Sensory experience selectively reorganizes the late component of evoked responses

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Abstract

In response to sensory stimulation, the cortex exhibits an early transient response followed by a late and slower activation pattern. Recent studies suggest that the early component represents features of the stimulus while the late component is associated with stimulus perception. In this work we study how patterns of evoked activity are modified by experience at mesoscale level using voltage and extracellular glutamate transient recordings over widespread regions of mice dorsal neocortex. We find that repeated tactile or auditory stimulation selectively modifies the spatiotemporal patterns of activity mainly of the late evoked response. This modification results not only in an increase in amplitude of the late response after repeated stimulation, but also the spatiotemporal pattern of the late evoked activity becomes more similar to the pattern of the early evoked response. These changes occur only for the evoked responses of the sensory modality that received the repeated stimulation and they persisted up to one hour. Thus, this selective long-lasting spatiotemporal modification of the cortical activity patterns provides new insights about how perception-related cortical activity changes with sensory experience.

Introduction

The ability to learn from and adapt to changes in their environments are crucial skills that allow organisms to survive. The neural correlates of such adaptive processes correspond to changes in patterns of brain activity. The study of how these changes are encoded and transformed by the brain is crucial to understand brain computation [1]. One of the main approaches to study the neural coding problem – to understand what features of brain activity encode information about the stimulus – is to analyze the dynamics of evoked brain responses. Recently, this approach has been used to study sensory perception for discrimination tasks in the somatosensory and visual cortices in rodents and humans [2–5]. In these experiments, it was shown that the evoked responses have two components, an early and a late evoked deflections. When compared, hit trials, in which the perception of the sensory stimulus is arguably better, show a larger late evoked deflection than in trials where the animals fail to discriminate the stimulus. Moreover, when the late evoked response is inactivated, the task performance decreases [2,4]. Therefore, these results suggest a causal role of the amplitude of the late evoked response for the performance in sensory discrimination tasks due to sensory perception being affected when the late evoked response is perturbed. In humans, the biphasic structure of the sensory evoked response has been reported and researched extensively using functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) [6,7] and magnetoencephalography (MEG) to study cognitive functions[7–13].

In addition to sensory perception, the study of evoked responses has also advanced our understanding of learning and brain plasticity [14,15]. In fact, pioneer *in vitro* electrophysiological studies of changes in synaptic efficacy are one of the pillars of the current understanding of brain plasticity [16,17]. More recent studies have demonstrated that experience can modify the temporal structure of firing patterns in different brain regions in freely behaving rodents [18,19] and even in anesthetized preparations [20,21]. One limitation of these studies is the spatial scale at which these processes are studied. Since complex cognitive functions such as perception and learning involve the interaction of multiple brain structures, the study of experience dependent changes of spatiotemporal patterns of sensory evoked responses at multiple scales can expand our understanding of the relationship between sensory perception and brain plasticity.

Here we present a study of the dynamics of the sensory evoked responses in different modalities in anesthetized and awake mice using wide-field Voltage-sensitive dye and glutamate imaging respectively. The spatiotemporal evoked pattern during the late response becomes more similar to the pattern during the early evoked response after repetitive stimulation. This modification lasted up to one hour after repeated stimulation. Similar changes were observed in extracellular glutamate recordings in awake mice receiving auditory stimulation.

In summary, our results show that the changes induced by experience to the perceptual signals in primary sensory cortices might be strongly related to the late evoked responses. A detailed study of the dynamics of cortical activity at multiple scales like the one presented here can help us to understand the brain mechanisms that are involved in perception and how they are modified by experience [22–24].

Materials and Methods

Animals

Twenty-seven C57Bl/6j adult (20-30 g, age 2-4month) mice were used for voltage-sensitive dye (VSD) experiments under anesthesia. For awake wide-field imaging experiments, 3 adult (>2 months) iGluSnFR transgenic mice (strain Emx-CaMKII-Ai85), expressing iGluSnFR in glutamatergic neocortical neurons [25,26], were used. For this, Emx-CaMKII-Ai85 transgenic mice were generated by crossing the homozygous B6.129S2-Emx1tm1(cre)Krj/J strain (Jax no. 005628) and the B6.Cg-Tg(CamK2a-tTA)1Mmay/DboJ strain (Jax no.007004) with the hemizygous B6;129S-Igs7 tm85(teto-gltI/GDP*) Hze/J strain (Jax no.026260). This crossing is expected to produce expression of iGluSnFR within all excitatory neurons across all layers of the cortex, but not in GABAergic neurons [27,28]. Brain sections of the positive transgenic mice confirmed robust expression in the neocortex and hippocampus. All procedures were performed following approved protocols by the University of Lethbridge Animal Care Committee (ACC) and in accordance with the standards of the Canadian Council on Animal Care (CCAC).

Surgery

Mice were anesthetized with 15% urethane in HEPES-buffered saline solution (1000-1250 mg/kg depending on the age and weight) and fixed in a stereotactic apparatus. Body temperature was maintained at 37°C with an electric heating pad regulated by a feedback thermistor throughout surgery and imaging. Mice were given Dexamethasone (80 µg) intramuscularly to prevent inflammation and Lidocaine (50 µl, at 0.2%) into the skin over the craniotomy area for local anesthesia. For Voltage-sensitive dye imaging experiments, a 7 × 6 mm unilateral craniotomy (bregma 2.5 to −4.5 mm, lateral 0 to 6 mm) was made and the dura mater was removed, as described previously [29–31]. In all cases for VSD imaging, mice were also given a tracheotomy to assist with breathing. For each hour under anesthesia, the mouse was given an intraperitoneal injection of 10 ml/kg of 0.5% dextrose and 0.9% saline solution to maintain hydration. For recordings in awake mice, a craniotomy of ~ 4mm of diameter was performed to expose the auditory cortex. This cranial window had the squamosal bone to its lateral end and its caudal end was 0.5mm anterior to the lambdoid structure. Finally, a stainless steel head-plate was fixed to the skull using metabond and dental cement, a glass coverslip was placed on top to keep the surface clear from accumulating debris from the environment (Fig. S4). After two weeks of recovery from this procedure,

these animals started to be habituated to the recording apparatus (see wide-field imaging procedure below).

Wide-field optical imaging

For VSDI experiments, the dye RH-1691 (optical Imaging, New York, NY) was diluted in HEPES-buffered saline solution (0.5mg/1ml), applied to the brain for 45 min and rinsed subsequently, which stained all neocortical layers as reported previously [29]. The brain was then covered with agarose in HEPES-buffered saline at 0.6% concentration and sealed with a glass coverslip. This procedure reduced the movement artifacts produced by respiration and heartbeat. VSD imaging began ~30 min after washing unbound VSD. For VSD data collection, 12-bit images were captured at 150 Hz during evoked activity and at 100 Hz during spontaneous activity with a charge-coupled device (CCD) camera (1M60 Pantera, Dalsa, Waterloo, ON) and an EPIX E8 frame grabber with XCAP 3.8 imaging software (EPIX, Inc., Buffalo Grove, IL). The dye was excited using a red LED (Luxeon K2, 627 nm center) and excitation filters of 630 ± 15 nm. Images were taken through a macroscope composed of front-to-front video lenses (8.6 x 8.6 mm field of view, 67 µm per pixel). Reflected VSD fluorescence was filtered using a 673-to-703 nm bandpass optical filter (Semrock, New York, NY). To reduce potential VSD signal distortion caused by the presence of large cortical blood vessels, the focal plane was set to a depth of ~ 1mm from the cortex surface [24]. To monitor extracellular glutamate concentration in iGluSnFR mice, the same camera and lenses were used as for VSD recordings. However, a blue LED (Luxeon K2, 473 nm) and an excitation filter (Chroma, 467-499 nm) were used to excite the glutamate fluorescent indicators. The reflected fluorescent signal from excited indicators was filtered using a (Chroma, 510 to 550 nm) band-pass optical filter (Semrock, New York, NY). To reduce potential artifacts caused by the presence of large cortical blood vessels, the focal plane was set to a depth of ~1 mm from the cortical surface [24]. For awake glutamate recordings, mice were habituated to the recording setup after two weeks of recovery from the head-plate implant. This consisted of putting the animals one by one on the recording platform with one or two pieces of Cheerios cereal. After a few days of becoming familiar with the apparatus, the animals were head-restrained in incremental daily periods starting from 20 min, and increasing 5 minutes per day, reaching a total restriction time of 1.5 hours. During the head-fixation period, each animal was placed inside a plastic tube to limit motion and encourage relaxation. In addition, the temperature of the platform was increased to room temperature using microwavable heat pads.

Sensory Stimulation

The experiment consisted of three different periods in time. The first period consisted of 20 single-pulse stimulation trials of 5 seconds each. Each trial consisted of a 0.9 s baseline period followed by a single pulse stimulation, followed by 4.1 s of activity after stimulus onset. Single-pulse stimulation was given to

three different sensory modalities in different experiments to evaluate changes in the evoked responses induced by repeated stimulation. To induce evoked responses in the somatosensory cortex (S1), a thin acupuncture needle was inserted into the paw and a 1ms of 0.2-0.3 mA electrical pulse was delivered. To induce visual evoked responses, a 1ms pulse of green light was delivered approx. 10 cm away from the contralateral eye of the recording hemisphere. To induce auditory evoked responses, a 12 kHz 60-80dB 50ms tone was played to the contralateral side of the recorded hemisphere at 15-20 cm of distance. All auditory stimuli were generated with custom software in MATLAB (MathWorks, Natick, MA) at a sampling rate of 192 kHz using the RX-6 multi-function processor and output to an Electrostatic Speaker driver (Part# ED1, Tucker Davis Technologies, Alachua, FL), which in turn delivered the sounds to an electrostatic speaker (Part# ED-10, Tucker Davis Technologies, Alachua, FL). The speaker was placed 10 cm directly to the left side of the animal's head (contralateral of the recorded hemisphere). The second period consisted of repeated continuous somatosensory or auditory stimulation for 30 min. For repeated somatosensory stimulation, electrical pulses were given to the hind paw as a continuous 20 Hz frequency stimulation train of pulses (0.2-0.3 mA, 1ms) (Figs. 1 and 2). For repeated auditory stimulation, a series of 12 kHz 1 sec tone followed by 1 sec of silence were given for 30 min. (Fig. 3). Finally, the third period consisted of a set of 20 single-pulse stimulation trials of 5 seconds each. Each trial was the same as in the first period (Fig. 1) [20].

Brain state manipulation

Before the experiment started, Methamphetamine (1mg/kg, Sigma-Aldrich) was injected to induce a desynchronized brain state to increase the chances to change the patterns of cortical activity of the sensory evoked responses [32]. To ensure that the brain reached a desynchronized brain state, data collection started 10-15 min after injection for the drug effect to stabilize and we visually verified that the cortical LFP showed increased content in high frequency and reduced amplitude. It is known that desynchronized brain states enhance changes induced by repeated stimulation during urethane anesthesia.

Data analysis of imaging data

To correct for time-dependent changes in VSD signals due to bleaching, 20 non-stimulation interleaved trials were used for normalization of the evoked data. A 10 s interval between each sensory stimulation trial was used. Although VSD fluorescence has been shown to have relatively high labeling at a depth of ~750 μm across the cortex [29], all VSD recordings were expressed as a percentage change relative to baseline VSD responses ($\Delta F/F0 \times 100\%$) to reduce regional bias in VSD signal caused by uneven dye loading or brain curvature where $\Delta F = (F - F0)$, F is the fluorescence signal at any given time and F0 is the average of fluorescence over baseline frames. Analogously, for glutamate imaging changes in glutamate concentration were also defined as a relative quantity to a baseline ($\Delta F/F0 \times 100\%$).VSD imaging of spontaneous activity

was continuously recorded in the absence of sensory stimulation at 100 frames per second. Slow, time-dependent reductions in VSD fluorescence were corrected in MATLAB® using a zero-phase lag Chebyshev bandpass filter (zero-phase filter) at 0.1 to 6 Hz. Ambient light resulting from VSD excitation (630 nm) was measured at 8.65×10^{-3} W⁄m2. In addition, for glutamate awake recordings, raw data were corrected using global signal regression to remove global hemodynamic and illumination fluctuations (Xie et al., 2016).

To measure the similarity between the early and late evoked responses, each stimulation trial was compared against a template constructed from the average evoked response during the first 33.3 ms (5 frames) after the time when the amplitude of the evoked response was 2 STD over the baseline (Fig. 1c and 1e). Similar results were obtained when using different templates lengths (data not shown). The similarity between the template and the evoked activity was calculated as the correlation coefficient between the template and each frame for each stimulation trial (Fig. 1b-c and 1e). To measure the similarity of the spontaneous activity to the early evoked response, the same template matching procedure was used. The similarity between the spontaneous activity and the template was calculated using a sliding window over 1.5 sec of the inter-stimulus interval after 2.5 sec of the stimulus onset of each trial (Fig. 1g).

To evaluate if the amplitude of the early and late components were related, the Fail ratio was defined as the division between the difference of the maximum amplitude of the early response and the maximum amplitude of the late response, divided by the sum of the maximum amplitude of the early response and the maximum amplitude of the late response (Fig. S1b).

Statistical tests.

One-sided paired t-tests and Wilcoxon signed-rank tests (when distributions were not normal) were used to determine if the similarity between the early and late evoked responses (in the same animal) increased after repeated stimulation across all animals. When more than two groups were compared, ANOVA with ad-hoc Bonferroni correction was used. All statistical tests were performed using MATLAB built-in functions.

Code and Data availability

All code was developed in Matlab (MathWorks) and is available on http://github.com. Data will be made available upon request.

Results

Early and late evoked response at the mesoscale level

Brain activity was monitored using VSD wide-field imaging over much of the dorsal cortex of the right hemisphere in anesthetized mice injected with amphetamine (Fig. 1a, see Methods). The reason to inject amphetamine was to induce a desynchronized brain state in which the chances of causing changes in the sensory evoked responses increases [32]. To evaluate the changes induced by experience, the evoked responses to single pulses of stimulation were recorded before and after repeated intermittent electrical stimulation of the hind paw (Fig.1b). As reported before [2–4,31], we found that the sensory evoked response to a single pulse stimulation consists of two components, an early and a late evoked responses. This organization is observed across different modalities (Fig. S3). The early evoked response lasts roughly 100 ms and appears approximately after 25 ms after stimulus onset. Usually, this early evoked response is attributed to thalamo-cortical connections [3]. The late component of the evoked response consists of a larger period of time that lasts around 200-250 ms and appears 200-300 ms after stimulus onset approximately (Fig. 1d).

After 30 min of repeated stimulation of the hind paw, a reorganization of the evoked response to single pulse stimulation was observed. The late evoked response increases in amplitude after repeated stimulation and the early component tends to decrease (Fig. 1d). In addition, after repeated stimulation, the late evoked pattern of activity resembles the early evoked response more closely than before (Fig. 1c). To quantify this spatiotemporal reorganization of the late evoked response, we calculated the similarity between the early and late evoked responses for each stimulation trial using template matching. The similarity was calculated as the correlation coefficient between a template from the average response during the first 33.3 ms and each stimulation trial (Fig. 1e i-ii). After repeated stimulation of the hind paw, the similarity between the early and late components increases.

To evaluate how long these changes last for, the evoked responses to single pulse stimulation of the hind paw were recorded up to 90 min after repeated stimulation (Fig. 1e and Fig. S1a). The similarity between the early and late evoked responses last for up to one hour after repeated stimulation compared to their similarity before (baseline) repeated stimulation (one-way ANOVA with Bonferroni correction, the stars represent $p<0.05$). Moreover, the early component of the evoked pattern of activity reverberates during the subsequent periods of spontaneous activity up to 15-30 min after the repeated stimulation stopped (Fig. 1g). This resembles the same timescale of reverberation reported in temporal patterns of single-unit activity in anesthetized rats [20]. The increase in amplitude of the late evoked response and its similarity to the early evoked response is observed even 1hr after repeated stimulation. These changes are not observed in animals that did not receive repeated stimulation (Fig. S1b-d).

Finally, we confirmed that similar results occur in awake head-fixed transgenic iGluSnFR mice [26]. In this experiment, auditory repeated stimulation was given to mice while recording the extracellular glutamate concentration over the auditory cortex (Fig. 1h (i)). The similarity between the early and late components of the spatiotemporal evoked response increase after repeated auditory stimulation (Fig. 1h (ii), paired t-test, $p<0.05$).

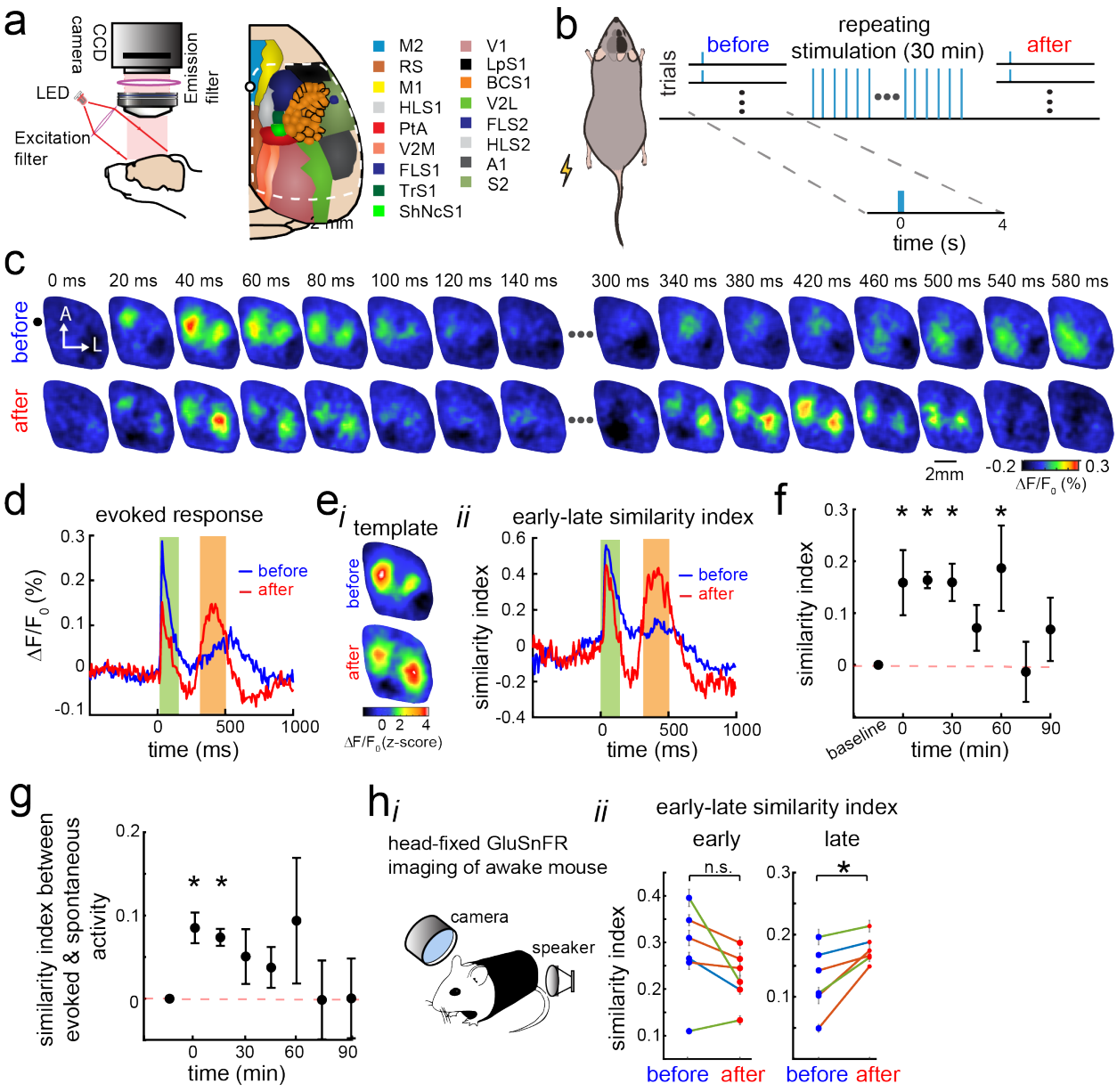

Fig. 1. Spatiotemporal changes in the early and late evoked responses induced by repeated stimulation. (a) Schematic of the VSD imaging apparatus (left). A CCD camera records changes in voltage over the imaged area. The voltage-sensitive dye is excited by a red LED, the reflected light is then filtered and finally captured by the camera. A craniotomy is performed over the right hemisphere to expose the cortical areas imaged (right). The white dashed line denotes a typical cranial window. The abbreviations denote the following cortices AC/M2: anterior cingulate / secondary Motor, RS: retrosplenial, M1: primary motor, HLS1: hindlimb primary somatosensory, PtA: posterior

parietal, V2M: secondary medial visual, FLS1: forelimb primary somatosensory, TrS1: trunk primary somatosensory, ShNcS1: shoulder/neck primary somatosensory, V1: primary visual, ULpS1: lip primary somatosensory, BCS1: primary barrel, V2L: secondary lateral visual, S2: secondary somatosensory, A1: primary auditory. **(b)** Experimental protocol. Anesthetized mice injected with amphetamine received 20 trials of single-pulse electrical stimulation to the hind paw followed by 30 min of rapid (repeated) stimulation of the hind paw at 20 Hz. Finally they received 20 trials of single-pulse stimulus to the hind paw. **(c)** Example of trial-average hind paw evoked activity before (top) and after (bottom) repeated stimulation of the hind paw for one mouse. **(d)** Trial-average evoked response in the HL area to hind paw stimulation for one mouse. The green and orange areas represent the early and late evoked responses, respectively. **(e)** Trial-average template **s**imilarity of evoked responses for the same mouse. (i) Templates of early evoked activity before and after repeated stimulation. (ii) Trial-average similarity between the templates and the evoked response before (blue) and after (red) repeated stimulation. **(f)** Dynamics of changes induced by repeated stimulation. Average similarity between the early and late components of evoked responses measured every 15 min for 1.5 hr. after repeated stimulation for multiple mice. n=8 animals, error bars represent S.E.M. and stars represent statistically different to baseline trials (one-way ANOVA with Bonferroni correction). **(g)** Average similarity of evoked patterns of activity during spontaneous activity measured up to 1.5 hours after repeated stimulation across animals (same as f). **(h)** Recordings in head-fixed awake mice (i). Similarity between template and early evoked responses (left) and late evoked responses (right) in awake animals before (blue) and after (red) repeated stimulation (ii). (n = 3 animals, colored lines represent sessions for different animals. Each session had 30 trials of stimulation each. Error bars denote S.E.M. and stars denote $p < 0.05$, paired t-test).

Stimulation dependent changes in the amplitude of the early and late somatosensory evoked responses

In order to evaluate whether the changes induced by stimulation occur only in the sensory modality that received the repeated stimulation, electrical pulses to the hind paw of the animal were interleaved with visual stimulation (see Methods), and repeated electrical pulses of stimulation (Fig. 2a) were only given to the hind paw (not repeated visual stimulation).

After repeated electrical stimulation of the hind paw, an overall increase in the average amplitude evoked response (across animals) was observed, mainly during the late component of the hind paw evoked response (Fig. 2c (i)) which is not observed in the visual evoked response (Fig. 2c (ii)). Interestingly, the opposite effect is observed in the visual evoked response. The quantification of the amplitude of the average evoked response shows that there is a significant increase (pair-wise t-test, $p < 0.05$, n=11) in the amplitude of the late hind paw evoked response after repeated stimulation (Fig. 2d (i)) but no significant changes in the visual evoked response (Fig. 2d (ii)).

Spatiotemporal reorganization of the late somatosensory evoked response after repeated stimulation

To evaluate whether the reorganization of the spatiotemporal late evoked response after repeated stimulation was exclusive to the sensory modality that was stimulated, the similarity between the late evoked response and the pattern of activity during the early response were compared for every trial of hind paw and visual stimulation.

The similarity between the early and late evoked responses was calculated using template matching as explained previously. After hind paw repeated stimulation, an increase in the mean similarity across

trials between the early and late hind paw evoked responses was observed (Fig 2e i) but not in the visually-evoked responses in the visual cortex (Fig 2e ii). In fact, there is a significant increase in the similarity between the early and late evoked responses after repeated stimulation for the hind limb area of the somatosensory cortex but not for the visual evoked responses (Fig. 2f, paired t-test, $p < 0.05$, n=11).

In summary, these results suggest that the spatiotemporal reorganization of the spatiotemporal evoked patterns after repeated sensory stimulation only occur in the cortical region corresponding to the sensory modality that was repeatedly stimulated.

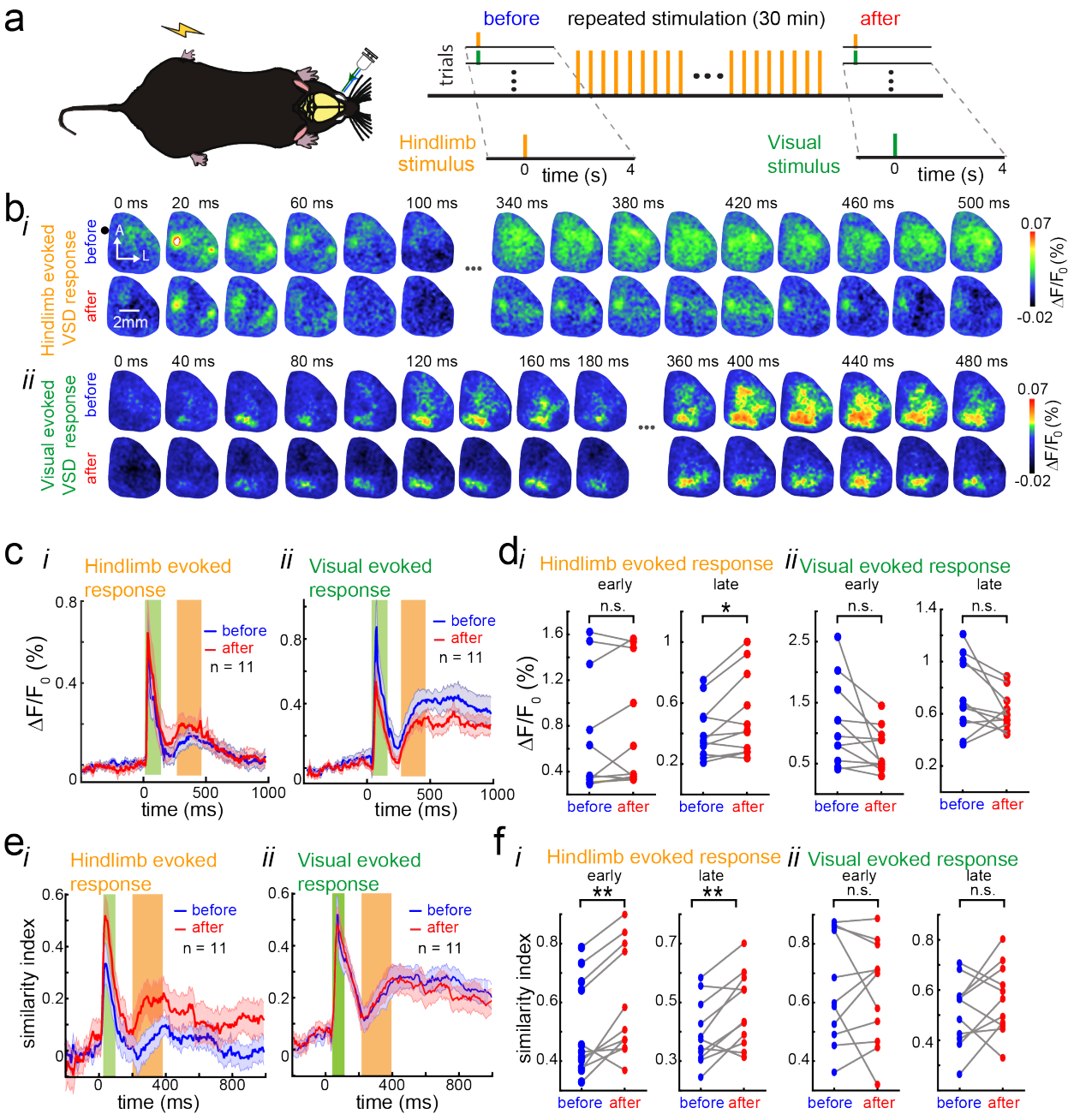

Fig. 2. Spatiotemporal changes in the early and late evoked responses are stimulation dependent. (a) Experimental protocol. The anesthetized mice injected with amphetamine received interleaved single-pulse electrical stimulation to the hind paw and a LED flash as visual stimulation (20 trials each) followed by 30 min of repeated stimulation of the hind paw at 20 Hz and finally received another interleaved single-pulse hind paw and visual stimulation (20 trials each). **(b)** Example of trial-average evoked activity dynamics in response to electrical hind paw stimulation (i) before (top row) and after (bottom row) repeated hind paw stimulation for one mouse. Example of trial-average evoked activity in response to visual stimulation (ii) before (top) and after (bottom) hind paw repeated stimulation in the same mouse. **(c)** Average evoked activity across animals before (blue) and after (red) HL repeated stimulation in response to HL electrical stimulation (i) and to visual stimulation (ii). The shaded regions denote the S.E.M. (n=11). Green and orange regions denote the early and late components of the evoked responses, respectively. **(d)** Paired comparison of the peak amplitude of the early and late evoked response before and after repeated stimulation for hind paw stimulation (i) and visual stimulation (ii) for all animals (n=11). **(e)** Average template

similarity between evoked response before (blue) and after (red) repeated hind paw (i) and visual stimulation (ii) across animals. **(f)** Paired comparison of the template similarity of early and late evoked responses to hind paw (i) and visual stimulation (ii) before (blue) and after (red) during early (left) and late components (right) for all animals. Stars represent significant increase in amplitude of the late evoked response after repeated hind paw stimulation (t-test, $p < 0.05$).

Spatiotemporal reorganization of the late evoked response in two sensory modalities

To evaluate whether the changes induced by the repeated stimulation were restricted to the somatosensory cortex or could be observed in other sensory cortices, auditory and hind paw repeated stimulation were provided in the same preparation (Fig 3a-b). As before, the single-pulse evoked responses were compared before and after repeated sensory stimulation. This time, a 50 ms 12 Khz tone was interleaved with 1 ms 300 μA pulse to the hind paw, separated by 10 seconds. These single pulse stimulations (20 trials each) were repeated three times: one before any repeated stimulation (denoted evoked1), one after repeated auditory stimulation (denoted evoked2) and one more after repeated hind paw stimulation (denoted evoked3) (Fig 3b). The spatiotemporal patterns of activity show that after single tone stimulation, the early response activity starts in the auditory cortex after 20 ms from stimulus onset and expands for approximately 120 ms. Similarly, the late auditory evoked response starts around 200 ms after the stimulus onset and lasts for 300 ms approximately (Fig. 3c (i)). Analogously, the hind paw early evoked response starts after approximately 20 ms after stimulus onset in the primary somatosensory cortex and lasts for 100 ms including the secondary somatosensory cortex and expanding to the midline areas (Fig. 3c (ii)).

We found that after repeated auditory stimulation the amplitude of the early auditory evoked responses does not change compared to baseline (Fig. 3d (i) and 3e (i), left). However, the amplitude of the late auditory evoked responses increased after repeated auditory stimulation (Fig. 3d (i), and 3e (i), right). In contrast, the amplitude of the late hind paw evoked responses did not increase after the auditory repeated stimulation (Fig 3d and 3e (ii) right). After repeated hind paw stimulation (evoked3), the amplitude of the auditory evoked response (red trace) decreased (Fig. 3d, left) and in contrast, the hind paw evoked response increased (Fig 3e, right), but it was not statistically significant (Fig 3e (ii), left).

To evaluate whether the similarity between the early and late spatiotemporal responses was modified after repeated sensory stimulation, the evoked responses were compared against a template built from the first 33.3 ms after the onset of the evoked response. As was done previously, we calculated the correlation coefficient between the spatiotemporal auditory evoked response for every trial and the template of the early auditory evoked response before (evoked1, black trace) and after auditory repeated stimulation (evoked2, blue trace). Analogously, we compared the similarity between the early and late hind paw evoked responses before and after repeated hind paw stimulation (evoked3, red trace) (Fig. 3f-g).

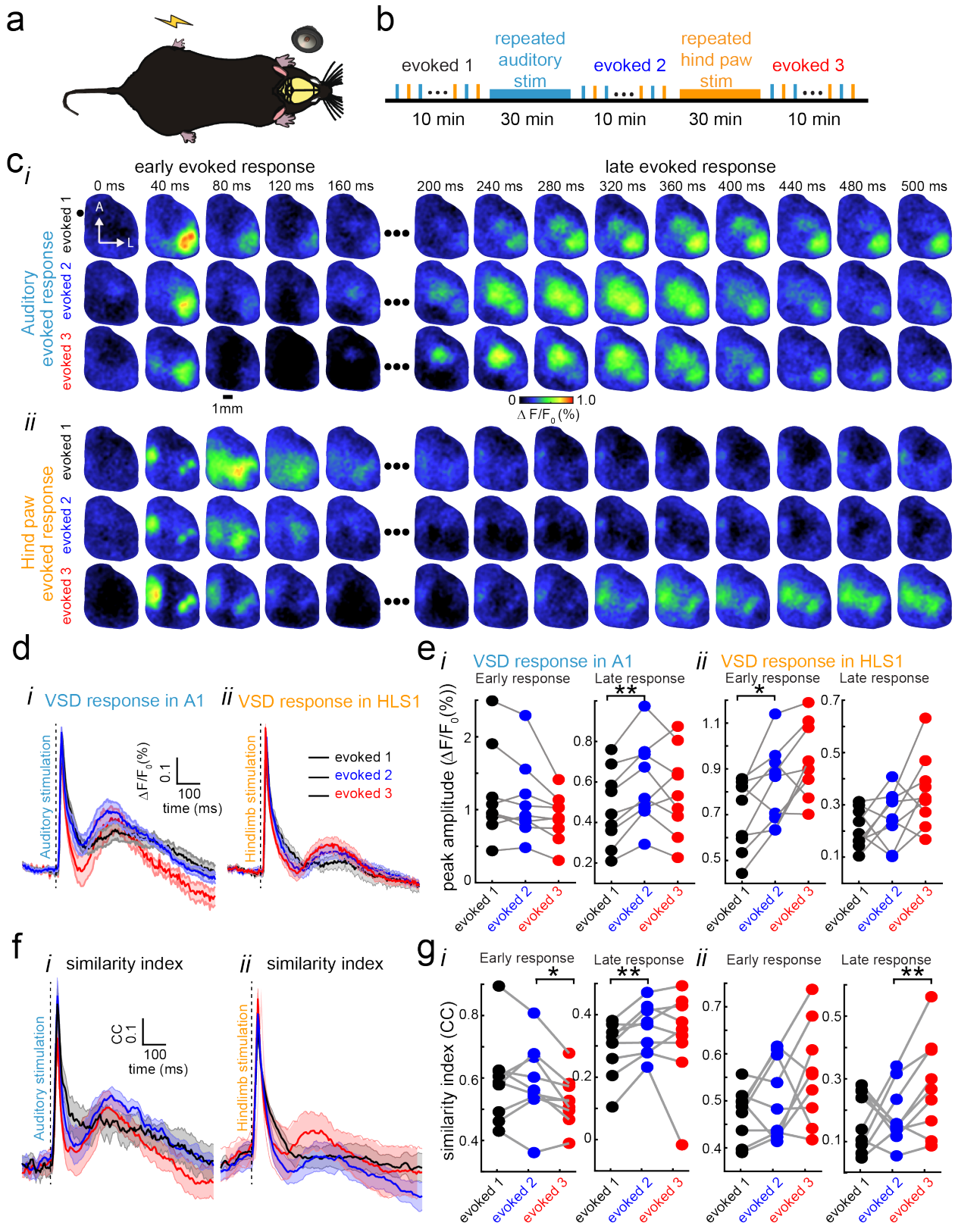

Fig. 3. Spatiotemporal changes in the early and late evoked responses are exclusive to the stimulated sensory modality. (a) Schematic of the experimental protocol. The anesthetized mice injected with amphetamine received

both repeated auditory and repeated hind paw stimulation. **(b)** Auditory and hind paw repeated stimulation in the same preparation. Single-pulse evoked responses to auditory and hind paw stimulation were interleaved every 10 sec before any repeated stimulation (evoked1), after auditory repeated stimulation (evoked2) and after hind paw repeated stimulation (evoked3). **(c)** Trial-average evoked responses to auditory stimulation (i) and hind paw stimulation (ii) before (evoked1) and after (evoked2) repeated auditory stimulation and after repeated hind paw stimulation (evoked3) for one mouse. **(d)** Average evoked response in the auditory cortex (i) and in the hind limb area of the somatosensory cortex (ii) across animals. **(e)** Pair-wise comparison between the peak amplitude of the responses to auditory stimulation (i) and hind paw stimulation (ii) during the early (left) and late (right) components for all animals. **(f)** Mean template similarity for auditory evoked responses (i) and hind paw evoked responses (ii) before any repeated stimulation (black), after repeated auditory stimulation (blue) and after repeated hind paw stimulation (red) across animals. **(g)** Paired comparison between the similarity between the early and late auditory (i) and hind paw (ii) evoked responses during the early (left) and late hind paw evoked responses (right) before any repeated stimulation (black), after auditory repeated stimulation (blue) and after repeated hind paw stimulation (red). (n=9, each line denotes an animal. * and ** denote $p<0.05$ and $p<0.01$, paired t-test respectively).

This time a significant increase in the similarity between the auditory late and early components of the evoked response was observed after repeated auditory stimulation but not after repeated hind paw stimulation (Fig. 3g (i), right). Analogously, a significant increase in the similarity between the late and early components of the hind paw evoked response was observed after repeated hind paw stimulation (Fig. 3g (ii), right) but in the auditory evoked responses. In summary, these results show that repeated sensory stimulation causes a reorganization of the evoked responses. This reorganization consists of an increase in the similarity between the spatiotemporal late evoked response and the early evoked pattern of activity. Moreover, this reorganization of the cortical activity occurs in more than one sensory modality at the mesoscale level.

In summary, these results show that the similarity of the temporal structure of the early and late components of the evoked response increases after repeated hind paw stimulation. In addition, most of the changes of the temporal structure after repeated stimulation occurs during the late evoked response. Altogether, our results show that the evoked response is formed by an early and a late component at the mesoscale and microcircuit levels. After repeated sensory stimulation, the late component changes its spatiotemporal structure to closely resemble the pattern of activity during the early evoked response. These selective changes occur in different sensory modalities, at different spatial scales, and in different rodent species.

Discussion

The sensory evoked response is formed by two components. The first component consists of the initial evoked response that lasts around 100 ms after stimulus onset (early evoked response). The second component consists of an additional ‘bump’ of activity that starts around 180 ms after stimulus onset and lasts for about 210 ms (late evoked response). A similar description of the evoked response in several

sensory areas has been reported [2–4,33]. In these studies, it is argued that the second component of the evoked response is associated with sensory perception.

In this work we demonstrate how repeated stimulation alters the dynamics of the evoked responses to sensory stimulation at the mesoscale level. We found that after repeated stimulation, the amplitude of the late evoked response increases after repeated sensory stimulation and the spatiotemporal pattern of the late evoked response becomes more similar to the pattern of the early evoked response. These experience dependent modifications of spatiotemporal brain activity occur in the sensory modality that was stimulated (i.e. somatosensory and auditory cortices) and can be induced in more than one modality in the same preparation and, in both, anesthetized and awake animals. In summary, we show that most of the changes induced by the repeated stimulation occur during the late component of the evoked responses and these changes make the patterns of activity of the late component more similar to the pattern of activity of the early evoked response.

The bimodal organization of the sensory evoked response

According to the literature, the two components of the sensory evoked response are related to different functions and are produced by different brain circuits. On the one hand, the early component is associated with sensory identity and is produced by the feedforward thalamocortical inputs to the corresponding sensory areas [3]. On the other hand, the second component is associated with sensory perception and it is produced by a combination of feedback cortico-cortical and thalamocortical inputs (Fig. 4) [3,4,34]. At least for the cortical feedback, it has been suggested that the late evoked responses in primary sensory areas is originated by a neuronal population in higher cortical areas and that this signal is associated to perceptual processing [5,35,36].

Apart from the perceptual content of the biphasic organization of cortical evoked responses, there are other ideas regarding the function of the biphasic organization of cortical activity. Recently, a biphasic organization of cortical activity has been proposed as an information organization in which brain activity is transmitted in packets. The early component is proposed to contain categorical (more general) information and be less variable. The late component is proposed to contain more specific information (identity) [37]. Even though more experiments are needed to clarify the information content of the early and late components of evoked responses, there are similarities in their organization described in this paper and the one in the packet-based theory of information transmission in the brain, which are important to highlight. One is that the two-part configuration of the packet-based theory, in which the first part shows less variability and a second part which carries a more complex information and seems to be more plastic after experience. The second part corresponds to the late component in our study, which resembles the spatiotemporal pattern of the early evoked response. In our experiments we observed that after repeated

stimulation, there is an enhancement of the evoked response organization into two components (Fig S1 c, f). This modification lasts up to two hours after repeated stimulation (Fig. S1 d). In addition, the slope of the early and late components of the evoked response after repeated sensory stimulation (Fig. S1 g). This result suggests that the repeated stimulation increases synaptic strengths in the brain circuitry involved in the early and late evoked responses. However, additional experiments are needed (e.g. NMDA blocking) to test this hypothesis.

Organization of the evoked responses in different sensory modalities

When we compared the cortical evoked responses to different sensory stimulation, we found that the temporal organization of the evoked responses, although similar, are not exactly the same across sensory modalities [24]. In particular, as shown previously [24], the stimulation of the forepaw and hind paw causes a rapid early evoked response in the corresponding areas of the somatosensory cortex that is comparable to the response in auditory cortex (around 20 ms after stimulus onset) but significantly faster than the response in the visual cortex (around 50 ms after stimulus onset) (Figs. 2 and S3 a). Remarkably, there is no difference in the temporal structure of the late component of the evoked response for different sensory modalities (Fig. S3 b).

Experience dependent changes in the sensory evoked response and their functional role

Why would the late evoked response increase after repeated stimulation? According to recent research, animals that are better learners in sensory discrimination tasks (visual or tactile) also show an increase in the late evoked response compared to animals that do not learn the task [2–4]. In our study, we observe this increase in the membrane depolarization (measured with voltage imaging) and synaptic activity (measured with glutamate imaging) signal at the mesoscopic level on average (Figs. 2d i, 3e i). This increase could be due to different mechanisms such as sensory adaptation[38,39]. For example, it could be due to physiological or morphological neuronal changes (e.g. lowering spiking threshold) or changes at population level such as an increase in the recruited neuronal population due to increases in the connectivity between the neurons that generate the late evoked response and the primary sensory neuronal population from which we are recording. All these are complex processes and require more experiments to be investigated.

This leads to the next question of why the late evoked response not only increases in amplitude but also in similarity to the early evoked response. Since we did not address this directly, we can only provide a speculative answer. A possible explanation for the increase in similarity between the late and early evoked responses after repeated presentation of similar stimulus could be provided from the neurophysiology of top-down modulation of evoked responses in primary sensory areas [40]. Similar modulation occurs in reinforcement learning mechanisms in the brain [41]. In this view, during learning, a feedback signal is necessary to perform the synaptic updates (strengthened or weakened) of the circuits according to rewards

or punishment associated with a selected action triggered by the received stimulus. Although, in our experiments, there was not explicit reward, the late component of the evoked response, arguably a signal generated in higher cortical areas, could serve this purpose (Fig. 4). That is, top-down modulation might be the mechanism to induce perceptual learning [42]. This perceptual learning might be implemented as modifications in the synaptic connections of the primary sensory circuitry. Another possible explanation that would not require reward-based learning, would be provided from a predictive coding perspective in which higher cortical areas provide a prediction signal observed during the late evoked response [40,43]. From this perspective, the repeated stimulation would shape such predictions via sensory adaptation processes [39,44]. Further experiments to test these ideas are necessary.

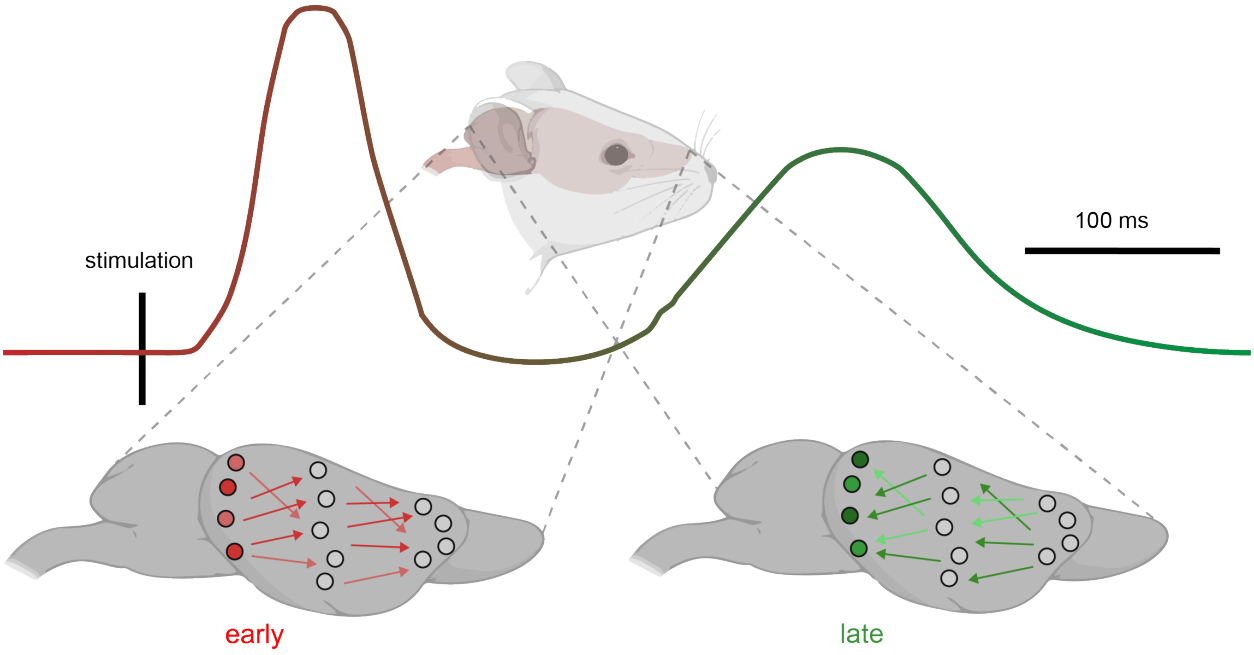

Fig. 4. Possible origin and role of the early and late components of the sensory evoked response. Schematic possible description of the origin of the early and late evoked components. The early component is originated by feedforward thalamocortical inputs to the sensory cortex (e.g. V1) and carries primary information about the stimulus (red). The late component might be a top-down signal either from higher cortical areas or thalamus and is associated with stimulus perception (green). After the initial cortical response (from the thalamus) reaches higher cortical areas (left) and the corresponding computations (i.e. motor selection and attentional tagging) occur, a signal goes back to primary sensory areas from higher cortical or subcortical regions.

Limitations and Future work

Different avenues are left unexplored in this work such as the relationship between cortical state and the organization of the evoked response. According to Curto et al., (2009), a similar structure of the evoked response can be largely explained by the cortical state preceding the stimulus presentation. However, in our

recordings, this was not the case. In our experiments, the brain state did not show a relationship with changes in the amplitude nor similarity between the early and late evoked components. However, we confirmed that the brain state was more desynchronized after amphetamine injection, and that changed in the brain state remain stable for at least one hour (Fig. S2). At the behavioral level, it has been shown that the cortical state preceding the stimulus presentation did not affect the performance in a simple sensory perception task in head-restrained mice [4]. A deeper understanding at the single trial level in a preparation in which the cortical state could be quantified and separated from behavior, such as the one presented here, could shed some light on this issue. On a similar note, with the approach presented here, it should be possible to study the relationship between the changes observed during the late evoked response and other cortical regions. For example, an analysis of the cortical activity propagation could reveal whether a cortico-cortical propagation of activity (travelling waves) could explain the increase in similarity between the early and late components at the single-trial level.

Finally, even though we show that repeated stimulation given to awake animals increases the similarity between the spatiotemporal patterns of activity during the early and late components of the evoked response in auditory cortex (Fig. 1g and Fig. S3), we believe that the perceptual content of this signal might be limited. We know that perception might be affected by cognitive processes such as attention and motivation [45]. Since the animals in our experiments were passively listening to tones, we believe that changes in the late evoked responses might be enhanced when the sensory stimulation has 'meaning' for the animal. Future experiments to test this would need to include a sensory-discrimination task in which sensory perception is directly associated to task performance.

Significance

The results presented in this work demonstrate how the late evoked response that is usually associated with perception in sensory cortices is modified by experience at the mesoscale level. This modification consists in a spatiotemporal reorganization of the early and late components of the evoked response becoming more similar to each other. The reported spatiotemporal changes of the late sensory evoked response induced by repeated stimulation occur in different sensory modalities and last up to one hour. This work expands the understanding of the content of the late evoked response and its relationship with experience.

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Author contribution: E.B.C., A.L. and M.H.M. designed the experiments. E.B.C. and A.G.P. conducted the experiments. E.B.C. performed data analysis. E.B.C and M.H.M. wrote the manuscript, which all authors commented on and edited. M.H.M. and A.L. supervised the project.

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Supplementary material

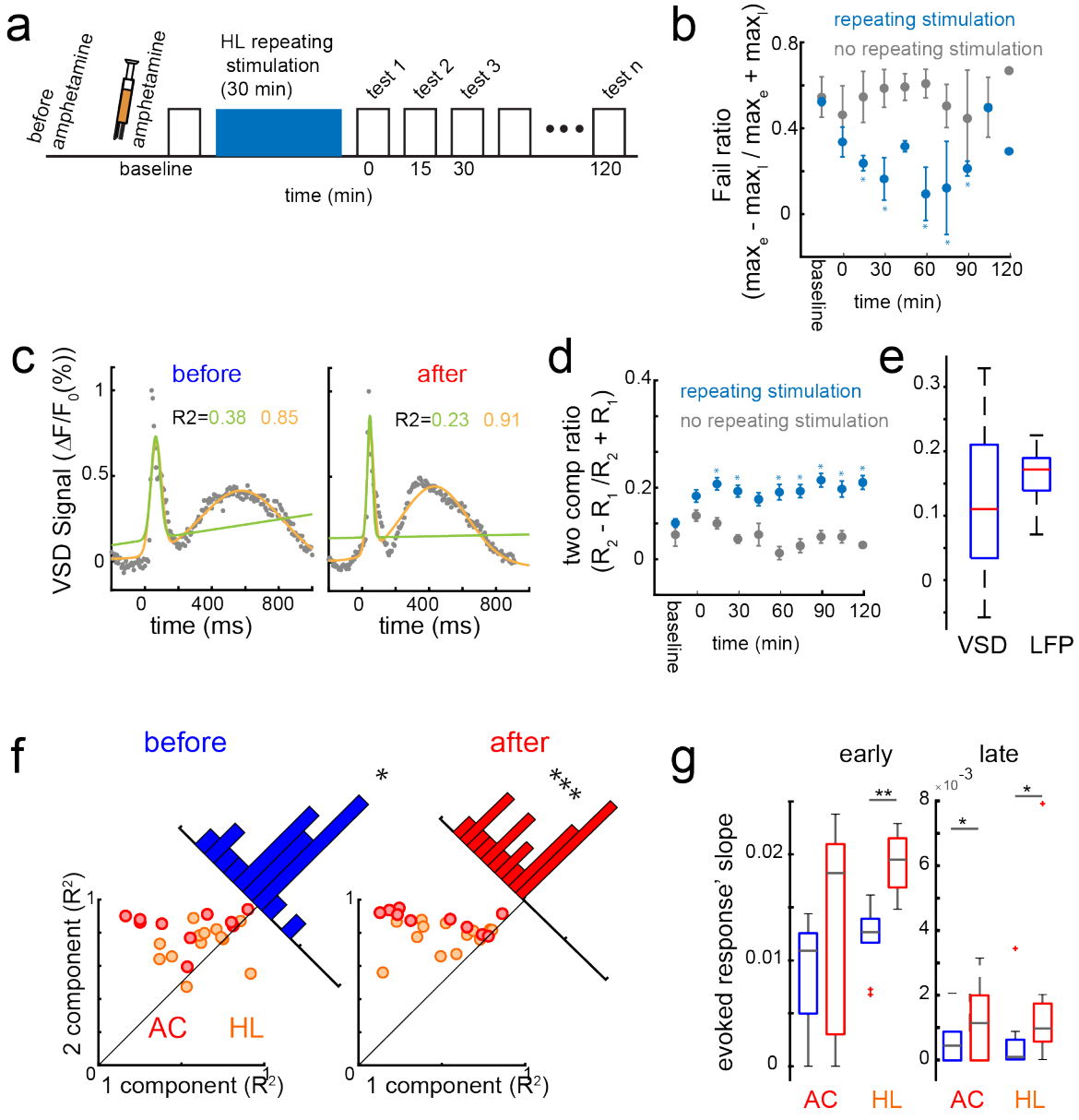

Fig. S1. Dynamics and organization of the evoked responses. (a) Experiment protocol. 30 trial evoked responses were collected after injecting amphetamine (baseline), repeated electrical pulses per second were given to the hind paw during 30 min. Subsequent tests of 30 trials each were collected every 15 min after repeated stimulation. **(b)** Dynamics of the Fail ratio between the early and late component for animals that received repeated stimulation (blue) and animals that did not receive repeated stimulation (grey). **(c)** Example of approximation of single-trial evoked responses using a Gaussian Mixture Model with one (green) or two (yellow) components before (left) and after (red) repeated stimulation. **(d)** Dynamics of the two-component ratio. Error bars denote the S.E.M. and stars denote trials that are significantly different to the baseline trials (ANOVA with Bonferroni correction, n= 90 trials). (**e**) Boxplot of the two component ratio for the HL evoked responses recorded with VSD and LFP. **(f)** Scatter plots of R-squared using one or two components before (blue) and after (red) repeated stimulation. Each dot denotes the mean R-squared for the corresponding model (1 or 2 components) across trials of each animal for hind paw (orange) or auditory (red)

stimulation. The stars represent p<0.05 and p<0.001, t-test). **(g)** Comparison of the slopes of the early (left) and late (late) evoked components for the evoked responses to auditory stimulation (AC) and hind paw stimulation (HL) before (blue) and after (red) repeated stimulation. Stars denote significant differences between the populations (p<0.05, p<0.01, Wilcoxon rank sum test or t-test depending whether the distributions were normal or not).

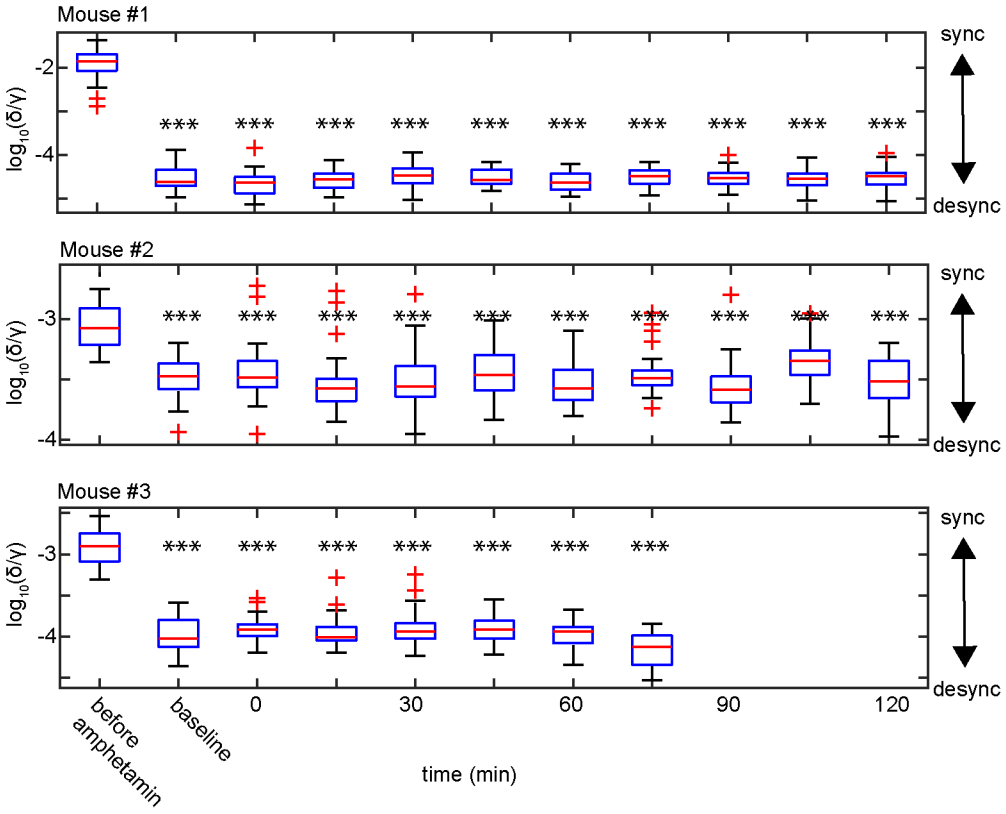

Fig. S2. Cortical state stability. Each panel shows the brain state for separate animals across the corresponding recordings. The brain state before amphetamine was injected was significantly different from every recording for up to two hours (one-way ANOVA, Bonferroni correction, *** represents p<0.001). In contrast, the brain state was not significantly different across recordings after amphetamine injection.

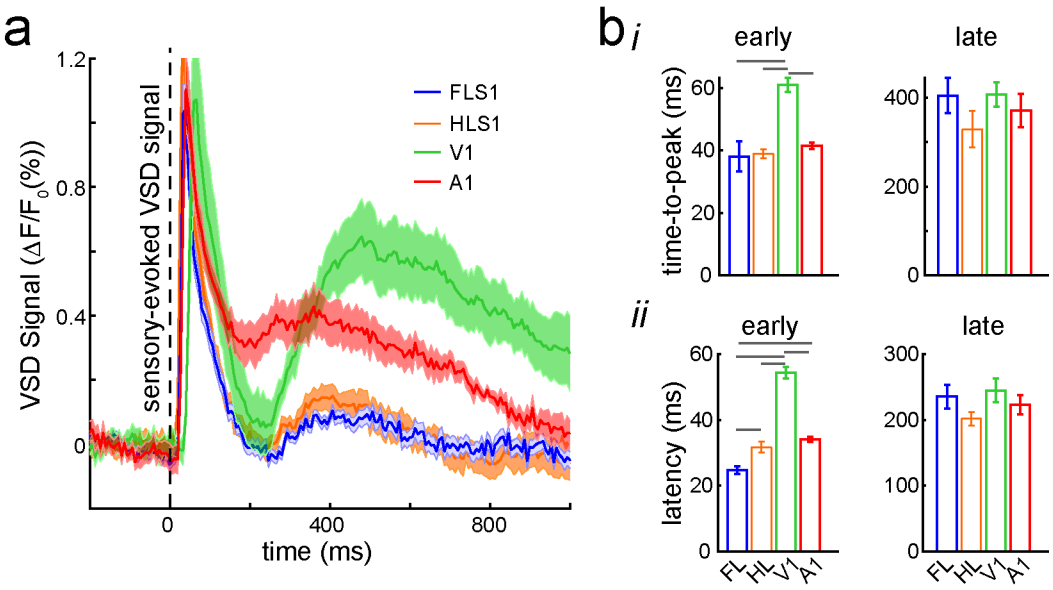

Fig. S3. Organization of the evoked response for multiple sensory modalities and its changes induced by repeated sensory stimulation. (a) Average evoked responses for stimulation of the fore paw (FL), hind paw (HL), visual stimulation (VC) and auditory stimulation (AC) before repeated stimulation. Solid lines represent the mean (n=7, 12, 7, 12 for each sensory modality respectively). The shadows represent the SEM across animals. **(b)** Temporal organization of the evoked response for different sensory modalities. (i) mean time-to-peak across animals for the early (left) and late (right) components of the evoked response. (ii) mean latency of the evoked response for the early (left) and late (right) components of the evoked responses. The error bars denote the SEM. The gray horizontal lines denote significant differences across the populations for the different stimulated peripheries ($p < 0.05$, one-way ANOVA).

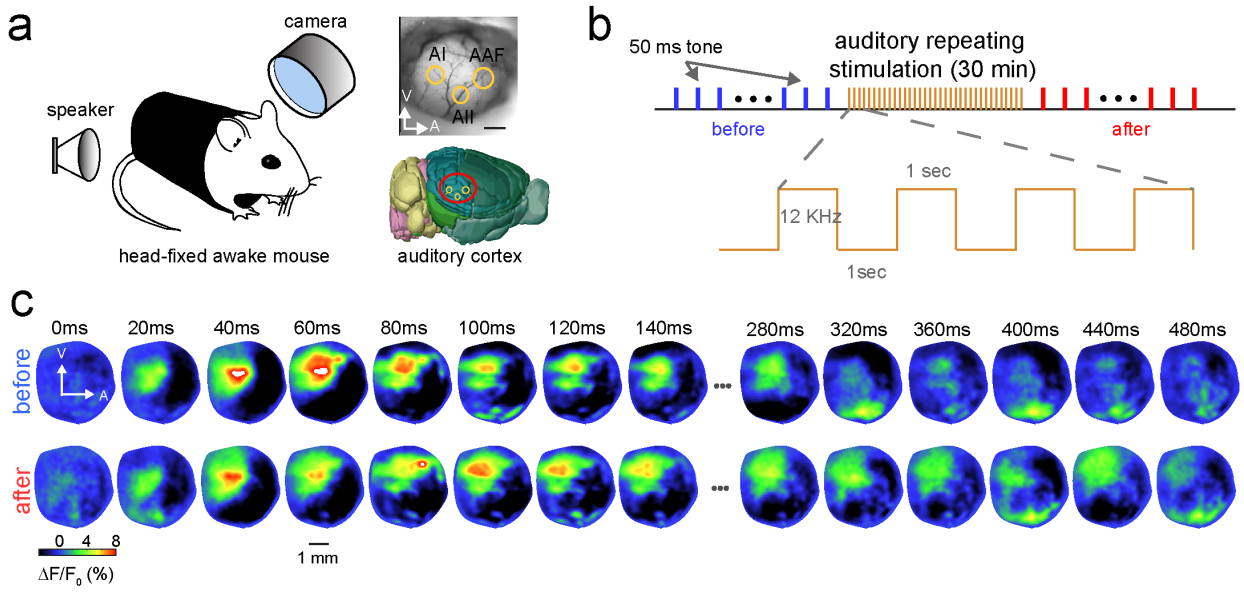

Fig. S4. Experience dependent changes in spatiotemporal patterns of evoked activity in awake animals. (a) Experimental setup. Animals are head-fixed passively listening to tones while brain activity is recorded in the auditory cortex. **(b)** Experimental protocol. Single 50 ms tone trials were recorded before (blue) and after (red) repeated auditory stimulation during 30 min. **(c)** Representative spatiotemporal pattern of trial-average evoked activity before (top row) and after (bottom row) repeated auditory stimulation for one mouse.