NeuroImage 202 (2019) 116082

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

The role of ongoing neuronal activity for baseline and stimulus-induced BOLD signals in the rat hippocampus

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ARTICLE INFO

Keywords:
BOLD fMRI
Electrophysiology
Negative BOLD

ABSTRACT

To understand how ongoing neuronal activity affects baseline BOLD signals, neuronal and resultant fMRI responses were simultaneously recorded in the right hippocampus of male rats during continuous low-frequency (2 or 4 Hz) pulse stimulation of the right perforant pathway. Despite continuously increased neuronal activity, BOLD signals only transiently increased in the hippocampus and subsequently returned to either the initial level (2 Hz) or even to a consistently lower level (4 Hz). Whereas the initially transient increase in BOLD signals coincided with an increased spiking of granule cells, the subsequent reduction of BOLD signals was independent of granule cell spiking activity but coincided with persistent inhibition of granule cell excitability, i.e., with reduced postsynaptic activity and prolonged population spike latency. The decline in BOLD signals occurred in the presence of an elevated local cerebral blood volume (CBV), thus the reduction of granule cell excitability is attended by high oxygen consumption. When previous or current stimulations lessen baseline BOLD signals, subsequent short stimulation periods only elicited attenuated BOLD responses, even when actual spiking activity of granule cells was similar. Thus, the quality of stimulus-induced BOLD responses critically depends on the current existing inhibitory activity, which closely relates to baseline BOLD signals. Thus, a meaningful interpretation of stimulus-induced BOLD responses should consider slowly developing variations in baseline BOLD signals; therefore, baseline correction tools should be cautiously used for fMRI data analysis.

1. Introduction

Variations in BOLD (blood oxygen level dependent) signals are generally accepted as a marker for changed neuronal activities in particular brain regions (e.g., during resting state fMRI) and furthermore, stimulus- or event-related changes in BOLD signals in particular brain regions (BOLD response) are considered as proof for an active role of these brain structures in the processing of the stimulus. However, variations in fMRI-BOLD signals only reflect complex hemodynamic responses (i.e., changes in blood flow, blood volume and blood oxygenation) to an altered neuronal activity. Furthermore, there is a wide difference between the speed of neuronal and related hemodynamic responses. Thus, a measured fMRI-BOLD response (in one particular voxel) does not only spatially average neuronal activities of all neurons (principal, interneurons) and related glia activities in this voxel, but also temporally sums up (or averages) all cell activities during the period in which one frame (usually 1–3 s) is acquired. For this reason, it is easier to predict from a given change in neuronal activity the resulting hemodynamic responses (convolution analysis) than to predict from a measured hemodynamic response the underlying changes in neuronal activities (deconvolution analysis). To develop, refine and verify convolution and deconvolution models, experimental approaches are required that are not only (relatively) simple in terms of limited population of neurons and connections, but that also allow for defined adjustments of neuronal activities and simultaneous measurement of the resulting BOLD response.

Such a suitable experimental approach originated from a well-established electrophysiological setup to monitor elicited neuronal activities in the dentate gyrus during stimulation of the perforant pathway (Andersen et al., 1966; Krug et al., 1983; McNaughton and Barnes, 1977), which was performed in a MRI scanner during acquisition of T_2*-weighted images (Angenstein et al., 2007). In this approach, neurons in the dentate gyrus, hippocampus proper and subiculum are monosynaptically activated during each applied electrical pulse. In addition, neurons in the hippocampus proper and subiculum are also activated by

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https://doi.org/10.1016/j.neuroimage.2019.116082
Received 19 March 2019; Received in revised form 18 June 2019; Accepted 6 August 2019
Available online 16 August 2019

intra-hippocampal connections, known as the trisynaptic circuit (Amaral and Lavenex, 2007). Thus, by defined adjustments of the stimulation parameters (e.g., variation in pulse width, intensity and number), neuronal activities in the hippocampus can be directly modified while the consequence on the generated fMRI-BOLD response is simultaneously measured. Using this approach, it became clear that the BOLD response is not linearly related to the input or output activity, but rather depends on the quality of localized signal processing (Angenstein et al., 2009). However, it also became obvious that other factors also control the quality of the formed BOLD response, namely the history of previous stimulations (Angenstein et al., 2010) or the current excitability of activated neurons (Angenstein, 2014). Certainly, these factors also affect the processing of the incoming stimulus, but their actual impact is often unknown in human fMRI studies using complex stimuli, e.g., during cognitive studies. Another crucial factor that may influence the formation of a stimulus-induced BOLD response is the amount neuronal background activity that concomitantly exists when the stimulus of interest arrives. Under conditions of ongoing increased neuronal activity, BOLD signals could also remain elevated; i.e., baseline BOLD signals would change. Thus, because of altered baseline BOLD signals a stimulus-induced increase in neuronal activity could potentially induce a different BOLD response as under conditions when no elevated neuronal activity is present. Such a mechanism could explain why identical stimuli can trigger different BOLD responses when it is given under different conditions. Therefore, we determined in a first step how ongoing elevated neuronal activity modifies baseline BOLD signals.

In a second step we then determined how an ongoing elevated neuronal activity affects the formation of BOLD responses to a superimposed short stimulation period. For that, neuronal and BOLD responses to short stimulation periods were measured in the presence or absence of continuous 2 or 4 Hz pulse stimulations. In case a stimulus-induced BOLD response mainly depends on the incoming activity the presence of continuously elevated neuronal activity should scarcely affect the BOLD response. On the other hand, when the amount of neuronal background activity is crucial for the formation of a stimulus-induced BOLD response, BOLD responses to identical stimuli should differ in presence or absence of continuous low frequency pulse stimulation even when an increase in induced neuronal activities were similar.

2. Material & methods

2.1. Animals and surgical procedure

For electrode implantation, nine to ten-week-old male Wistar rats were anesthetized with pentobarbital (40 mg/kg intraperitoneal) and placed into a stereotactic frame. A bipolar stimulation electrode (114 μm in diameter, made from Teflon-coated tungsten wire) was placed into the perforant pathway in the right hemisphere at the coordinates AP: −7.4, ML: 4.1 mm from the bregma, DV: 2.0–2.3 mm from the dural surface. Stimulation of the perforant pathway at this position elicited field potentials in the dentate gyrus that were characteristic for medial perforant pathway fiber stimulation. When the bipolar stimulation electrode was placed in the right hemisphere at the coordinates: AP: −7.4, ML: 5.0 mm from the bregma, DV: 2.6 mm from the dural surface, stimulation elicited field potentials that were characteristic for lateral perforant pathway stimulations. A monopolar recording electrode (114 μm in diameter, made from Teflon-coated tungsten wire) was lowered into the granule cell layer of the right dentate gyrus AP: −4.0 mm, ML: 2.3 mm from the bregma, DV: 3.0–3.2 mm from the dural surface. Monitoring the monosynaptic-evoked field potentials during implantation controlled the correct placement, especially with regard to electrode depth. Grounding and indifferent electrodes (silver-wires) were set on the dura through the left side of the cranium, and fixed to the skull with dental cement and plastic screws. Following surgery, the animals were housed individually and given seven days for recovery, with *ad libitum* food and water.

The experiments were approved by the animal care committee of the State Saxony-Anhalt (No. 42502-2-1406 DZNE).

2.2. Combined fMRI and electrophysiological measurements

The experimental setup used for simultaneous fMRI and electrophysiological measurements during electrical stimulation of the perforant pathway are already previously described in detail (Angenstein, 2014). All animals were initially anesthetized with isoflurane (1.5–2%; in 50:50 N_2:O_2; v:v) and the anesthesia was switched to deep sedation by application of medetomidine (Dorbene, Pfizer GmbH, bolus: 50 μg/kg s.c. and after 15 min 100 μg/kg per h s.c.) after animals were fixed into the head holder and connected to recording and stimulation electrodes. For the fMRI experiment, the anesthetized animals were connected to the stimulation and recording electrode after fixation of the head. Heating was provided from the ventral side and heart rate, breathing rate and oxygen saturation were monitored during the entire experiment using an MRI-compatible pulse oximeter (MouseOx™, Starr Life Sciences Corp. Pittsburgh PA, USA). All recorded physiological parameters remained stable during the combined fMRI-electrophysiology experiment.

To determine the appropriate stimulation intensity for the subsequent fMRI experiment, an input/output curve for the population spike was first constructed for each individual animal. For that, the perforant pathway was stimulated with single test pulses at increasing intensities (i.e., three test pulses at 10 s intervals with the following intensities: 100, 200, 300, 400, 500, 600, 800 μA; recordings were made with an interval of 2 min, except the 600–800 μA, which were taken at 4 min intervals). The electrophysiological responses were filtered with an antialiasing filter, i.e., a low cut (<1 Hz) and a high cut filter (>5000 Hz) using an EX4-400 amplifier (Science Products, Hofheim, Germany), transformed by an analog-to–digital interface (power-CED: Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer with a sampling rate of 13000 Hz. No further processing of the electrophysiological data was necessary because the gradient artifacts were minor in comparison to the neurophysiological responses (see Fig. 1B).

The intensity that evoked 50% of the maximum population spike amplitude (250–400 μA) was then used for the subsequent experimental stimulations.

All fMRI experiments were performed on a 4.7 T Bruker Biospec 47/20 scanner, equipped with a BGA09 (400 mT/m) gradient system. A 50 mm Litzcage small animal imaging system (Doty Scientific Inc., Columbus, SC, USA) was used for RF (radio frequency) excitation and signal reception. Initially, ten horizontal anatomical spin-echo-images (T_2-weighted) were obtained using a *RARE* (rapid acquisition relaxation enhanced) sequence (Hennig et al., 1986) with the following parameters: TR 4000 ms; TE 15 ms, slice thickness 0.8 mm, FOV 37 × 37 mm, matrix 256 × 256, RARE factor 8, averages 4. The total scanning time was 8 min 32 s. Functional MRI (BOLD-fMRI) was performed using a gradient-echo EPI (echo planar imaging) sequence with the following parameters: TR 2000 ms, TE 24 ms, slice thickness 0.8 mm, FOV 37 × 37 mm, matrix 92 × 92 (in plane resolution 402 × 402 μm). The slice geometry, i.e., ten horizontal slices, was identical to the previously obtained anatomical spin-echo-images. Trigger pulses that were generated by the scanner at the beginning of every volume, i.e., every 2 s, were used to synchronize fMRI-image acquisition and electrophysiological stimulations. Each fMRI measurement started with an initial period without any stimulation (to determine baseline BOLD signals) and then the appropriate stimulation protocol was applied (Fig. 1).

In addition, local blood volume changes were determined after intravenous injection of 300 μl Molday ION solution (30 nm iron based superparamagnetic nanoparticle, USPIO (10 mg Fe/ml), BioPAL Inc., Worcester, MA, USA). For monitoring local changes in USPIO distribution and by that blood volume changes, a gradient echo with flow compensation (GEFC) imaging sequence with the following parameters was used: TR: 62.5 ms TE: 3.2 ms, FOV: 25.73 × 25.73 mm, matrix 64 × 64 (in plane resolution 402 × 402 μm) slice thickness 1 mm. Scan time for one volume (i.e., four images) was 4 s (Figure S1-1).

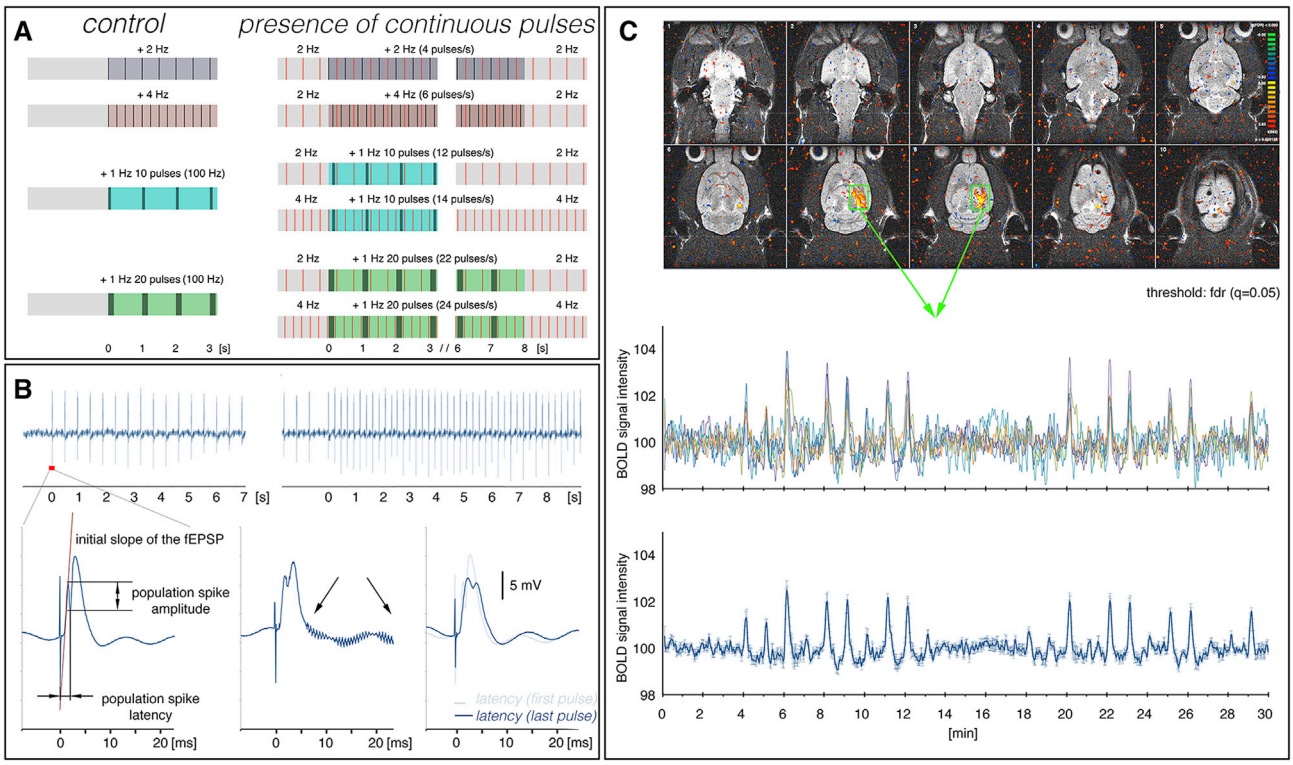

Fig. 1. Overview of the stimulation protocol and measured field responses and fMRI signal changes. **A** The perforant pathway was stimulated with 8 s long trains in the absence (left side) or presence (right side) of continuous low-frequency pulse stimulations. **B** Local field potentials were continuously recorded in the right dentate gyrus (upper part). To monitor variations in synchronized spiking activity of granule cells, population spike amplitudes and latencies were measured for each consecutive pulse (left side, lower part). Artifacts generated by switching gradient were minor in comparison to pulse-induced local field potentials (middle, lower part). Comparison of field potential elicited at the beginning (characteristic medial perforant pathway response) and at the end of continuous 4 Hz pulse stimulation (characteristic lateral perforant pathway response). Note the difference in population spike latency (indicated by horizontal bars) and field potential width (right side lower part). **C** Concurrently measured BOLD signals in the entire brain. Spatial distribution of all significantly activated voxels (upper part) induced by repetitive short 2 Hz and 4 Hz pulse trains (see also Fig. 3). Individual BOLD time series of all significantly activated voxels in the right dorsal hippocampus were averaged to visualize and compare the development of individual BOLD responses to consecutive stimulation trains (lower part).

2.3. Data analysis

2.3.1. fMRI data

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, Maastrich, the Netherlands), such as slice scan time correction, three-dimensional (3D) motion correction (trilinear interpolation and reduced data using the first volume as a reference) and temporal filtering (Gaussian filter, FWHM 3 data points) were applied to each data set. Because the reconstruction of the fMRI images resulted in a 128×128 matrix (instead of the 92×92 imaging matrix), spatial smoothing (Gaussian filter of 1.4 voxel) was added.

Volume of interest (VOI) analysis was used to analyze variations in baseline BOLD signals in the right dorsal hippocampus, septum and medial prefrontal cortex during continuous low frequency pulse stimulations. For that, 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. The right dorsal hippocampus and the other two structures (VOIs) were marked in the 3D standard rat brain. The average BOLD time series of all voxels located in one VOI was then calculated for each individual animal using the VOI analysis tool implemented in the BrainVoyager QX 2.6.1 software. Each individual BOLD time series was normalized using the averaged BOLD signal intensity as 100. All normalized BOLD time series were then averaged and depicted as mean BOLD time series ± SD (Fig. 1).

Significant changes in baseline BOLD signals were calculated using paired t-tests. For each animal BOLD signal intensities measured between frame 6–58 (i.e., the period between 12 and 116 s) were averaged. These values were then compared to individual values at later time points. Significant differences in stimulus induced BOLD responses were calculated using a two-sample equal variance *t*-test. Differences were considered significant at a calculated p-value < 0.05.

To analyze BOLD responses induced by repetitive short stimulation trains a linear regression analysis (general linear model (GLM) implemented in the BrainVoyager QX 2.6.1) was performed. This means that signal intensity changes in each voxel were correlated with the given stimulus protocol. Based on this setup, an appropriate activation map was generated. To account for the hemodynamic delay, the stimulus representing block design was modified by 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 false-discovery rate (FDR) with a q-value of 0.05 (which corresponds to a t-value > 3 or $p < 0.005$) was used as the threshold.

To determine whether changes in postsynaptic activity (measured as initial slope of the fEPSP), spiking activity (measured as population spike amplitude) or the excitability (measured as population spike latency) relates to measured changes in BOLD signals, cerebral blood volume (CBV) or to calculated changes in oxygen consumption ($CMRO_2$) basic multiple regressions (http://vassarstats.net/index.html) were calculated. To determine what electrophysiological parameter predicts variation in BOLD signals bivariate Granger causality tests were performed using a free statistics software for bivariate time series analysis, i.e., bivariate Granger causality (Wessa, 2018).

Measured BOLD signals and CBV values were used to roughly estimate corresponding changes in $CMRO_2$. For that variations in BOLD

signals were related to variations in oxygen concentration, whereas Molday ION related signal decreases were related to increases in CBV. Changes in CMRO2 were related to the difference between CBV and related BOLD signals.

2.3.2. Electrophysiological data

The amplitude of the population spike was measured in mV (from the first most positive point to the following most negative point) and the latency in ms (from the middle of the stimulus artifact to the most negative point, see Fig. 1B). Postsynaptic activity was measured as initial slope of the field excitatory postsynaptic potential (fEPSP, mV/ms) as it is linearly related to synaptic conductance (Johnston and Wu, 1995). All absolute measurements were averaged and depicted as arithmetic mean ± SEM.

3. Results

To study how elevated neuronal activity modifies stimulus-induced BOLD responses we performed two sets of experiments. In the first set of experiments we monitored neuronal and corresponding hemodynamic responses in the right dorsal hippocampus during continuous low frequency pulse stimulation of the right perforant pathway. Then, in a second set of experiments, we quantified BOLD responses in the right dorsal hippocampus that were induced by short stimulation trains in absence or presence of continuous low frequency pulse stimulations of the perforant pathway.

3.1. Development of BOLD signals and CBV in the right dorsal hippocampus during continuous 2 Hz pulse stimulation of the right perforant pathway

At the beginning of continuous 2 Hz pulse stimulation, the population spike amplitude varied and reached a transient maximum after about 30 s. During the next 10 min, the amplitude declined to a level below the initial response and then remained at this level (Fig. 2). Although suppressed, population spikes were still induced by consecutive pulses until the end of the 2 Hz pulse stimulation period. The corresponding population spike latency increased by about 1 ms during the initial 30 s and remained at this level. With onset of continuous 2 Hz pulse stimulation the initial slope of the fEPSP declined and then remained almost stable (Fig. 2).

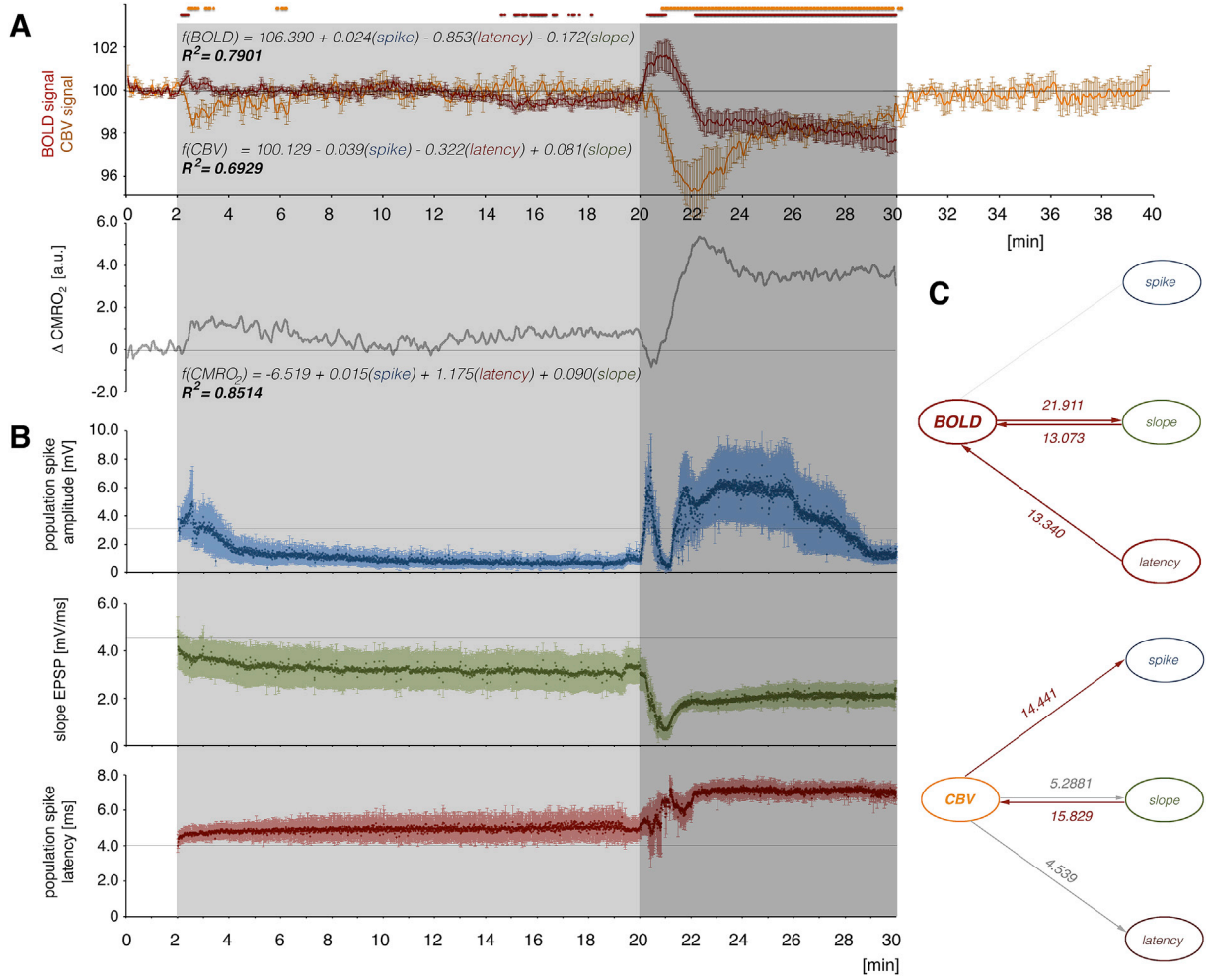

Fig. 2. Summary of detected variations in fMRI signals and concurrently measured electrophysiological responses. **A** Development of BOLD signals (red graph, n = 6), CBV signals (orange graph, n = 7) and calculated $CMRO_2$ values (gray graph) during stimulation of the perforant pathway with continuous 2 Hz pulses (depicted by a light gray box) and subsequent continuous 4 Hz pulses (depicted by a dark gray box). The $CMRO_2$ graph equates the difference between CBV (as negative values) and corresponding BOLD signal, thus it depicts no absolute values but only relative changes. Significant changes to the corresponding initial baseline level (i.e., the first 2 min) are depicted by the colored dots above the graph (red dots: BOLD signals; orange dots: CBV). **B** Electrophysiological parameters of neuronal activations recorded during the fMRI experiment. Continuous stimulation of the right perforant pathway causes variation in: granule cell spiking activity, measured as population spike amplitudes (blue graph), granule cell postsynaptic activities, measured as initial slope of the fEPSP (green graph) and granule cell excitability, measured as population spike latency (red graph). **C** Summary of Granger causalities between measured parameters of neuronal activity and fMRI signals. Depicted are only significant predictions (the origin of the arrow indicates what factor predicts the resulting measured parameter and the number indicates the F value).

Similar to the initial increase in spiking activity in the right dentate gyrus during the first 30 s, the corresponding BOLD signals in the right dorsal hippocampus also significantly increased after the onset of perforant pathway stimulation and reached a maximum after 24 s. Then BOLD signals in this region declined, returned 80 s after stimulation onset to the initial value and then remained stable until it eventually decreased at the end of the 2 Hz pulse stimulation period (Fig. 2). The increase in BOLD signals was accompanied by a transiently increased blood volume, as detected by USPION-mediated changes in MR-signals. A maximal increase in local blood volume was observed 36 s after stimulation onset and similar to the development of BOLD signals, signal intensities returned to the initial level; however, with a longer delay of 6 min (Fig. 2).

A second increase of the continuous pulse stimulation, again by 2 Hz (i.e., to a total of 4 Hz), also resulted in a transient increase of population spike amplitudes, followed by the appearance of neuronal afterdischarges, which in turn caused an almost collapse of population spikes and fEPSPs after 60 s (Fig. 2). Then, population spikes recovered during the next 60 s and remained elevated for about 300 s before they eventually decreased to a similar level seen at the end of the continuous 2 Hz pulse stimulation period. With the recovery of population spikes, the latency increased by more than 2.5 ms and remained at this level until the end of the 4 Hz pulse stimulation, although the corresponding population spike amplitudes varied (Fig. 2). With the change in population spike latency, the width of the fEPSP increased too, so that these field potentials resemble responses of lateral perforant pathway stimulation, whereas the initial response resembles those of medial perforant pathway stimulation (Fig. 1B). The initial slope of the fEPSP also partly recovered after about 60 s and then remained stable at a low level. Thus, the slope of fEPSP decreased before the collapse of population spikes. Eventually, for about 30 s, continuous 4 Hz pulse stimulation elicited almost no detectable postsynaptic or spiking activity of the principal cells in the dentate gyrus (Fig. 2).

The concurrent measured hemodynamic responses in the right dorsal hippocampus did not follow the observed variations in neuronal responses in the dentate gyrus. BOLD signals only transiently increased after the onset of continuous 4 Hz pulse stimulation. During the period when neuronal afterdischarges appeared, BOLD signals increased further (Figure S2-1). When neuronal afterdischarges ceased and almost no granular cell responses were detectable, BOLD signals remained at this maximal level (Fig. 2). Only with the recovery of granular cell responses, BOLD signals decreased and eventually reached a lower level as seen before the onset of any stimulation. Similarly, increasing the continuous stimulation frequency to 4 Hz resulted in a transient strong increase in local blood volume, which then declined but still remained at a slightly elevated level until the stimulation ceased (Fig. 2). Thus, reduced BOLD signals and increased local blood volume were present, although substantial postsynaptic and spiking activity of granular cells was recorded. The decline of BOLD signals coincided with an increase in population spike latency and a decrease in the initial slope of the fEPSP.

Continuously recorded fMRI signals (i.e., BOLD and CBV) and corresponding evoked field potentials were used to determine what parameter of neuronal activity correlates best with the observed fMRI signal. According to a multiple regression analysis all three measured parameters of neuronal activities affect the BOLD signal ($R^2 = 0.7901$; $F = 1048.54$; $p < 0.0001$), although to a different degree (Fig. 2, Table S1). Single correlations of individual parameters of neuronal activities with BOLD signal revealed a strong relation between spike latency and BOLD $r = -0.767$ and lower relation between fEPSP ($r = -0.620$) and spiking ($r = -0.492$) and BOLD signals (Table S1). Because there exist a delay between changes in neuronal activity and resultant hemodynamic response Granger causality tests were used to determine which parameter of neuronal activity predicts best the resultant variations in hemodynamic signals. Considering the entire period of stimulation variations in population spike latencies and in the initial slope of the fEPSP similarly predicted the development of BOLD signals in the dorsal right hippocampus (Fig. 2, Table S1). In contrast, only variations in the initial slope of the fEPSP predicted corresponding changes in CBV (Fig. 2, Table S2). When CBV and BOLD signals were used to roughly calculate $CMRO_2$, it turned out that variation in the initial slope of the fEPSP and to a lesser extend population spike latencies predicted the development of $CMRO_2$ (Table S3).

Considering only the continuous 2 Hz pulse stimulation period, BOLD signals were predicted by variations in all recorded parameters of neuronal activities (Table S1). All three measured parameters of neuronal activity were predictive to changes in the CBV, with the best prediction by the spiking activity (Table S2). Considering only the entire 4 Hz pulse stimulation period then variations in population spike latencies and initial slopes of the fEPSPs again predicted the development of BOLD signals, whereas all three parameters predicted the development of CBV in the right hippocampus (Tables S1 and S2).

3.2. *Development of BOLD signals in the right dorsal hippocampus during continuous 4 Hz pulse stimulation of the right perforant pathway*

In the initial experiment continuous 4 Hz pulse stimulation followed 18 min of continuous 2 Hz pulse stimulation, thus started when previous stimulation already affected hippocampal signal processing. To check whether doubling the input activity (i.e., from 2 to 4 Hz) or in fact activation with continuous 4 Hz pulses is responsible for the strong baseline BOLD shift, an additional experiment was performed with another group of animals. In this experiment the perforant pathway was only stimulated with continuous 4 Hz pulses and neuronal and BOLD signals were monitored during 18 min of continuous 4 Hz pulse stimulation and the subsequent 10 min in which perforant pathway fibers were not stimulated.

Under this condition BOLD signals also only increased transiently, which was also followed by a subsequent decrease to a lower level when compared to the initial BOLD signals (Fig. 3). A decrease in BOLD signals during prolonged 4 Hz pulse stimulation was again observed in the right dorsal hippocampus but not in the septal area (Fig. 3); thus, it is not a general hemodynamic phenomenon. In contrast to changes in local blood volume (Fig. 2), BOLD signals remained at a lower level for at least 10 min after the stimulation ceased (Fig. 3). Although no neuronal activities were induced during this period, granule cells would still respond differently to single test pulses. Thus, an identical test pulse after the fMRI experiment caused a population spike with lower amplitude, longer latency and lower initial slope of the fEPSP when compared to the response immediately before the fMRI experiment (Fig. 3D).

Regional BOLD signal analysis (VOI analysis) revealed that continuous 4 Hz pulse stimulation of the right perforant pathway did not only affect BOLD signals in the right dorsal hippocampus but also BOLD signals in the medial prefrontal cortex (Figure S3-1). The transient increase of BOLD signals in the medial prefrontal cortex was not contemporaneous but clearly delayed. Thus, in the medial prefrontal cortex the increase of BOLD signals coincided with the appearance of neuronal afterdischarges in the dentate gyrus (Figure 2 and Figure S3-1).

Similar to continuous 2 Hz pulse stimulation multiple regression analysis revealed that all parameters of neuronal activity affect the resultant BOLD response, however again with a different impact (Fig. 3, Table S4). Single correlation analysis with individual parameters of neuronal activities again revealed the strongest correlation between variations in population spike latencies and BOLD signals. In contrast to the previous experiment, bivariate Granger Causality indicate that only population spike latencies significantly predict changes in BOLD signals, whereas the development of BOLD signals seems to predict variations in the initials slopes of fEPSP (Table S4).

In summary, both continuous 2 and 4 Hz stimulations of the right perforant pathway caused only transient increases in BOLD signals, which then returned either to baseline level (during 2 Hz pulse stimulation) or decreased to values below the baseline level (during 4 Hz pulse stimulation). The decrease in BOLD signals started after neuronal

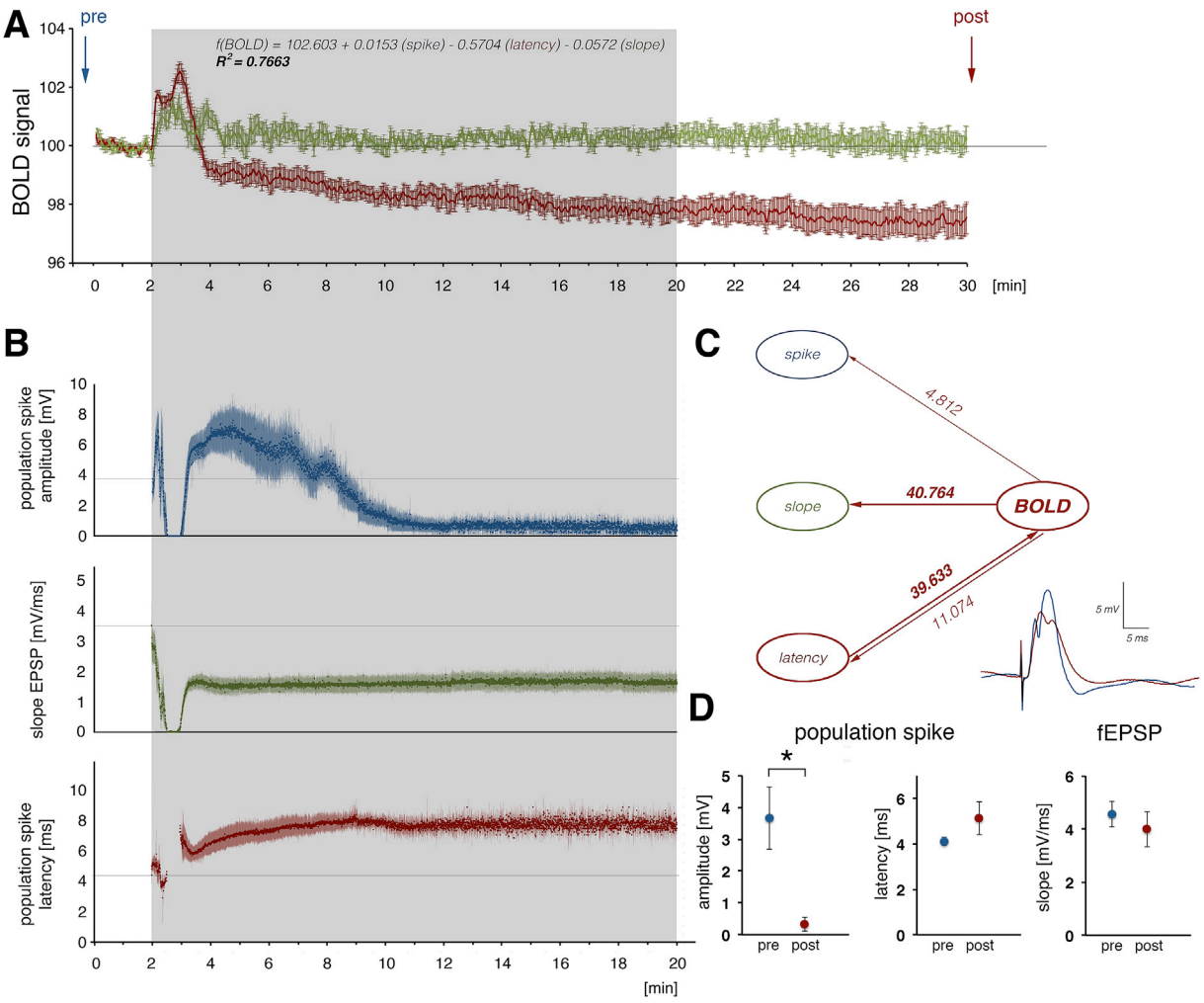

Fig. 3. Summary of detected variations in fMRI signals and concurrently measured electrophysiological responses during continuous 4 Hz pulse stimulation of the perforant pathway. **A** Development of BOLD signals in the right dorsal hippocampus (red graph) and septum (green graph) during 18 min of stimulation (depicted by a gray box) and the subsequent period without stimulation. Note that BOLD signals in the right dorsal hippocampus did not returned to the initial level during the last 10 min. **B** Summary of neuronal responses in the dentate gyrus during continuous 4 Hz pulse stimulation. Granule cell spiking activity initial increase, collapsed, transiently recovered and eventually declined to a constant low level after 10 min (blue graph). In contrast, postsynaptic activities initially declined, disappeared and recovered to a stable lower level until the end of stimulation (green graph). The population spike latency initially declined, increased after the collapse of spiking and remained at this higher level until the end of stimulation (red graph). **C** Summary of significant Granger causalities. During continuous 4 Hz pulse stimulation only variations in population spike latencies predicted changes in BOLD signals in the right hippocampus. **D** Electrophysiological responses in the dentate gyrus before (pre) and after (post) the combined electrophysiology/fMRI experiment. For each rat the response to a test pulse was recorded immediately before (blue arrow in A) and after the fMRI (red arrow in A) experiment. After the fMRI experiment a population spike was only in detected 3 out of 7 animals, therefore calculation of statistic significant differences in population spike latencies were not performed.

afterdischarges ended and coincided with an increase in population spike latencies, decrease in postsynaptic responses and increase in $CMRO_2$.

3.3. Lateral versus medial perforant pathway stimulation

Continuous 4 Hz pulse stimulations of mainly medial perforant pathway fibers caused an increase in the population spike latency and a widening of the fEPSP; thus the resulting field potential resembles a characteristic response to lateral perforant pathway stimulation. To exclude that observed changes in BOLD signals did not simply reflect a switch in the susceptibility threshold between medial and lateral perforant pathway fibers the same experiment was repeated with another group of animals that had a stimulation electrode implanted into mainly lateral perforant pathway fibers. Stimulation of the lateral perforant pathway, with similar pulse intensities (350 μA), resulted in lower population spike amplitudes that further declined after isoflurane anesthesia was switched to medetomidine sedation. Thus, in this group, continuous 2 Hz pulse stimulation elicited fEPSPs with minor or even absent population spike components. The increase to continuous 4 Hz pulse stimulation also resulted in the generation of neuronal afterdischarges and a transient increase in population spike amplitudes. Nevertheless, the resultant BOLD time series was almost similar to the experiment (Figure S3-2), in which the medial perforant pathway was stimulated. Thus, the observed changes in baseline BOLD signals were not related to changes from medial to lateral perforant pathway activations.

3.4. BOLD responses to low frequency pulse trains in presence of continuous stimulation of the perforant pathway

During continuous 2 Hz pulse stimulations, BOLD signals in the right dorsal hippocampus returned within 2 min to the initial baseline level, although elevated neuronal activities were still present. To test if under this condition an additional stimulus causes a similar BOLD response as in a condition without stimulation, we performed a second set of

experiments. Similar to the first set of experiments, the perforant pathway was again stimulated with continuous 2 Hz pulses, but this time short 2 Hz and 4 Hz pulse trains were superimposed (Fig. 1A).

Under control conditions, i.e., without additional continuous 2 Hz pulse stimulations, these short pulse trains induced significant BOLD responses in the right dorsal hippocampus, which declined during consecutive presentations. Stimulation with 4 Hz pulse trains elicited stronger BOLD responses than stimulation with 2 Hz pulse trains (Fig. 4A and B). Repetitive stimulations with alternating 2 and 4 Hz stimulation trains did not significantly affect baseline BOLD signals. During consecutive stimulation trains, the average population spike amplitudes decreased whereas corresponding population spike latencies remained almost constant (Fig. 4C). Similarly, the initial slopes of the fEPSP, i.e., the synaptic responses, remained almost constant during the entire stimulation session.

The onset of continuous 2 Hz pulse stimulation caused a transient increase of BOLD signal intensity in the right dorsal hippocampus that again returned to the initial baseline level after about 2 min. Overlaying the continuous 2 Hz pulse stimulation with short 2 and 4 Hz pulse trains (i.e., periods in which the perforant pathway was actual stimulated with 4 and 6 pulses per second) resulted initially in similar BOLD responses as observed during the control experiment (Fig. 4A). However, there was a faster adaptation of BOLD responses to consecutive stimulation trains, i.e., a stronger decline of maximal BOLD responses was observed (Fig. 4B). After a short interval of 5 min the presentation of a second period of 2 and 4 Hz pulse trains resulted in an almost complete absence

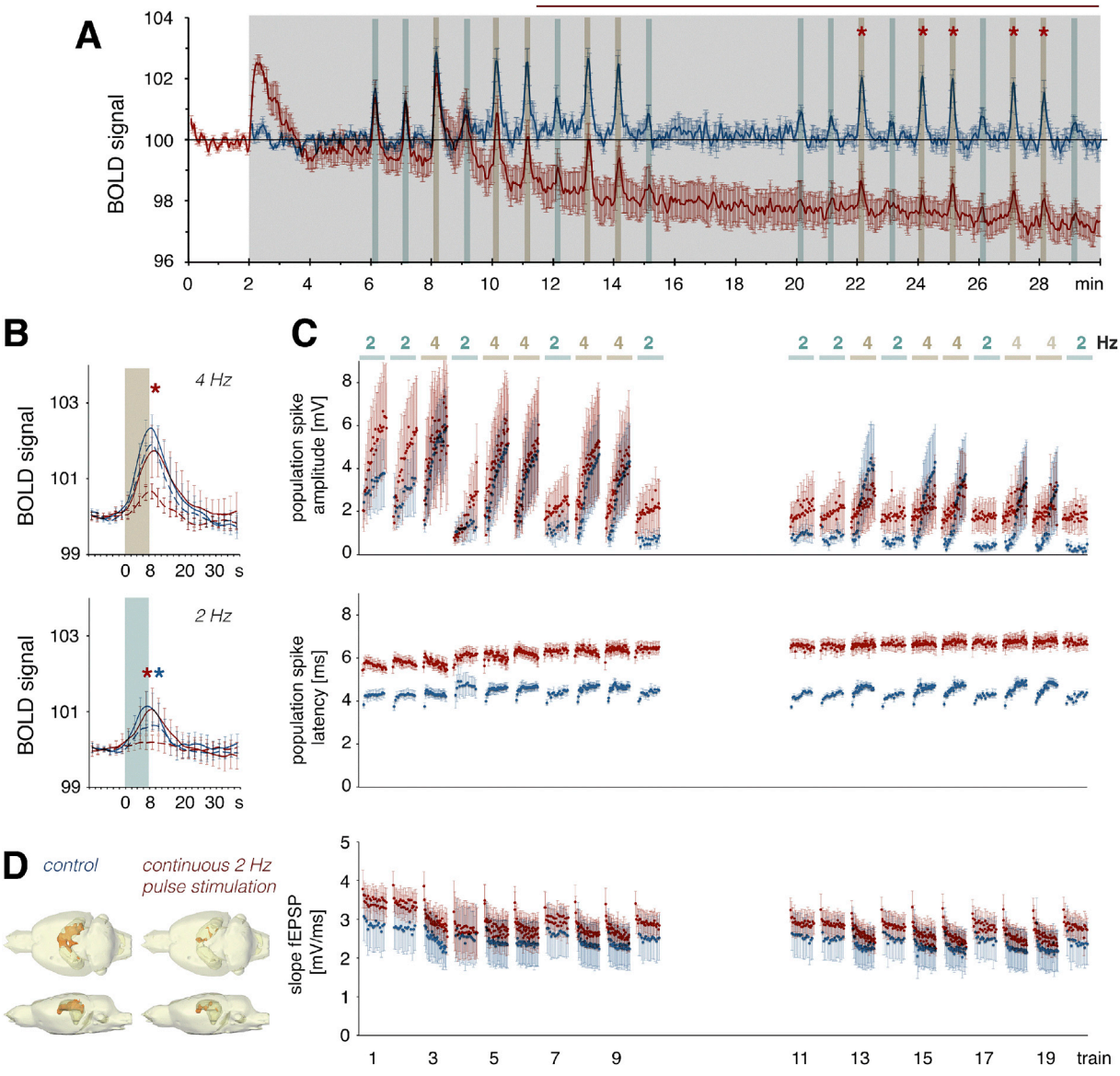

Fig. 4. BOLD and electrophysiological responses during repetitive short low-frequency stimulation trains in the absence (blue graphs, n = 6) or presence (red graphs, n = 5) of continuous 2 Hz pulse stimulation. **A** BOLD time series in the right dorsal hippocampus. The time period of continuous 2 Hz pulse stimulation is indicated by the gray box, 2 Hz stimulation trains are indicated by blue bars, 4 Hz stimulation trains by ocher bars. When short low-frequency stimulation trains were applied in presence of continuous 2 Hz pulse stimulation then baseline BOLD signals gradually decline and were significantly lower after the 6th stimulation train (significant decrease in baseline BOLD signals are depicted by the red line above the graph). Red asterisks indicate significantly different BOLD responses in presence of continuous 2 Hz pulse stimulations. **B** Average BOLD responses (blue graphs: control condition; red graphs: in presence of continuous 2 Hz pulses) during the first stimulation period (i.e., trains 1–10, solid lines) and during the second stimulation period (trains 1–20, dashed line) for 2 Hz (upper graph) and 4 Hz (lower graph) stimulation trains. **C** Concurrently measured electrophysiological responses in the right dentate gyrus. Note that in the presence of continuous 2 Hz pulse stimulation the average population spike amplitude was not reduced, whereas the latency was increased. The initial slope of the fEPSPs remained similar. **D** Spatial distribution of significant BOLD responses induced by repetitive low frequency stimulation in absence (left side) or presence of continuous 2 Hz pulse stimulation (right side).

of significant BOLD responses (Fig. 4A and B). In contrast to the sole presentation of 2 and 4 Hz pulse trains or sole continuous 2 Hz pulses, the combination of the two stimulation protocols resulted in a steady decline of BOLD baseline signals, i.e., BOLD signals between individual trains. Thus, after the sixth stimulation train baseline BOLD signals significantly differed between control and continuously stimulated animals. The reduced baseline BOLD signals did not recover during a 5-min-long stimulus free interval; thus, the second round of 2 and 4 Hz pulse trains was applied in the presence of reduced baseline BOLD signals (Fig. 4A). Similar to the control conditions, i.e., without presentation of continuous 2 Hz pulses, neuronal responses adapted during consecutive stimulation trains; however, in contrast, this was paralleled with a continuous increase in the population spike latency (Fig. 4C). In contrast to the population spike latency, the initial slopes of the fEPSPs were not significantly affected by the presence of continuous 2 Hz pulses.

To test if the attenuation of stimulus-induced BOLD responses during the second stimulation period depends on the existing reduced baseline BOLD signals or on the ongoing additional low frequency pulse stimulations, continuous 2 Hz pulses were only applied during the beginning. Under this condition again almost no BOLD responses were generated during the second stimulation period, although at this time no background activity was present (Fig. 4-1Figure S4-1A, B). Again, neuronal responses during the second stimulation period were characterized by low or absent population spikes and significantly prolonged latencies (Figure S4-1C).

In summary, the presence of continuous 2 Hz pulses did not affect the formation of initial BOLD responses to short low-frequency pulse stimulation trains but caused stronger adaptation of the hemodynamic response, in terms of a faster decline during repetitive stimulations and a reduction of baseline BOLD signals. The reduction of BOLD baseline intensities coincided with an increase of population spike latencies.

3.5. BOLD responses to high frequency pulse trains in presence of continuous stimulation of the perforant pathway

A previous study (Angenstein, 2014) indicates that the duration of inter-pulse intervals controls the adaptation of BOLD responses to consecutive stimuli. To check if continuous 2 Hz pulse background activity generally affects the adaptation of BOLD responses to consecutive stimulations, the stimulation protocol was changed from low-frequency pulse trains to bursts of high-frequency pulse trains.

For that, two related high-frequency stimulation protocols were applied. First, the perforant pathway was stimulated with trains consisting of four bursts of ten pulses with an inter-pulse interval of 10 ms (Fig. 1A). This stimulation protocol induced almost uniform neuronal responses and no neuronal afterdischarges (Riemann et al., 2017).

Under the control condition, i.e., in the absence of continuous low-frequency pulse stimulation, this stimulation protocol caused almost similar BOLD responses during all consecutive stimulation trains and BOLD baseline signals remained unchanged (Fig. 5A and B). Presentation of short high-frequency pulse bursts in one stimulation train resulted in increasing population spike amplitudes. The overall responses increased during the second stimulation train and then returned to the initial level during subsequent trains. The corresponding population spike latencies increased within one train but decreased during consecutive trains. The initial slopes of all fEPSPs, as marker for postsynaptic activities, remained almost stable during the entire stimulation session (Fig. 5C).

When the same stimulation protocol was applied in the presence of continuous 2 Hz pulse stimulation, initially, a similar BOLD time series was induced. The onset of continuous 2 Hz pulse stimulations again caused transiently increased BOLD signals in the right hippocampus that returned within 2 min to baseline level. Subsequently applied repetitive high-frequency stimulation trains initially caused similar BOLD responses and a steady decline of baseline BOLD signals, thus after the 7th stimulation train baseline BOLD signals were significantly reduced (Fig. 5A). Concurrent with the decline of BOLD baseline signals, individual BOLD responses became smaller, thus at the end significantly smaller BOLD responses were induced (Fig. 5A and B).

Although identical BOLD responses were initially induced, the underlying neuronal responses in the dentate gyrus were completely different. The previous onset of continuous 2 Hz pulse stimulation caused a strong depression of neuronal responses, i.e., population spike amplitudes to individual test pulses decreased and the corresponding latencies increased by less than 2 ms. Similarly, the initial slopes of all fEPSP, i.e., the postsynaptic activation, were reduced. Under this condition, additional applied bursts of high-frequency pulses did not cause an augmentation of responses during consecutive bursts (Fig. 5C).

A rerun of the same stimulation protocol in the presence of continuous 4 Hz pulse stimulation resulted in a completely different BOLD time series (Fig. 5A). Again, BOLD signals only transiently increased after the onset of continuous 4 Hz pulse stimulation and then decreased significantly below the initial level before 15 trains with high-frequency pulse bursts were presented. Under this condition, both the first train and all subsequent trains induced significantly smaller BOLD responses (Fig. 5A and B). Continuous 4 Hz pulse stimulation first caused an augmentation of neuronal responses that was then followed by a depression (Fig. 5C). Independent of the development of the population spike amplitudes, the corresponding latencies substantially increased and remained at this highly elevated level (Fig. 5C). The initial slopes of the fEPSPs were in the same level as observed during continuous 2 Hz pulse stimulation, thus postsynaptic activity was also suppressed when compared to control conditions. Superimposed bursts of high-frequency pulses, similar to the control condition, initially caused an augmentation of responses to the first pulse of consecutive bursts that then rapidly declined during subsequent trains (Fig. 5C).

In summary, stimulation of the perforant pathway with short bursts of high-frequency pulses elicited similar neuronal response patterns and almost uniform BOLD responses without modifying baseline BOLD signals. Concurrent stimulation with continuous 2 Hz pulses did not affect initial high-frequency pulse-induced BOLD responses, but caused a continuous decline in baseline BOLD signals, which in turn was accompanied by reduced BOLD responses. Continuous 4 Hz pulse stimulation by itself caused a strong reduction of baseline BOLD signal intensity and under this condition the same high-frequency pulse burst stimulation only caused attenuated BOLD responses. The strong decline in BOLD baseline intensity again coincided with longer population spike amplitudes.

In a second related experiment the number of bursts and pulses per train were doubled (i.e., 8 bursts of 20 pulses). This stimulation protocol is known to induce neuronal afterdischarges after the first stimulation period (Helbing et al., 2013) and might therefore already affect baseline BOLD signals. Applying this stimulation protocol elicited clear BOLD responses and, as expected, also a decline of BOLD baseline signals. Thus, after ten stimulation trains, baseline BOLD signals remained significantly reduced for 5 min (Fig. 6A). After this stimulus free interval, a second period of identical stimulations caused almost similar BOLD responses, except that the first train only caused an attenuated response when compared to the initial stimulation train (Fig. 6A, C).

Electrophysiologically recorded neuronal responses in the dentate gyrus revealed a reduced synchronized spiking activity during the second stimulation train with increased latencies, probably as a result of afterdischarges that were induced during the first stimulation train. During the third stimulation train, population spikes recovered and the corresponding latencies decreased. Then, population spike amplitudes gradually decreased whereas corresponding latencies remained almost stable. Within each individual train, however, population spike amplitudes and latencies increased during consecutive bursts. During the second stimulation period (i.e., trains 11–20), reduced population spike amplitudes and latencies were observed (Fig. 6B) when compared to the initial stimulation trains (i.e., trains 1–10). Although after train 11 occasional neuronal afterdischarges were observed, coinciding with the sustained elevation of BOLD signals, population spike latencies were not altered

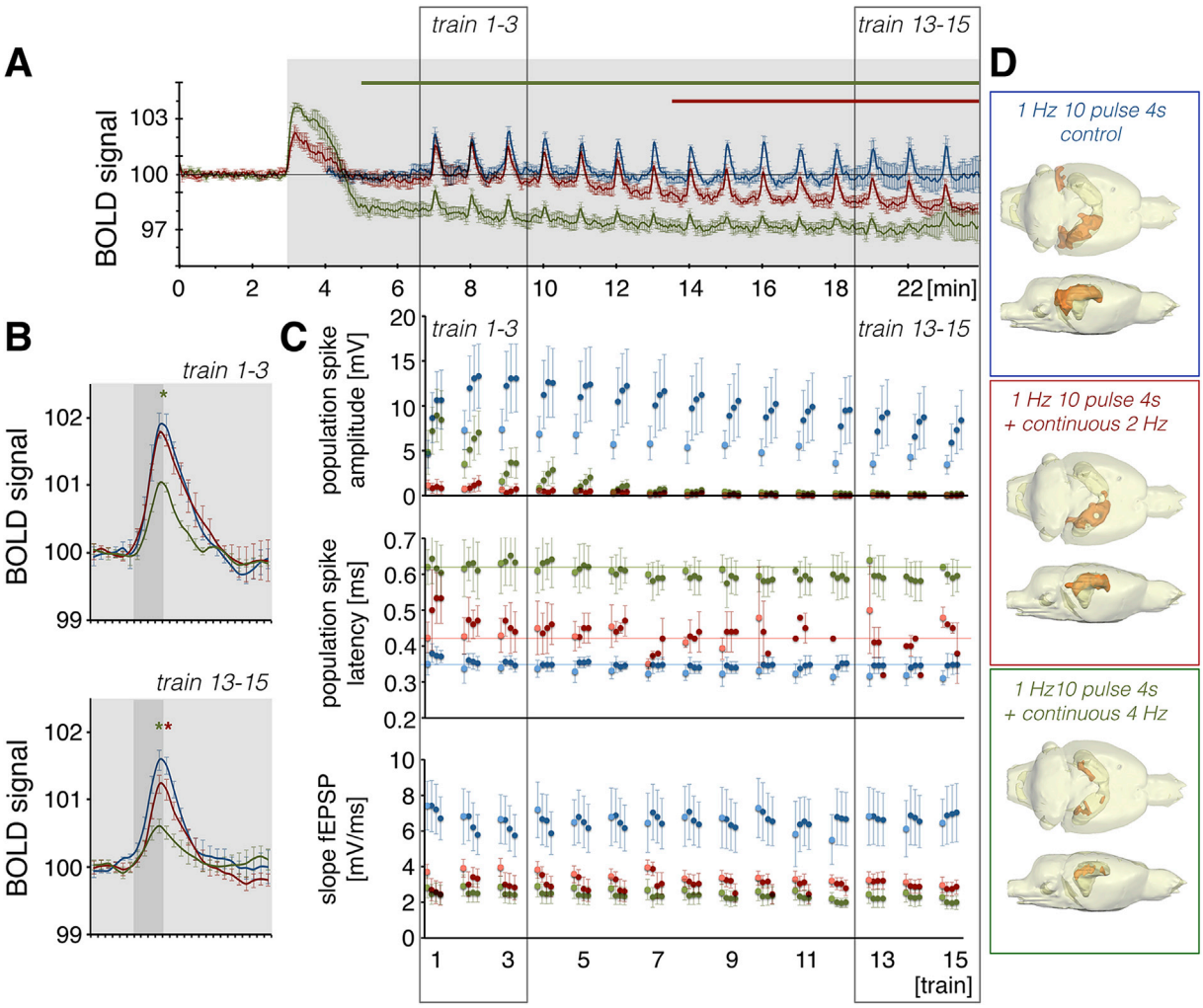

Fig. 5. BOLD and electrophysiological responses elicited by short high-frequency burst pulse stimulation in the absence (blue graphs, n = 7) or presence of continuous 2 Hz (red graphs, n = 7) or 4 Hz pulse (green graphs, n = 7) stimulation. **A** BOLD time series in the right dorsal hippocampus. The gray box indicates the presence of continuous low-frequency pulse stimulation. The red (during continuous 2 Hz pulse stimulation) or the green (continuous 4 Hz pulse stimulation) line indicate the presences of significantly reduced baseline BOLD signals. **B** Average BOLD responses during early (trains 1–3) and late (trains 13–15) stimulation trains. Asterisks depict significantly reduced BOLD responses when compared to control condition. **C** Comparison of neuronal responses in the dentate gyrus induced during high-frequency pulse burst stimulation. **D** Effect of continuous low-frequency pulse stimulation on the distribution of significantly activated voxels during identical high-frequency pulse burst stimulation.

during the subsequent stimulation train. In contrast to the spiking activity of granule cells, the corresponding postsynaptic activities remained almost similar after the second stimulation trains; only within each individual train a continuous decline was observed.

A rerun of the same stimulation protocol in the presence of continuous 2 Hz pulse stimulations resulted in an altered BOLD time series. The onset of continuous 2 Hz pulse stimulation again resulted in a transient increase in BOLD signals that returned to the initial level after about 2 min. Superimposed trains with long bursts of high-frequency pulses resulted in significant BOLD responses that developed similar to responses seen under the control condition without additional continuous 2 Hz stimulations, except that baseline BOLD signals significantly declined after the third stimulation train (Fig. 6A). Thus, during the stimulus free interval after stimulation train 10, BOLD baseline signals were significantly lower than under the control conditions. Nevertheless, subsequently induced BOLD responses during the second stimulation period (i.e., trains 11–20) were similar, i.e., BOLD responses during consecutive stimulation trains were not attenuated. (Fig. 6A, C).

Similar to previous experiments, ongoing 2 Hz pulse stimulation modified granule cell responses to additional stimuli, i.e., population spike amplitudes to subsequently applied high-frequency pulses were reduced, whereas the corresponding latencies increased by about 2 ms (Fig. 6B). These changes in stimulus-induced neuronal responses where not related to altered BOLD responses but only to decreased baseline BOLD signals.

A rerun of the same stimulation protocol in the presence of continuous 4 Hz pulse stimulations again resulted in another BOLD time series. The onset of continuous 4 Hz pulse stimulation again caused a transient increase of BOLD signals that was followed by a decline of BOLD signals to a level below the initial value. The delayed second increase in BOLD signals again coincided with the appearance of neuronal afterdischarges. Subsequent stimulations of the perforant pathway with high-frequency pulse burst stimulation trains resulted in the appearance of uniform BOLD responses. This means that the BOLD response to the first high-frequency stimulation train was not characterized by a sustained elevation of BOLD signals, which in turn was paralleled with the absence of neuronal afterdischarges. Furthermore, under this condition, stimulation with high-frequency pulses did not further modify BOLD baseline intensities, which remained almost stable. Thus, after the first stimulation period (i.e., trains 1–10), it was similar to the level that was observed when the stimulation was applied in the presence of continuous 2 Hz pulse stimulations (Fig. 6A).

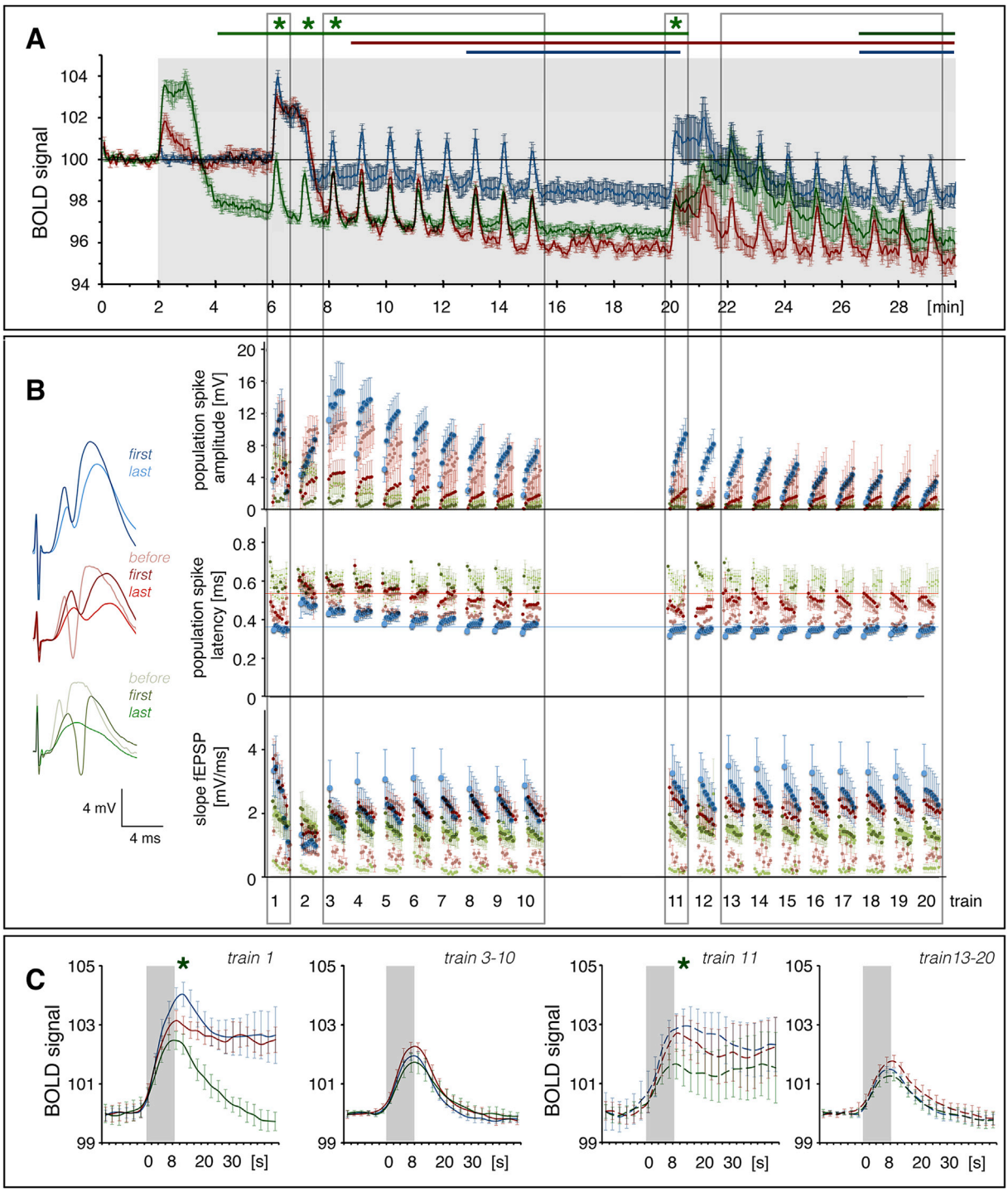

Fig. 6. BOLD and electrophysiological responses elicited by long high-frequency burst pulse stimulation in the absence (blue graphs, n = 6) or presence of continuous 2 Hz (red graphs, n = 7) or continuous 4 Hz pulse (green graphs, n = 5) stimulation. **A** BOLD time series in the right dorsal hippocampus. The gray box indicates the presence of continuous low-frequency pulse stimulation. The colored lines above indicate significant declines in baseline BOLD signals. Green asterisks depict significantly different BOLD responses in presence of continuous 4 Hz pulse stimulation when compared to control condition. **B** Neuronal responses in the dentate gyrus induced during high-frequency pulse burst stimulation. Left side: Comparison of the neuronal response to the first pulse of the first high-frequency stimulation train (dark graphs) with the response to the last pulse of the last train (bright graphs). Right side: Summary of neuronal responses in the right dentate gyrus during consecutive stimulation trains. **C** Summary of BOLD responses to the first and subsequent stimulation trains during the first and second stimulation period. Green asterisks depict significantly different BOLD responses in presence of continuous 4 Hz pulse stimulation when compared to control condition.

The combination of continuous 2 or 4 Hz pulse stimulation with superimposed high-frequency pulse burst stimulation eventually caused a similar decline of BOLD baseline intensities (i.e., >4%), which would indicate that the drop in baseline BOLD signal intensity is not simply an additive effect of these two stimulation protocols, but rather depends on a common mechanism that was induced when high-frequency pulse stimulation followed an already existing low-frequency pulse stimulation.

To confirm this assumption, we applied continuous 2 or 4 Hz pulse stimulation after ten high-frequency pulse stimulation trains, i.e., at a

time point when responses to high-frequency pulse stimulation were adapted and stabilized. When continuous 2 or 4 Hz pulse stimulations were applied after several high-frequency stimulation trains, BOLD signals were not affected, i.e., neither during the onset of continuous low-frequency pulse stimulation nor at later time points. Furthermore, subsequently applied high-frequency pulse stimulation trains generated similar BOLD responses; thus, the onset and presence of continuous low-frequency pulse stimulation was not assignable in the BOLD time series (Fig. 7A). Although stimulus-induced BOLD responses were almost identical, stimulus-induced neuronal responses in the dentate gyrus differed. In particular, the onset of continuous 4 Hz pulse stimulation caused an almost complete suppression of granule cell spiking activity during high-frequency pulse burst stimulations (Fig. 7C).

4. Discussion

In the current study, neuronal activities in the dentate gyrus and BOLD signals in the dorsal hippocampus were simultaneously recorded during continuous 2 or 4 Hz pulse stimulation of the perforant pathway. All pulses in one session were applied with identical physical parameters (i.e., intensity, pulse width); thus, during one experiment an identical set of fibers was always activated. The main findings of the study can be summarized as follows: (I) *Continuous* 2 Hz *pulse stimulation of the right perforant pathway only causes a transient increase in BOLD signals in the right hippocampus*. Thus, although increased neuronal activity persists, the resultant hemodynamic response quickly normalizes. (II) *Continuous* 4 Hz *pulse stimulation elicited transient neuronal afterdischarges that were followed by a concurrent strong (> 2%) and stable decline in baseline BOLD signals.* This decline in baseline BOLD signals (negative BOLD response) was independent of granule cell spiking activity but coincided with an increase in population spike latency and, to a lesser extend, reduction in the initial slope of the fEPSP, thus with signs of increased inhibitory activity. (III) *Stimulus-induced decline in baseline BOLD signals can persist for at least* 10 min *after stimulation ceased.* Similarly, the excitability of granule cells remains reduced, thus cellular mechanisms that control the excitability coincide with reduced baseline BOLD signals. (IV) *BOLD responses to a particular pulse pattern remained similar when they are applied in the presence of continuous low-frequency (*2 Hz*) pulse stimulation.* This means as long as

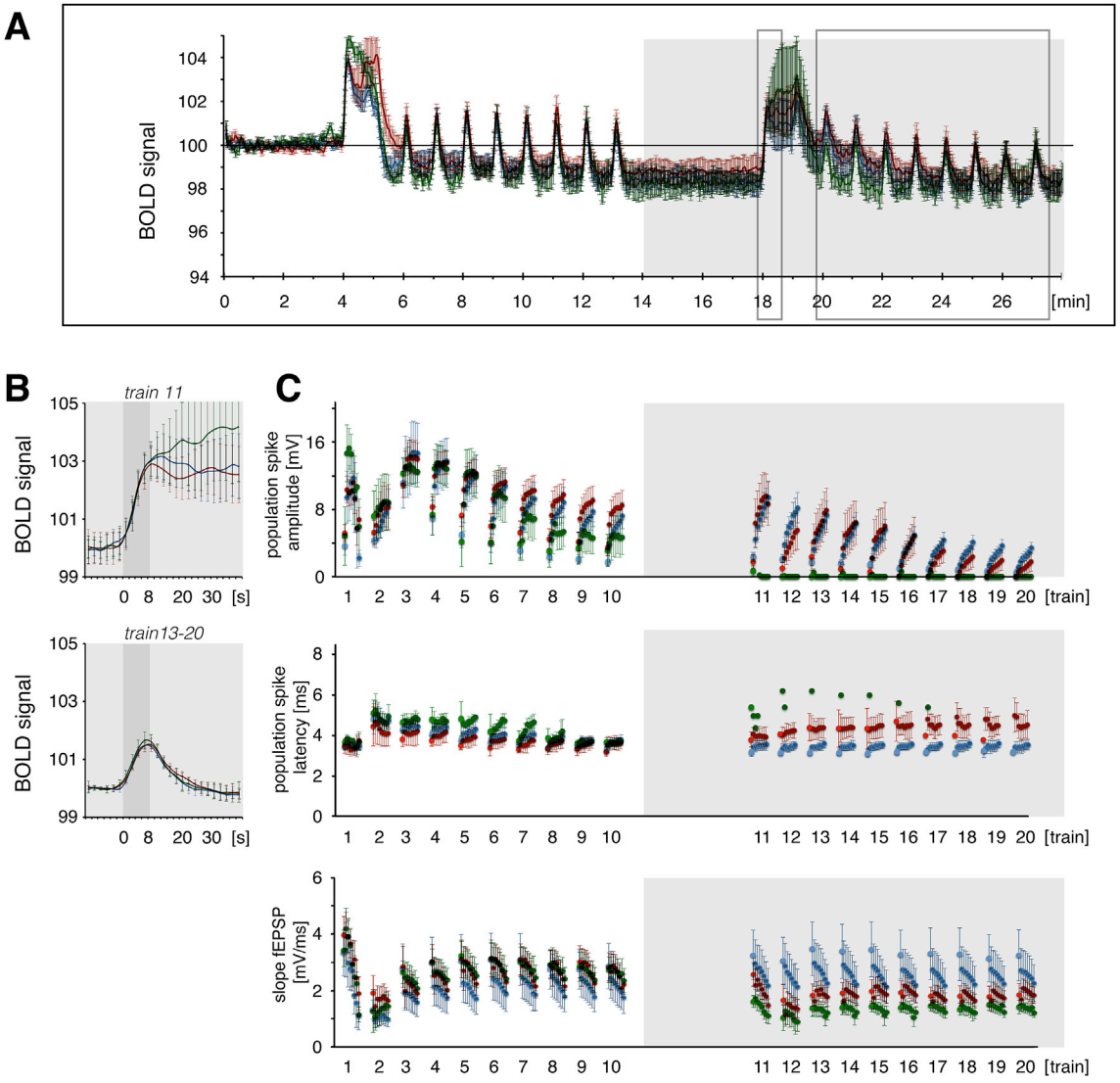

Fig. 7. BOLD and electrophysiological responses elicited by long high-frequency burst pulse stimulation in the absence (blue graphs, n = 6) or presence of continuous 2 Hz (red graphs, n = 6) or continuous 4 Hz pulse (green graphs, n = 5) stimulation. Continuous low-frequency stimulation started after ten long high-frequency pulse burst stimulation trains. **A** BOLD time series in the right dorsal hippocampus. Note that the late onset of low-frequency stimulation (indicated by the gray box) did not alter the BOLD time series. **B** BOLD responses to the first and subsequent high-frequency pulse burst stimulation trains during the second stimulation period (trains 11–20). Despite different neuronal background activities, stimulus-induced BOLD responses were almost identical. **C** Neuronal responses in the right dentate gyrus during consecutive stimulation trains.

baseline BOLD signals remain stable, measured BOLD signal variations remain specific for the incoming activity and do not reflect total neuronal activity. (V) *BOLD responses to identical stimulations are attenuated when previous stimulations caused a declined of baseline BOLD signals*. This means when long lasting inhibitory mechanisms are activated stimulus-induced BOLD responses are reduced even when principal neuron spiking activity remains similar, thus the measured BOLD responses is not anymore specific for the incoming activity.

4.1. Control of baseline BOLD signal intensity

Stimulus- or event-related BOLD responses describe differences in BOLD signals in the absence or presence of an applied stimulus. Theoretically, there exists a maximal BOLD signal (i.e., when no deoxygenated hemoglobin is present); thus, the currently existing "baseline" BOLD signal, i.e., before an applied stimulus, determines the subsequently maximal inducible BOLD response. This means that the higher "baseline" BOLD signals are, the lower are, theoretically, inducible positive BOLD responses to an applied stimulus.

The current results reveal that continuous 2 Hz pulse stimulations only cause a transient increase in BOLD signals and blood volume, which quickly returns to the initial level although neuronal activity and the related energy consumption remained elevated (Fig. 2). Thus baseline BOLD signals do not necessarily disclose the presence or absence of ongoing elevated neuronal activities. It appears that vasoactive substances are only released or effective at the beginning of an elevated activity, i.e., concurrently with the strongest change in synchronized granule cell spiking activity. Subsequently postsynaptic activity slightly decrease, the granule cell spiking activity declined and persisted at a lower level. Under this condition, the normal blood and oxygen supply was sufficient to support the energy demand required by this continuously increased neuronal activity.

A second increase of ongoing neuronal activities by a further 2 Hz again caused a transient elevation of BOLD signals and blood volume that was followed by a strong decline of BOLD signals below the initial level. The corresponding blood volume in the right dorsal hippocampus also declined but still persisted at an elevated level when compared to the initial level. This means that during continuous 4 Hz pulse stimulation oxygen consumption eventually exceeded the ongoing increased supply.

Neither the amount of postsynaptic nor spiking activity of granular cells relates to the steady high oxygen demand. First, BOLD signals remained consistently reduced, although the spiking activity considerably varied during the entire 4 Hz pulse stimulation period. Second, granule cell postsynaptic activity declined but was still consistently present, i.e., postsynaptic activity was still elevated when compared to the non-stimulated period at the beginning of the experiment. Third, BOLD signals remained at this lower level when stimulation ceased, although no further synaptic or spiking activity of the granule cells was present. The decrease in baseline BOLD signals was more prominent when onset of consistent 4 Hz pulse stimulation caused clearly detectable neuronal afterdischarges (Figure S2-1). Thus, mechanisms that are induced during neuronal afterdischarges to cease and/or prevent the (re) appearance of these afterdischarges may initiate and mediate the drop in baseline BOLD signals.

This confirms previous findings showing that during and after bicuculline-induced seizures negative BOLD signals develop in the hippocampus (Schridde et al., 2008). In this study, the effect of interneuronal activity was blocked by the GABA-A receptor antagonist bicuculline and therefore principal neurons developed epileptic discharges for up to 8 min. Evidently, after cessation of bicuculline-induced epileptic discharges, the BOLD signal increased but subsequently decreased again although no further discharges were observed. Although not discussed in this paper, the mechanism responsible for the second delayed decrease in BOLD signals could be the same as in the current study, namely a prolonged activation of inhibitory mechanisms to prevent further discharges of hippocampal principal neurons.

Reduction of postsynaptic responses and the extension in population spike latency are likely mediated by inhibitory interneurons. This agrees with previous observations that long-lasting recurrent inhibition of hippocampal neurons is accompanied by increased glucose metabolism (Ackermann et al., 1984) and that inhibitory interneurons are characterized by high energy utilization (Kann et al., 2014). Therefore, the continuously reduced BOLD signals might reflect a sustained increased activity of GABA-ergic interneurons. This would mean that, in the hippocampus, short-lasting positive BOLD responses are mainly mediated by (excitatory) principal neurons whereas the concurrently existing baseline BOLD signal is mainly controlled by a persisting activity of (inhibitory) interneurons. Consequently, negative BOLD signals can develop during strong interneuronal activity even in the presence of elevated principal neuron activity. A negative BOLD response might, therefore, also occur when a major input system is strongly inhibited allowing a strong excitation of principal neurons by another, normally unimportant input system. Of note, cessation of continuous stimulation was never accompanied by a decrease in baseline BOLD signals; thus, only a simple reduction of principal neuronal activity appears not to be sufficient to reduce BOLD signals.

Statistical analysis indicates that especially mechanism(s) controlling the excitability of principal neurons affect baseline BOLD signal. BOLD signals always correlated best with variations in population spike latencies (Tables S1 and S4) and, furthermore variations in population spike latencies always predicted subsequent changes in baseline BOLD signals (Figs. 2 and 3).

In contrast to the excitability, the correlation between postsynaptic activity (measured as initial slope of fEPSP) and BOLD signal was not consistent. On one hand a positive correlation ($r = 0.568$) was observed during the initial continuous 2 Hz pulse stimulation period and on the other hand a negative correlation ($r = -0.666$) was present during the following continuous 4 Hz pulse stimulation period (Table S1). The negative correlation mainly reflects the fact that with the appearance of neuronal afterdischarges BOLD signals increases whereas postsynaptic responses collapses. Thus, it appears that postsynaptic activities affect BOLD signals but this effect is mainly obscured by mechanisms that control principal neuron excitability. As previously observed, spiking activity of principal neurons has only minor effects on BOLD signals; only during continuous 2 Hz pulse stimulation a weak correlation between spiking activity and BOLD signals was found. Based on these statistical values it appears that baseline BOLD signals mainly reflect the activity of mechanisms that reduces the excitability of principal neurons, i.e., the stronger the inhibition the lower are baseline BOLD signals.

It should be noted, that continuous 2 and 4 Hz pulse stimulation of the perforant pathway, although this stimulation is similar to the natural occurring beta and low theta frequency range, remains an artificial stimulation. Nevertheless, other fundamental hippocampal functions, such as e.g., long-term depression of synaptic efficacy, were revealed by similar electrophysiological approaches, i.e., long lasting perforant pathway stimulations (Manahan-Vaughan, 2019).

4.2. When does elevated neuronal background activity affect stimulus-induced BOLD responses?

As long as ongoing activity does not alter population spike latencies and postsynaptic activities additional incoming periods of increased activity are going to trigger similar BOLD responses. Thus, during continuous 2 Hz pulses stimulation all applied stimulus pattern initially generated similar BOLD responses as they did without concurrent background activity. This means that BOLD responses mainly relate to the incoming activity (and resultant local processing of these signal) but not to relative changes in total neuronal activity, i.e., the BOLD response is stimulus specific. One might speculate that these short transient positive BOLD signals are mainly initiated by excitatory (glutamatergic) principal neurons. In contrast, BOLD responses to identical stimuli are attenuated when previous or ongoing stimulations reduced baseline BOLD signals,

i.e., when previous or ongoing stimulations trigger long-lasting (protective) inhibitory mechanisms to reduce principal neuronal excitability. Under this condition, BOLD responses no longer mainly depend on incoming activities but rather on modified local processing, i.e., the BOLD response is not anymore input specific. Thus, although an identical stimulus may cause similar or even an increased spiking activity of principal neurons, the resultant BOLD response becomes smaller (Figs. 4, 6 and 7). It appears that interneurons not only affect principal cell spiking activity but also control the efficacy of neurovascular coupling mechanism(s) triggered by principal neurons. Thus, when ongoing neuronal activity alone or in combination with a superimposed stimulus activates inhibitory (protective) mechanisms (that in turn causes a decline in baseline BOLD signal) then subsequent BOLD responses are reduced (Fig. 8).

The observations that: (I) the decline of baseline BOLD signals during continuous low frequency pulse stimulation coincides with an increased oxygen consumption and (II) only attenuated BOLD responses were elicited in presences of reduced baseline BOLD signals agree with the hypothesis that a formed BOLD response crucially depends on the actual coupling ratio n, i.e., on the stimulus-induced ratio between blood flow changes and changes in oxygen consumption ($n = \mathrm{CBF}/\mathrm{CMRO_2}$) (Buxton et al., 2014; Griffeth and Buxton, 2011; Hoge et al., 1999).

A decline in baseline BOLD signals does not only affect the formation of positive BOLD responses but also negative BOLD signal changes. When baseline BOLD signals were reduced then subsequent stimulations only caused an attenuated or even no negative BOLD baseline shift. Thus, stimulations with 8 bursts of 20 high-frequency pulses (Fig. 6) or onset of continuous 4 Hz pulses after high-frequency pulse stimulation (Fig. 7) did not caused a further decline in baseline BOLD signals although they did when baseline BOLD signal were previously not affected. This could indicate that the initial stimulation already activated these inhibitory (protective) mechanisms, or more likely that the triggered neurovascular coupling mechanisms are also less effective. The latter assumption is supported by the fact that when low frequency pulse stimulation started after several high-frequency pulse stimulation trains, it still lengthened population spike latencies and reduced postsynaptic activities but did not affected baseline BOLD signals (Fig. 7). Thus, not only variations in neuronal activities but also the characteristics of baseline BOLD signals, i.e., the current activity of inhibitory mechanisms, determine the strength of a stimulus-induced BOLD response.

Thus, to consider variation in baseline BOLD signals during an entire fMRI experiment is not only informative for the interpretation of underlying neuronal activities but also important for the valuation of formed BOLD responses. Unfortunately, baseline BOLD signals are no absolute but relative values and therefore baseline BOLD signals are not unambiguously assignable. However, monitoring the time course of baseline BOLD signals during an entire fMRI experiment will reveal slowly developing changes in baseline BOLD signals, provided that no baseline correction tools are used for processing the data. One should be cautious, because drifts in baseline BOLD signals are not unusual during gradient echo EPI fMRI experiments (Evans et al., 2015) and may relate to scanner instabilities (Smith et al., 1999) or incomplete motion corrections (Bandettini et al., 1993). On the other hand variations in baseline BOLD signals were already related to neurophysiological processes

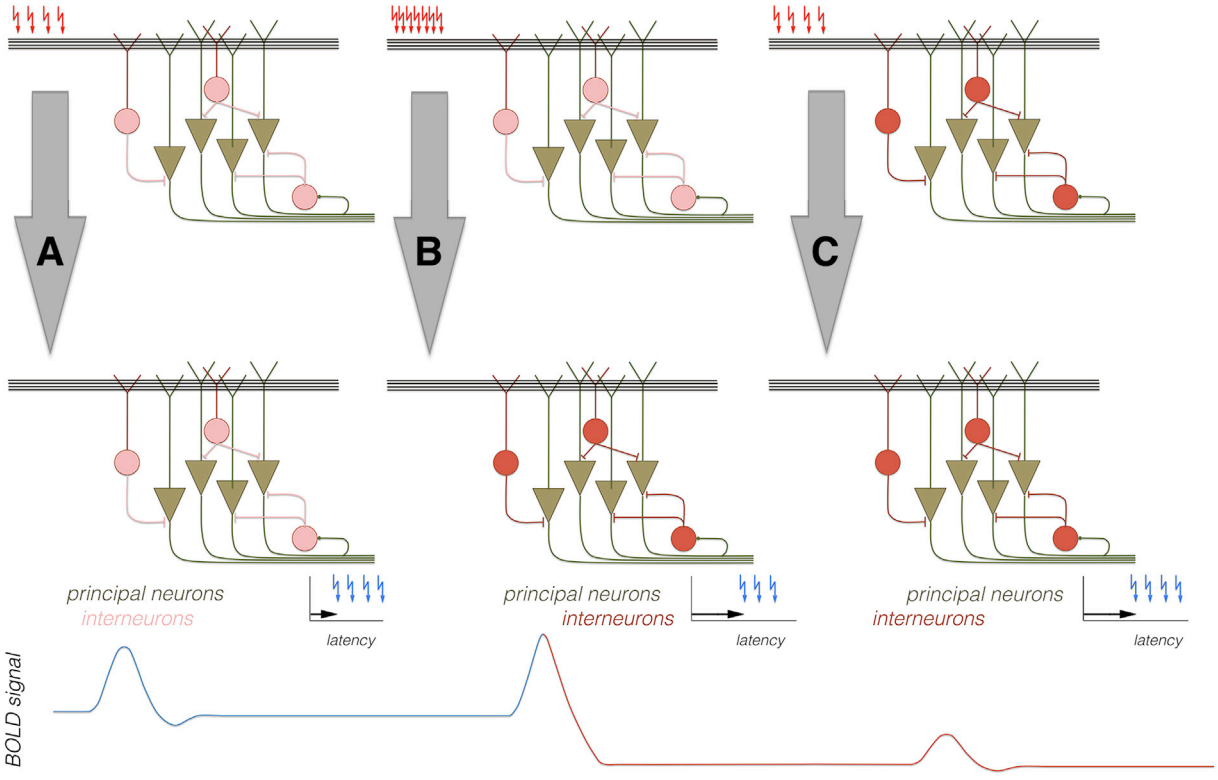

Fig. 8. Stimulus-induced BOLD responses are controlled by the currently existing inhibitory mechanisms that define the excitability of principal neurons. **A** Schematic view of a local neuronal network that processes incoming pulses without long-lasting activation of inhibitory mechanisms. Under this condition the corresponding BOLD response mainly reflects the activity of principal neurons, e.g., in the dentate gyrus the granular cells. Because stimulation did not activated long-lasting inhibitory mechanism(s) baseline BOLD signals remain unaffected. **B** Schematic view of a local neuronal network that processes incoming pulses with a concurrent sustained activation of inhibitory mechanisms to prevent hyperexcitability and afterdischarges. As a result, the latency of granule cell spiking is increased. The ongoing activity of inhibitory mechanisms is metabolically demanding and requires more oxygen than actually supplied; as a result, baseline BOLD signals decline during or after stimulation. **C** Schematic view of a local neuronal network that processes incoming (low-frequency) pulses when previous stimulation caused a sustained activation of these inhibitory mechanisms. Under this condition the induced BOLD response is lower, although the actual spiking activity of principal neurons may not differ (except that the latency increases). Thus, an identical stimulus and identical spiking activity of principal neurons induce smaller BOLD responses depending on the quality of previous stimulations.

(Bovet-Carmona et al., 2019; Yan et al., 2009), thus appropriate controls are always necessary to distinguish between physiological and technical related baseline BOLD signal variations.

4.3. Methodological implications and conclusions

Analysis of raw fMRI data usually requires several steps of pre-processing. Among them, baseline correction is often included. The data presented here suggest that variations in baseline BOLD signals may include valuable information about changing (inhibitory) interneuronal activities and therefore, general baseline correction tools should be used cautiously. Interpretation of observed short- (i.e., negative BOLD responses) and, in particular, long-lasting declines in BOLD signals should not exclude the possibility of an existing increased principal spiking activity. This might be of specific relevance when inputs through a minor efferent fiber system gain importance after ongoing inputs from a major efferent fiber system are effectively inhibited.

Conflicts of interest

The author declares no competing financial interests.

Acknowledgments

I thank Karla Krautwald for her excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2019.116082.

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