Low frequency pulse stimulation of Schaffer collaterals in Trpm4$^{-/-}$ knockout rats differently affects baseline BOLD signals in target regions of the right hippocampus but not BOLD responses at the site of stimulation

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Abstract

Electrical stimulation of right Schaffer collateral in Trpm4$^{-/-}$ knockout and wild type rats were used to study the role of Trpm4 channels for signal processing in the hippocampal formation. Stimulation induced neuronal activity was simultaneously monitored in the CA1 region by *in vivo* extracellular field recordings and in the entire brain by BOLD fMRI measurements. In wild type and Trpm4$^{-/-}$ knockout rats, consecutive 5 Hz pulse trains elicited similar neuronal responses in the CA1 region and similar BOLD responses in the stimulated right hippocampus. Stimulus-related positive BOLD responses were also found in the left dorsal hippocampus. In contrast to the right dorsal hippocampus, baseline BOLD signals in the left hippocampus significantly decreased during consecutive stimulation trains. Similarly, slowly developing significant declines in baseline BOLD signals, in absence of any positive BOLD responses, were also observed in the right entorhinal, right piriform cortex, right basolateral amygdala and right dorsal striatum whereas baseline BOLD signals remained almost stable in the corresponding left regions. Furthermore, significant declines in baseline BOLD signals were found in the prefrontal cortex and prelimbic/infralimbic cortex. Because significant baseline BOLD declines were only observed in target regions of the right dorsal hippocampus, it might reflect functional connectivity between these regions. In all observed regions the decline in baseline BOLD signals was significantly delayed and less pronounced in Trpm4$^{-/-}$ knockout rats when compared to wild type rats. Thus, either Trpm4 channels are involved in mediating these baseline BOLD shifts or functional connectivity of the hippocampus is impaired in Trpm4$^{-/-}$ knockout rats.

Keywords: In vivo electrophysiology, fMRI, BOLD, TRPM4, CA1

Introduction

The hippocampal formation, which encompasses the entorhinal cortex, dentate gyrus, hippocampus proper and subiculum, is crucially involved in a multitude of complex brain functions, such as learning, memory, pattern separation/completion and navigation. In order to purposefully target a loss or impairment of these brain functions, for example by deep brain stimulation in patients with neurodegenerative diseases, the significance of these hippocampal substructures as well as their mutual interactions must be understood. In the past, electrophysiological measurements have been successfully used to characterize changes in neuronal signal processing of specific hippocampal subfields upon selective stimulation. However, to detect how several substructures of the hippocampal formation interact with each other is still a challenging endeavor for electrophysiological studies, since they only record neuronal responses in the vicinity of the recording electrode. Functional MRI, in contrast, can monitor brain-wide neuronal activities, but only indirectly by measuring hemodynamic parameters, such as blood flow/volume or blood oxygenation level (BOLD), which in turn are controlled by changes in neuronal activity. Therefore, combining *in-vivo* electrophysiology and fMRI is a promising approach to overcome inherent limitations of each individual approach. To date, signal processing in substructures of the hippocampal formation and their impact on global network activities have been studied by four combined electrophysiology/fMRI approaches: (1) stimulation of the perforant pathway and electrophysiological recordings in the ipsilateral dentate gyrus (Angenstein et al., 2007; Canals et al., 2008), (2) stimulation of the CA3 region and recording in the contralateral CA1 (Moreno et al., 2016; Scherf and Angenstein, 2015), and recently (3) stimulation of the lateral entorhinal cortex and electrophysiological recording in the ipsilateral dentate gyrus (Krautwald et al., submitted) and (4) stimulation of the Schaffer collaterals and electrophysiological recordings in the ipsilateral CA1 region (Bovet-Carmona et al., 2018). The challenging part of the last approach is the close distance

between stimulation and recording electrode. Nevertheless, we were recently able to demonstrate that the first high frequency stimulation of the Schaffer collaterals elicited different BOLD responses in the ipsilateral hippocampus in wild type and *Trpm4*$^{-/-}$ knockout rats. Because this stimulation also differently affected expression of long-term potentiation in the CA1 region in wild type and *Trpm4*$^{-/-}$ knockout rats, it remained uncertain whether the observed difference in the initial BOLD responses reflects different signal processing or rather different existing baseline conditions.

In the current related study, we therefore stimulated Schaffer collaterals with repetitive pulses at 5Hz and monitored signal processing in and propagation through the hippocampus to examine an electrical stimulation that has been reported not to induce long-lasting changes in synaptic efficacy (Bienenstock et al., 1982).

Material and methods

Animals were cared for and used following the regulations of the European Community Council Directive (2010/63/EU). The experiments were approved by the animal care committees of the State of Saxony-Anhalt (No.: 42502-2-1218 DZNE) and were performed according to the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines. Trpm4 knockout rats (described in Bovet-Carmona et al…) of both gender and their respective wildtype controls were housed in conditions of constant temperature (23°C) and maintained on a controlled 12:12 h light/dark cycle, with food and tap water available ad libitum. For all experiments, 18 rats (9 *Trpm4* knockout rats, 9 wild type rats) were scanned.

Chronic implantation of electrodes in the hippocampal CA1-region

The animals were anesthetized using an Isoflurane/oxygen pump system (Iso-vet Surgivet 1000mg/g at a rate of 3% for induction and 1-2% maintenance; oxygen rate: 1/min). A drop in body temperature was prevented by a ventral heat pad during the whole surgery. Additional subcutaneous anesthetics were

applied on the shaved head (Xylocaine 2%, 1:200000 Adrenaline, AstraZeneca). Then, the animals were chronically implanted with custom-made stimulation- and recording electrodes as described earlier (Seidenbecher, Balschun, & Reymann, 1995). They consisted of Teflon-coated Tungsten wire (inner core diameter 0.05 μm and 0.075 μm, respectively; Advent Research Materials), and were connected to a miniature socket. The stereotaxic coordinates were determined by a dual standard digital stereotaxic instrument (Stoelting Co) and referenced to the Bregma for anterioposterior (AP) and mediolateral (ML) distance, and to the brain surface for dorsoventral (DV) distance. The bipolar stimulation electrode was implanted in the *Schaffer collaterals* of the right hippocampus (AP -4.4, ML 3, DV. 2-3) to evoke a field excitatory post-synaptic potential (fEPSP) that was recorded by a monopolar recording electrode implanted in the apical dendritic layer of *stratum radiatum* of the hippocampal CA1 area (AP -3.4, ML 2, DV 2-3). The depth of the electrodes was adjusted such that the fEPSP amplitude and slope were maximal, by using biphasic test-pulses of 200μA (0.2 ms pulse width). Additionally, silver wire was fixed on the contralateral side of the skull, serving as reference and ground electrodes. Once the fEPSP was stable, the whole assembly was fixed on the skull with dental cement (Paladur). Pain relief was ensured for 24h with 0.06 mg/kg Vetergesic (Ecu phar). The rats were group-housed until the surgery, after which they were placed individually to promote recovery and protect the head post. The correct position of the electrodes was observed in the magnetic resonance images (as exemplified in Figure 1A) and confirmed a posteriori via post-mortem histological analysis.

Electrical stimulation and functional MRI (fMRI)

All combined electrophysiology/fMRI measurements were performed on a 4.7 T Bruker Biospec 47/20 animal scanner (free bore of 20 cm) equipped with a BGA 09 (400mT/m) gradient system (Bruker BioSpin GmbH, Ettlingen, Germany). A 50mm Litzcage small animal imaging system (DotyScientific Inc., Columbus, SC, USA) was used for the RF signal reception.

All animals were initially anesthetized with isoflurane (2.0%; in 50:50 N_2:O_2; v:v), fixed into the head holder and connected to recording and stimulation electrodes. Then isoflurane concentration was reduced to 1.5% and a bolus of medetomidine (Dorbene, Pfizer GmbH, 50μg/kg) was subcutaneously injected. Ten minutes later isoflurane concentration was further reduced to 0.4% and continuous application of medetomidine (100μg/kg per h s.c.) started. Five minutes later isoflurane application was completely switched off.

All necessary MRI and electrophysiological adjustments for the simultaneous fMRI experiment were set in parallel before the measurements began. Breathing, heart rate and oxygen saturation were monitored throughout the experiment by an MRI-compatible pulse oximeter (MouseOX™; Starr Life Sciences Corp., Pittsburgh, PA, USA). Heating was provided from the ventral site. All measured physiological parameter remained constant during the fMRI session, i.e., electrical Schaffer collateral stimulation had no effect on breathing (about 60 breath/min), heart rate and systemic blood oxygen saturation.

The right Schaffer collaterals were stimulated with 20 consecutive stimulation trains; the first train was presented 2min after starting the fMRI session. Each train lasted 8s and consisted of 40 pulses with an interpulse interval of 200 ms (i.e., 5 Hz). Bipolar pulses with of 0.2 ms duration and an intensity of 150μA were used to stimulate the Schaffer collaterals. Trigger pulses that were generated by the scanner at the beginning of every volume, i.e., every 2 s, were used to synchronize fMR-image acquisition and electrophysiological stimulation. The total scanning time for one fMRI experiment was 22 min.

The electrophysiological responses during stimulation were recorded with a sample rate of 5000 Hz, filtered between 1 and 5000 Hz by using a differential amplifier EX 4-400 (Science Products, Hofheim, Germany), transformed by an analogue-to-digital interface (power-CED, Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer. No further processing filter was needed because the artifacts caused by the imaging system were very small in comparison with the recorded field potential.

For anatomical images, 10 horizontal T_2-weighted spin-echo images were obtained with a *RARE* sequence (rapid acquisition relaxation enhanced (Hennig et al., 1986)) using the following parameters: TR 4000 ms, TE 15 ms, slice thickness 0.8 mm, FOV 37x37 mm, matrix 256x256, RARE factor 8, and number of averages four. The total scanning time was 8min 32 s. Functional MRI (fMRI) was performed with a gradient-echo EPI (echo planar imaging) sequence with the following parameters: TR 2000 ms, TE 24 ms, slice thickness 0.8 mm, FOV 37x37 mm, matrix 92x92, and total scanning time per frame 2 s. The slice geometry, i.e., ten horizontal slices, was identical to the previously obtained anatomical spin-echo-images.

Data processing and analysis

The functional data were loaded and converted into BrainVoyager data format. A standard sequence of preprocessing steps implemented in the BrainVoyager QX 2.6.1 software (Brain Innovation, Maastricht, The Netherlands), such as slice scan time correction, 3D motion correction (trilinear interpolation and reduced data using the first volume as a reference) and temporal filtering (FWHM 3 data points), were applied to each data set. Because the reconstruction of the fMRI images resulted in a 128 x 128 matrix (instead of the 92 x 92 imaging matrix), a spatial smoothing (Gaussian filter of 1.4 voxel) was added.

GLM analysis: each individual functional data set was used for a multi-subject GLM analysis implemented in BrainVoyager QX 2.6.1 software. Functional activation was analyzed by using the correlation of the observed BOLD signal intensity changes in each voxel with a predictor (hemodynamic response function), generated from the given stimulus protocol (see above); based on this, the appropriate 3D activation map could be generated. To calculate the predictor, the square wave representing stimulus on and off conditions was convolved with a double gamma hemodynamic response function (onset 0 s, time to response peak 5 s, time to undershoot peak 15 s). To exclude false-positive voxels, a correction for serial correlation (csc) was performed (implemented in the BrainVoyager QX 2.6.1 software) and we only considered those with a significance level (p) above the threshold set by

calculating the false discovery rate (FDR) with a q-value of 0.01 (which corresponds to a t value greater than 3.5 or $p < 5.1x10^{-4}$).

VOI analysis: each individual functional imaging data set was aligned to a 3D standard rat brain using the 3D volume tool implemented in BrainVoyager QX 2.6.1 software. Individual VOIs, i.e., right/left hippocampus, right/left dorsal hippocampus, anterior cingulum/prefrontal cortex region, were marked in the 3D standard rat brain. The averaged BOLD time series of all voxels located in one VOI was then calculated for each individual animal using the volume-of-interest-analysis tool implemented in the BrainVoyager QX 2.6.1 software (Figure 1C). Each individual BOLD time series was normalized using the averaged BOLD signal intensity of 100%. All normalized BOLD time series were then averaged and depicted as mean BOLD time series ± SEM. Based on the calculated BOLD time series, event-related BOLD responses were calculated by measuring the signal intensities starting six frames before stimulus onset (-12 s until 0 s), during stimulus presentation (between 0 s and 8 s, which corresponds to four frames) and the following 15 frames (8s to 38 s) after the end of the stimulus. To avoid the confounding effect of putative variations in baseline BOLD signal intensities on the calculated BOLD response (i.e., BOLD signal $_{stimulus}$/BOLD signal $_{baseline}$ * 100%), each BOLD response was related to BOLD signal intensities of the stimulus over the preceding 12 s.

Results

In the present study we electrically stimulated the right Schaffer collaterals to directly activate the adjacent right hippocampal CA1 subregion. The presence of two closely positioned electrodes only caused small artifacts (Figure 1A, red arrows)similar to two electrodes with further distance to each other (Scherf and Angenstein, 2015). As shown in Figure 1B, artifacts in electrophysiological recordings that are induced by the gradient system during acquisition were minor in comparison to the recorded neuronal response. Furthermore, when the scanner triggered the stimulation onset, these artifacts can be easily placed outside the recorded neuronal response, thus no

preprocessing of the electrophysiological recordings was required. BOLD signal changes were calculated for individual regions of interest (i.e., right and left dorsal hippocampus and medial prefrontal cortex) or for clusters of significantly activated voxels. Figure 1C depicts an example is for the right hippocampus.

Repetitive stimulation of the Schaffer collaterals with 20 trains of 40 pulses with an inter-pulse interval of 200 ms (i.e., 5 Hz) did not cause long-lasting changes in synaptic responses of pyramidal cells in the CA1 region (WT mean = 117.52 ± 10.26, *Trpm4* knockout mean = 96.33 ± 8.77; no significant difference between the two groups, t = 1.378, p = 0.19, Student t-test; Figure 2A, upper panel). Thus, the fEPSP slopes to a given test pulse after the fMRI session were not significantly different from the responses to an identical test pulse given immediately before the fMRI session (individual traces for each group in Figure 2A, lower panel).

Within each individual train neuronal responses to consecutive pulses varied (Figure 2B). In general, the neuronal response pattern during the first train differed from the response pattern during all subsequent stimulation trains, i.e., the incoming pulses were differently processed during the first stimulation period when compared to all subsequent trains. During the first stimulation train 40 consecutive pulses only caused a transient augmentation of postsynaptic responses (Figure 2C left), whereas during all subsequent stimulation trains a continuous augmentation of postsynaptic responses was induced (Figure 2C right). The stimulus-induced increase in postsynaptic activity did not persist during the following 52s, the time between two stimulation trains, thus the response to the first pulse in the subsequent train was not elevated. During the first and all subsequent stimulation trains, stimulus-induced neuronal responses in the CA1 were similar in wild type and $Trpm4^{-/-}$ knockout rats.

Concurrently with neuronal responses in CA1, 5 Hz stimulation of the right Schaffer collaterals elicited significant BOLD responses in a cluster that included the entire right dorsal hippocampus and left dorsal hippocampus. However, in contrast to the pyramidal cell responses induced in the CA1 region,

the development of BOLD responses apparently differed between wild type and Trpm4$^{-/-}$ knockout rats. In wild type rats all 20 consecutive stimulation trains elicited similar BOLD responses in the right dorsal hippocampus whereas in the left dorsal hippocampus the first stimulation train elicited a stronger BOLD response than almost all subsequently applied stimulation trains (Figure 3, left in blue). In contrast to that, the same stimulation procedure triggered in the right dorsal hippocampus of Trpm4$^{-/-}$ knockout rats first a significantly stronger BOLD response than during almost all subsequent stimulation trains. The concurrently induced BOLD responses in the left dorsal hippocampus of Trpm4$^{-/-}$ animals remained, however, similar (Figure 3, left, in red). Performing a second level analysis to detect differences in the distribution of significant BOLD responses between the two groups revealed a significantly larger spreading in Trpm4$^{-/-}$ knockout rats. This larger spread of significantly activated voxels occurred mainly in regions adjacent to the dorsal right hippocampus such as the mediodorsal thalamus (Figure 3, top right). Individual BOLD responses in this region were significantly stronger in Trpm4$^{-/-}$ knockout rats when compared to wild-type rats and varied during repetitive stimulation trains (Figure 3, bottom right). Thus voxels outside the dorsal right and left hippocampus confound the results that are attributed to the dorsal hippocampus in the GLM analysis.

In addition, the different development of BOLD responses in Trpm4$^{-/-}$ knockout rats were obviously not related to congeneric differences in pyramidal cell activities as they were similar in these two groups. To check whether measured BOLD responses in fact reflect dorsal hippocampal activity, we subsequently performed a VOI analysis to assign BOLD signal changes to the dorsal hippocampus and other anatomical structures. As Trpm4 channels are also assumed to control myogenic responsiveness of parenchymal arterioles (Li et al., 2014), we performed this analysis without any baseline correction algorithms to detect possible stimulus-induced variations in baseline BOLD signals, i.e., BOLD signals between individual stimulation periods. According to the VOI analysis, repetitive 5 Hz pulse stimulations of the right Schaffer collaterals caused in wild type rats uniform BOLD responses in the left and right dorsal hippocampus (Figure 4).

Baseline BOLD signals in the stimulated right dorsal hippocampus of wild-type animals were almost stable and only significantly declined after the 19th stimulation train. In contrast, baseline BOLD signal intensities rapidly declined in the left (i.e., contralateral) dorsal hippocampus (significant after the third stimulation train), right entorhinal cortex (significant after the 5th stimulation train), right piriform cortex (significant after the 6th stimulation train), right basolateral amygdala (significant after the 8th stimulation train) and dorsal striatum significant after the 9th stimulation train). In contrast to the left dorsal hippocampus no clear decline of baseline BOLD signal intensities were observed in all other corresponding left (contralateral) regions. In midline structures baseline BOLD signal intensities declined in the medial prefrontal cortex (after train 15) and in the prelimbic/infralimbic cortex (after train 16), whereas in the septum baseline signal intensities remained stable during the entire experiment (Figure 4, blue lines).

In Trpm4$^{-/-}$ knockout rats the same stimulation protocol also caused uniform BOLD responses in the right and left dorsal hippocampus and a similar development of baseline BOLD signals in the stimulated right dorsal hippocampus. In contrast to wild type rats, the decline of baseline BOLD signals in the left hippocampus was less distinguished and delayed (significant only after train 8). A similar time course was detected in the right entorhinal cortex (significant only after train 7). In the right basolateral amygdala only a transient decline in baseline BOLD signal intensities was observed and no decline in medial prefrontal and prelimbic/infralimbic cortex. In all other analyzed regions, the development of baseline BOLD signal intensities was similar although always slightly delayed (Figure 4, red lines).

Discussion

In this study we combined *in vivo* electrophysiology and fMRI to monitor the development and spreading of neuronal activity in the CA1 and entire hippocampal formation during low frequency stimulation of Schaffer collaterals in wild type and Trpm4$^{-/-}$ knockout rats. The frequency of 5 Hz was chosen

because different lines of evidence indicate that such electrical stimulation has only minor effects on long-lasting changes in synaptic efficacy (Bienenstock et al., 1982). By using this rather 'plasticity-neutral' frequency, the current study complements a recent similar study in which high-frequency pulse stimulations were used to induce long-lasting changes in synaptic efficacy of Schaffer collateral CA1 pyramidal cell synapses (Bovet-Carmona et al., 2018).

The main findings can be summarized as follows: (1) consecutive stimulations of Schaffer collaterals with short 5 Hz pulse trains elicited similar pyramidal cell responses in the ipsilateral CA1 region of wild type and Trpm4$^{-/-}$ knockout rats. (2) In line with the induced neuronal activities, the development of corresponding BOLD responses in the right dorsal hippocampus was similar in wild type and Trpm4$^{-/-}$ knockout rats. (3) Repetitive short lasting 5Hz pulse stimulations caused a sustained significant decrease in baseline BOLD signals in several target regions of the right dorsal hippocampus, but only marginally in the right dorsal hippocampus. (4) Declines in baseline BOLD signals were delayed in Trpm4$^{-/-}$ knockout rats and less pronounced in the left dorsal hippocampus and right entorhinal cortex.

Electrical stimulation of Schaffer collaterals activates orthodromic pyramidal cells in the ipsilateral CA1 region and antidromic pyramidal cells in the ipsilateral CA3 region. These direct neuronal activations may then be propagated to regions that in turn receive projections from CA1 neurons (i.e., subiculum, deep layers of the entorhinal cortex) or CA3 neurons (i.e., contralateral CA3). Eventually, electrical stimulation of right Schaffer collaterals activates neuronal circuits in the hippocampal formation as well as brain wide neuronal networks. Conceptually, this approach is closely related to previous studies that performed fMRI during electrical stimulation of the CA3 region and recordings at the contralateral CA1 region (Moreno et al., 2016; Scherf and Angenstein, 2015, 2017). In both approaches the interaction between CA3 and CA1 pyramidal cells was targeted by electrical stimulations of CA3 pyramidal cells or their axons. Accordingly, the spatial distribution of significant BOLD responses was similar, i.e., significant BOLD responses appeared in the

ipsilateral (i.e., stimulated) and contralateral (i.e., unstimulated) dorsal hippocampus. However, in contrast to Schaffer collateral stimulation, repetitive stimulation of the CA3 region with pulse frequencies higher than 2 Hz often results in the appearance of neuronal afterdischarges (Angenstein, 2018). Similarly, continuous strong neuronal activities were observed in the CA1 region after direct stimulation of the CA3 region with 5, 10, 20 or 40 Hz pulse trains. Thus neuronal activity in the CA1 region during CA3 stimulation was virtually indistinguishable from the neuronal activity during the subsequent stimulus free interval (see Figure S3 in (Moreno et al., 2016). In the current study, electrical stimulation of Schaffer collaterals with pure 5 Hz pulses induced one synaptic response to each pulse (Figure 1) and no synchronized pyramidal cell activity after cessation of stimulation. Likewise, no neuronal afterdischarges were observed in the CA1 region after stimulation of Schaffer collaterals with several short bursts of 200Hz pulses (Bovet-Carmona et al., 2018). Thus, Schaffer collateral stimulation appears to be more useful to study frequency-related propagations of signals through the hippocampus proper, because of its lower propensity to generate neuronal afterdischarges.

In contrast to the previous study that used 3 consecutive trains with 200 Hz pulses (Bovet-Carmona et al., 2018), consecutive trains of 5 Hz pulse stimulation caused similar BOLD responses in Trpm4$^{-/-}$ knockout rats and wild type rats (Figure 3). Although consecutive pulses during the first stimulation train again elicited a qualitatively different neuronal response pattern than during all subsequent stimulation trains, the resulting BOLD response remained similar. Our results also indicate that defining the anatomical structure of interest by VOI analysis (e.g., the dorsal hippocampus) is more informative than only considering clusters of significantly activated voxels. These clusters are obviously not restricted to a defined anatomical structure and therefore a meaningful interpretation of cluster activation data in terms of dorsal hippocampus activation is not unambiguous.

Unlike 200 Hz pulse burst stimulations, short lasting 5 Hz pulse stimulations did not induce long-lasting changes in synaptic efficacy. Thus, it is reasonable

to assume that metabolic changes that mediate the induction of long-lasting changes in synaptic functions were not activated during the current experiments and in accordance with that induced BOLD responses remained similar. Although the relation between input activity was similar during all stimulation trains, while the resultant postsynaptic activation differed after the first stimulation period, the corresponding BOLD responses were similar. The pattern of postsynaptic activities should remain similar as long the input activity is processed in an identical manner, but should differ when the postsynaptic processing is altered. Thus, during this stimulation condition the size of the BOLD response depends on the summarized input and/or the summarized postsynaptic activity (which remains on average similar) rather than on the quality of signal processing in the CA1 region (which is different between the first and all subsequent stimulation trains). During 200 Hz pulse burst stimulation the response to the first stimulation period differed when compared to subsequent identical stimulations (Bovet-Carmona et al., 2018). Thus it appears that during high-frequency stimulation mechanisms that mediate long-lasting changes in synaptic efficacy also affect the formation of BOLD responses, whereas mechanisms that only control short-lasting (i.e., less than 60s) changes in synaptic efficacy are not effective.

In *Trpm4*$^{-/-}$ knockout rats and their controls, stimulation of Schaffer collaterals with consecutive 5 Hz pulse trains only generated positive BOLD responses in the ipsilateral (right) and contralateral (left) dorsal hippocampus but not in any other region outside the hippocampal formation. As mentioned above, orthodromically activated CA1 neurons mainly project to the adjacent subiculum, which in turn is the main output structure of the hippocampal formation, and to deep layers of the entorhinal cortex, which project to numerous cortical and subcortical structures (Amaral and Lavenex, 2007). Antidromically activated CA3 neurons are likely responsible for the efficient transfer of activity to the contralateral hippocampus via commissural fibers.

Without concurrent electrophysiological measurements, one can only speculate about induced neuronal activities in target regions of the right CA1,

but they were obviously below the threshold for triggering significant positive BOLD responses. Nevertheless, in some of these target regions, namely the right entorhinal cortex, basolateral amygdala, nucleus accumbens, medial prefrontal, prelimbic/infralimbic cortex, the stimulation induced a slowly developing significant decline in baseline BOLD signals. A decline in baseline BOLD signals, if physiological and not artificially induced during image acquisition, primarily reflects a decrease in oxygenated hemoglobin, which in turn may be induced by several mechanisms. Drifts in baseline BOLD signals were often observed during gradient echo fMRI experiments (Evans et al., 2015) and were originally attributed to scanner instabilities (Smith et al., 1999), incomplete motion correction (Bandettini et al., 1993), but later also to neurophysiological processes (Yan et al., 2009). In our experiments we detected both, regional and group specific drifts in baseline BOLD signals (Figure 4), thus general effects on the hemodynamic (e.g., as a result of prolonged sedation by medetomidine) or scanner-related instabilities are unlikely. In particular, the strongest decline in baseline BOLD signals occurred in the right entorhinal cortex of wild type rats and to a significantly lower degree in Trpm4$^{-/-}$ knockout rats, whereas in the left entorhinal cortex baseline BOLD signals remained unchanged in the two groups. Similarly, in the left dorsal hippocampus baseline BOLD signals significantly declined, whereas this was not observed in the right dorsal hippocampus.

The two regions, right entorhinal cortex and left dorsal hippocampus, are primary targets of activated CA1 and CA3 principal neurons, thus the slowly developing decline of baseline BOLD signals in these two regions is controlled by these periodic (i.e., 8s long) activation of neurons in the right dorsal hippocampus. The neurophysiological basis for the regional specific development of baseline BOLD signals remains unknown but may also partially depend on TRPM4 channels, because in absence of functional TRPM4 channels the decline was significantly delayed and less pronounced. Furthermore, stimulus-induced activity in CA1 pyramidal cells, i.e., the principal neurons in this region, is unlikely the origin for baseline BOLD signal decline in regions outside the stimulated right hippocampus proper. The

electrophysiological CA1 pyramidal responses to 5Hz pulse stimulation were virtually indistinguishable in wild type and *Trpm4*$^{-/-}$ knockout rats but the baseline BOLD drift in the left hippocampus and right entorhinal cortex differed. Thus, it appears that other projecting neurons are involved. Among them could be GABAergic long-range projecting neurons. These neurons are located in the CA1 region, project either to the medial septum and/or subiculum and retrosplenial cortex, and fire at frequencies between 4-10 Hz during theta oscillations, i.e. in the same frequency range as used in the current experiment (Jinno, 2009; Jinno et al., 2007; Katona et al., 2017). TRPM4 channels are expressed in GABAergic interneurons in the medial prefrontal cortex (Riquelme et al., 2018), but it is still not known if TRPM4 channels are also expressed in these GABAergic long-range projecting neurons. When GABAergic long-range projecting neurons are involved in mediating this decline of baseline BOLD signals their sole activation is not sufficient. One of the target regions of these neurons is the medial septum, a region in which the most stable baseline BOLD signal was detected in the current study. Thus, for the development of baseline BOLD signals, it appears more important how GABAergic inputs affect ongoing local neuronal activity rather than the amount of input activity itself.

In addition to the left dorsal hippocampus and right entorhinal/retrosplenial cortex significant declines in baseline BOLD signals were detected in the right piriform cortex, right nucleus accumbens, right dorsal striatum, right basolateral amygdala as well as in the medial prefrontal sng prelimbic/infralimbic cortex. Neuronal activities in these regions are likely affected by subicular and/or entorhinal projecting neurons, thus the mediating mechanism might be similar as in the left dorsal hippocampus and entorhinal/retrosplenial cortex. That would also mean that the decline in baseline BOLD signals is not necessarily related to reduced neuronal activities (Shmuel et al., 2006) but only indicates an altered neuronal baseline activity. Obviously, in the left dorsal hippocampus all stimulation trains caused increased neuronal activity, as indicated by significant positive BOLD responses, even though baseline BOLD signals continued to decline.

These differences in the decline of baseline BOLD signals between wild type and *Trpm4*$^{-/-}$ knockout rats resulted in genotype differences in the correlation of BOLD time series between these regions. If we suppose that this correlation of BOLD time series represents a valid measure of functional connectivity, it indicates that in Trpm4$^{-/-}$ knockout rats repetitive short 5Hz pulse stimulations activate a smaller brain-wide neuronal network than in wild type rats (Table 1, 2). If we further assume that the decline in baseline BOLD signals is at least partly related to the activity of GABAergic long-range projecting neurons, these correlations would reflect differences in global acting inhibitory circuits. The 5Hz pulse stimulation used in our study is in the theta frequency range, a frequency assumed to be involved in synchronizing neuronal activities in the hippocampus and prefrontal cortex during working memory tasks (Benchenane et al., 2011; Colgin, 2011; Fujisawa and Buzsaki, 2011; Harris and Gordon, 2015; Hasselmo, 2005). Hence, an altered level of brain wide inhibitory neuronal network during theta oscillations might also affect learning performance which would fit to the observation that Trpm4$^{-/-}$ knockout rats show distinct deficits in spatial working and reference memory tests (Bovet-Carmona et al., 2018).

Conclusions

Analyzing BOLD time series in wild type and *Trpm4*$^{-/-}$ knockout rats during repetitive short lasting 5Hz pulse stimulation of Schaffer collateral revealed distinct changes in various target regions of the right dorsal hippocampus but not in the stimulated right dorsal hippocampus itself. Whereas in the right dorsal hippocampus repetitive stimulation elicited uniform positive BOLD responses with minor effects on baseline BOLD signals, baseline BOLD signals significantly declined in several but not in all target regions of the right dorsal hippocampus. The decline was significantly delayed and less pronounced in *Trpm4*$^{-/-}$ knockout rats. The development of baseline BOLD signals is unlikely to be related to a general decrease in neuronal activity as in the left dorsal hippocampus the decline occurred parallel to the appearance of positive stimulus-related BOLD responses. Thus, to characterize putative effects of

hippocampal stimulation on global neuronal networks in different experimental groups, such as genetically modified rats or mice, the analysis of baseline BOLD signals variations in various brain regions should complement stimulus-induced positive BOLD responses.

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Figure Legends

Figure 1

Experimental setup and recorded data. During fMRI the right Schaffer collaterals were stimulated with 8s long 5Hz pulse trains and the neuronal responses were recorded in the ipsilateral CA1 region. **A** The presence of two closely spaced electrodes (red arrow) results in minor artifacts in GE-EPI. **B** Field potentials recorded in the ipsilateral CA1 region during fMRI. Each of the 80 pulses elicited one fEPSP (upper panel). Magnification revealed the ratio between synaptic response and gradient artifact (gray arrows). The recordings are superimposed by 50Hz noise. **C** Stimulus-induced BOLD signal changes were calculated by a general linear model (GLM) analysis or by volume of interest (VOI) analysis. The resultant BOLD time series only considers BOLD signal changes of either significantly activated voxels (GLM, blue graph) or all voxels (VOI red graph) in the right hippocampus.

Figure 2

Electrical Stimulation of right Schaffer collaterals with repetitive 5Hz pulse trains elicited similar field responses in the right CA1 of wild type and Trpm4$^{-/-}$ knockout rats. **A** The stimulation protocol caused no long-lasting changes in the fEPSP slopes. Top: Relative changes in synaptic responses after repetitive stimulation of the Schaffer collaterals during the fMRI experiment in wild type (blue) and *Trpm4* knockout rats (red) when compared to a test pulse given before the fMRI experiment. Bottom: Representative examples of analogue traces after receiving a test pulse before (black graph) and after the fMRI

experiment (colored). **B** Relative changes of fEPSP slopes in wild type and Trpm4$^{-/-}$ knockout rats during the entire fMRI experiment. A consistent response pattern developed after the first stimulation period. Note that the response to the first pulse in each train remained almost stable during all consecutive stimulation trains. Depicted are only the responses to all pulses during the stimulation period (i.e., 8 s), the time between individual stimulation periods are not included. **C** Magnification of the development of consecutive fEPSP slopes during the first and last stimulation train.

Figure 3

GLM analysis to map spatial distribution of significant BOLD responses induced by repetitive trains of 5 Hz pulses. Stimulation of the right Schaffer collaterals with consecutive 5 Hz pulse trains induced significant BOLD responses in the right and left dorsal hippocampus. Calculating the difference between BOLD activations in wild type and *Trpm4* knockout revealed a spatially significant larger activation in *Trpm4* knockout rats when compared to wild type rats (top right side). BOLD time series of all significantly activated voxels in the left (left side top) and right dorsal hippocampus (left side bottom) of wild type (blue graphs, n=9) and *Trpm4* knockout rats (red graphs, n=9). The black line indicates significant different BOLD response to the first BOLD response. Note that these BOLD time series results from GLM analysis that corrected for baseline drifts. Right side superimposes the first BOLD response with the average BOLD response of train 3-20). BOLD time series of the region that is additionally activated in Trpm4-/- knockout rats (see 3D image above) is depicted at the right bottom. Note that in this region that is located outside the hippocampal formation BOLD responses also vary during consecutive stimulation trains.

Figure 4

Volume of interest analysis to depict BOLD time series in individual structures during the entire fMRI experiment in wild type (blue graphs) and Trpm4$^{-/-}$ knockout rats (red graphs). A significant decline of BOLD signals below the initial value is depicted by a horizontal bar above the graph. A green bar indicates significant different BOLD signals in wild type and Trpm4$^{-/-}$ knockout rats (in the left dorsal hippocampus and right entorhinal cortex). These BOLD time series were used for the correlation analysis summarized in Table 1 and 2.