1 and α4 subunits. Immunoreactivity for the α1 subunit but not for the α4 subunit was present in the plasma membrane of Cre^+ δ subunit-expressing INs. Comparing the immunoreactivity for α1 subunit in Cre^+ and Cre^- δ subunit-expressing cells revealed no apparent change in fluorescent intensity. To reveal if the δ subunit can replace the γ2 subunit in GABAergic postsynaptic specializations, triple-labelling with the α1 and δ subunits, and gephyrin was made. Interestingly, gephyrin clusters were absent from the somatic plasma membrane of δ subunit-containing Cre^+ INs within the injection zone. In contrast, gephyrin clusters were present in the somata of Cre^- δ subunit-containing INs, where they co-localized with the α1 subunits but not with the δ subunit. Despite the absence of the major inhibitory synaptic scaffold molecule in Cre^+ putative FSINs, spontaneous IPSCs were present in Cre^+ FSINs (recordings performed by Mark Eyre). The presence of spontaneous IPSC in δ subunit-containing cells indicates that synaptic-like currents are preserved despite the absence of γ2 subunits in these cells. Although from these experiments one cannot conclude that the δ subunit is absent from the GABAergic synapses of Cre^+ putative FSINs, it is worth noting that THDOC and DS2 (both acting on δ subunit-containing receptors) did not have any effect on the amplitude and decay time constant of mIPSCs recorded from Cre^+ PCs and INs. Therefore, I suggest that the δ subunits present in the somato-dendritic compartments of Cre^+ putative FSINs are most likely localized to the extrasynaptic membrane. Indeed, tonic current recordings performed by Mark Eyre revealed that Cre^+ FSINs showed a larger outward shift in the holding current following SR95531 application compared to other cell types. Furthermore THIP, which selectively acts on δ subunit-containing $GABA_A$Rs, potentiated the tonic currents in Cre^+ FSINs. Although statistical comparisons did not show significant difference between Cre^+ and Cre^- cells ($p = 0.26$ for SR95531, $n = 9$; $p = 0.36$ for THIP application respectively, $n = 8$; Factorial ANOVA, *post-hoc* comparisons with Tukey's Unequal n HSD test), a trend towards larger tonic currents in Cre^+ FSINs was visible.

To provide direct evidence for the synaptic enrichment of $GABA_A$Rs in γ2 subunit-lacking cells, I carried out SDS-FRL of two $GABA_A$R subunits. Based on immunofluorescent data, the lack of the γ2 subunit in a given neuron is a good indicator of the presence of Cre-recombinase in the nucleus. For quantifications, I concentrated on perisomatic GABAergic

synapses, as paired recordings performed by Mark Eyre showed that unitary IPSCs could originate from the perisomatic synapses.

First, I randomly selected somata within the layer 2/3 of replica made from the virus-injected cortex, where I could find clusters of gold particles labelling for NL-2, indicating GABAergic synapses. Quantification of the γ2 subunit content of perisomatic synapses on the mirror half of the replica revealed two cell populations: γ2-negative ($\gamma2^-$) and γ2-positive ($\gamma2^+$) cells. These results provide evidence that the absence or presence of the γ2 subunit can be used to categorize cells within the injection zone as putative Cre^+ or Cre^-, respectively. After the cells were categorized as either $\gamma2^+$ or $\gamma2^-$, I quantitatively assessed the density of NL-2, α1 and β3 subunits, and compared the synaptic area in these two cell populations. The differences in synaptic density between $\gamma2^+$ and $\gamma2^-$ cells for NL-2, $GABA_AR\alpha1$ and $GABA_AR\beta3$ subunits were not statistically significant ($p > 0.05$; One-way ANOVA), despite the slight reduction in the mean synaptic densities found in $\gamma2^-$ cells. Interestingly, I found that the area of GABAergic somatic synapses was significantly ($p < 0.03$; Tukey's *post-hoc* test) smaller in $\gamma2^-$ cells ($0.018 \pm 0.008\ \mu m^2$; n = 113 synapses on 21 cells from two mice, pooled across α1 and β3 subunit experiments) compared to $\gamma2^+$ cells ($0.025 \pm 0.014\ \mu m^2$; n = 239 synapses on 18 cells from two mice, pooled across α1 and β3 subunit experiments). This indicates that the total number of synaptic NL-2, α1 and β3 subunits is somewhat reduced in $\gamma2^-$ cells.

In addition to the quantitative analysis of synaptic α1 and β3 subunits, I assessed the extrasynaptic labelling for these two subunits by counting gold particles outside the synaptic areas delineated on the P- or E-face membranes of the quantified cells. Extrasynaptic gold particle density for the α1 subunit was significantly reduced in the $\gamma2^-$ cells compared to $\gamma2^+$ cells ($p = 0.03$; One-way ANOVA), while the extrasynaptic β3 density did not change ($p = 0.75$; One-way ANOVA).

CONCLUSIONS

Conclusions for the first part of my dissertation are:

1. Distribution of immunogold particles for the Kv4.2 subunit shows homogenous distribution pattern along the proximo-distal axis of rat CA1 PCs.
2. The steep increase in I_A current density cannot be explained by a corresponding increase in channel number.
3. Probably other mechanisms are involved in the generation of I_A current density: such as interactions with auxiliary subunits (KChIPs and DPP6) and phosphorylation.

4. Distribution of immunogold particles for the Kir3.2 subunit shows a quasi linear increase from the soma towards the distal dendrites of CA1 PCs.
5. There is no significant difference in Kir3.2 density between the main apical dendrites, oblique dendrites or dendritic spines at approximately the same distance from the soma.

In the first part of my dissertation I revealed a previously unseen subcellular distribution pattern and density of two functionally different potassium channel (i.e. Kv4.2 and Kir3.2) subunits in the rat CA1 PCs. My results suggest that potassium channels regulate neuronal excitability in a compartment-specific manner.

Conclusions for the second part of my dissertation are:

1. Both α and β subunits are concentrated in inhibitory synapses following virus-mediated γ2 subunit deletion.
2. The density of gold particles labelling the synaptic α1 and β3 subunits is unchanged in cells lacking the γ2 subunit.
3. Pharmacological experiments performed by my colleague Dr. Mark D. Eyre indicate that in Cre^+ cells the γ3 subunit replaced the γ2 subunit in the heteropentameric receptor channels.
4. The $GABA_A$ receptor δ subunit is upregulated in the somato-dendritic plasma membrane of Cre^+ PV INs.

Experiments conducted in the second part of my dissertation revealed that postsynaptic $GABA_A$ receptor clustering can still occur in mouse cortical layer 2/3 neurons following γ2 subunit deletion

PUPLICATIONS

Publications that formed the basis of the dissertation:

Kerti-Szigeti K., Nusser Z., Eyre M.D. (2014) Synaptic clustering without the γ2 subunit. J. Neurosci., 34: 10219-10233.

Kirizs T., **Kerti-Szigeti K.**, Lorincz A., Nusser Z. (2014) Distinct axo-somato-dendritic distributions of three potassium channels in CA1 hippocampal pyramidal cells. Eur. J. Neurosci., 39: 1771-1783.

Kerti K, Lorincz A, Nusser Z. (2012) Unique somato-dendritic distribution pattern of $K_v4.2$ channels on hippocampal CA1 pyramidal cells. Eur. J. Neurosci., 35: 66-75.

Other publications:

Eyre MD, **Kerti K**, Nusser Z.s (2009) Molecular diversity of deep short-axon cells of the rat main olfactory bulb. Eur. J. Neurosci., 29: 1397-1407.