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Conditioning-induced changes in sensory cortical maps detected in mice by intrinsic signal optical imaging

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Intrinsic signal optical imaging (ISOI) has been used previously for the detection of changes in sensory processing in the somatosensory cortex in response to environment alteration or after deprivation of sensory information. To date, there have been no reports of ISOI being used in learning-induced changes in the somatosensory cortex. In the present study, ISOI was performed twice in the same mouse: before and after conditional fear learning. The conditioning paradigm consisted of pairing sensory stimulation of vibrissae with electric tail shock. In order to map the cortical representation of the vibrissa B1 with ISOI, we deflected the vibrissa with an intensive stimulation (frequency of 10 Hz for 6 s). After conditioning, we found that the cortical representation of vibrissa B1 had expanded by an average of 44%, compared with pre-learning, by using images obtained with ISOI. Previously, we demonstrated an enlargement of the cortical representation of the vibrissae stimulated by the same behavioral training paradigm but using [^{14}C]2-deoxyglucose. This current investigation provides the first ISOI-based evidence of learning-induced changes in plasticity in the barrel cortex. The results indicate that irrespective of physiological mechanisms used for visualization of the vibrissae representation or subject's testing state (aware or anesthetized animal), the conditioning induced changes in each case in the cortical processing of intensive stimuli. This suggests specific functional reorganization of the neuronal circuits. Moreover, ISOI as a noninvasive method of mapping cortical activation in the same animal before and after behavioral training could serve as a very useful tool for precise manipulation within the cortex and for assessing the resulting effects on experience-dependent cortical plasticity.

Key words: intrinsic signal optical imaging, barrel cortex, classical conditioning, learning-induced plasticity, experience-dependent plasticity, mice

INTRODUCTION

Classical associative conditioning results in changes in the perception of sensory stimuli. Specifically, a neutral stimulus serves as the conditioned stimulus (CS) that, as a result of conditioning evokes an involuntary reflexive response specific to the biologically relevant unconditioned stimulus (US). We have designed a classical conditioning paradigm for mice, in which stimulation of one row of mystacial vibrissae (CS) is paired with a mild tail shock (UCS). Conditioning evoked the behavioral change of a reduction in head movements in response to the CS, termed "minifreezing" (Cybulska-Klosowicz et al., 2009).

This well-expressed conditioning-induced alteration in the animal's behavior is accompanied by a change in the areal extent of the cortical representation receiving the conditioned stimulus, as we have shown with [14C]-2-deoxyglucose (2DG) mapping in the barrel cortex (Siucinska and Kossut, 1996; Liguz-Lecznar et al., 2011; 2015; Posluszny et al., 2015). The change in the extent of cortical representation was observed only in

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the CS+UCS group, but not in the pseudoconditioned animals or in the CS-only control group. Mice in the control groups did not develop minifreezing during the course of training (Cybulska-Klosowicz, 2016). These results confirmed that the association between the UCS and CS was formed and accompanied by an expansion of the cortical representation of the "trained" whiskers. A gain in the cortical representation responding to a CS has also been shown using a microelectrode array in the auditory cortex after instrumental conditioning (Rutkowski and Weinberger, 2005; Blake et al., 2006; Bieszczad and Weinberger, 2010).

However, these methods have limitations, as 2DG mapping enables only post-mortem assessments and the use of a multielectrode array is an invasive approach. Non-invasive intrinsic signal optical imaging (ISOI) of brain tissue in rodents, being performed through the skull, provides maps that can also be used for quantitative assessment of the areal extent of functional cortical units (Chen-Bee et al., 1996). As it enables repeated imaging in the same animal, ISOI seems to be especially well suited for the detection of changes in cortical sensory representations, i.e., cortical maps; this would include, for example, changes resulting from learning-induced plasticity or, more generally, experience-dependent plasticity. Indeed, spatial reorganization of the representational area in the visual cortex has already been demonstrated using ISOI in experiments on modulation of ocular dominance plasticity or after chronic exposure to a single orientation (Cang et al., 2005; O'Hashi et al., 2007; Seibt et al., 2008; Greifzu et al., 2014; Kalogeraki et al., 2017). Cortical plasticity was also detected by ISOI in the somatosensory cortex after specific vibrissae deprivation or after the placement of an animal in an enriched environment (Polley et al., 1999; 2004; Drew and Feldman, 2009).

Until recently, there was no available data on ISOI visualization of cortical plasticity induced specifically by learning. Goltstein et al. (2018) have reported a spatial reorganization in the visual cortex induced by appetitive conditioning detected with ISOI. However, to date, there is a lack of reports on the use of ISOI for the visualization of learning-induced plastic changes in the barrel cortex, a rodent vibrissae cortical representation. Located within the primary somatosensory cortex, the vibrissae cortical representations have a unique structural feature – the neuronal aggregates representing particular vibrissae – referred to as barrels (Fig. 1A). Clearly visible in formalin-fixed brain tissue without staining, a set of barrels reflects the vibrissae arrangement within the mystacial pad. Barrels constitute an anatomical cortical representation of the facial vibrissae, whereas a functional cortical representation is an overall cortical area responding to the stimulation of a particular vibrissa. In the current study, we imaged the functional cortical representation of vibrissae. We provide the first ISOI-based visualization of plastic changes in the barrel cortex, resulting from learning in a classical conditioning paradigm. The pairing of a whisker deflection (CS) with an electric tail shock (US) caused an enlargement of the functional vibrissa cortical representations.

METHODS

Subjects

The experiments were performed on 14 four-week-old C57BL/6J strain mice. The animals were housed in standard conditions: a temperature-controlled room with a light/dark cycle (12h/12h) and food and water available ad libitum. All procedures conformed to the European Community Council Directive (2010/63/UE) and were approved by the Local Ethical Commission No.1 in Warsaw (381/2017). All efforts were made to minimize the number of animals used and their suffering. The experiments were performed on two groups of mice: the experimental group, which underwent conditioning (conditioned group, n=7), and the control group, without conditioning (n=7).

Habituation and conditioning

The experimental design is shown in Fig. 1. The conditioned group mice had been habituated to the test room and a restraining holder (10 min per day) over a three-week period prior to conditioning. The restraining holder was necessary to keep the mouse in one place while allowing free movement of the head. Conditioning was comprised of three sessions, with one 10-min session/day and each session comprising 40 trials. During each trial, three 3 s smooth strokes of the row B vibrissae on one side of the snout applied manually with a fine brush (CS) were coupled with a mild tail shock (US; 0.5 mA, 0.5 ms, administered at the end of the third vibrissae stroke when the brush was still touching the vibrissae). Stimuli coupling was separated by a 6 s interval. All conditioning sessions were recorded with a video camera.

ISOI

In the conditioned group, the first ISOI acquisition was performed two days before conditioning (the day after the initial ISOI was designed as a recovery day for

the animal, and the second day was used for a habituation reminder; Fig. 1B). Then, conditioning was carried out over the next 3 days. The second ISOI acquisition was performed on the day after the final conditioning session. The control group animals were imaged at the same time interval, with the omission of behavioral training. Preparation for imaging was performed as follows. Mice were placed in a plexiglass box and initially anesthetized by inhalation of 3% isoflurane (Aerrane, Baxter). Throughout the surgery approximately 2% isoflurane, and throughout optical imaging approximately 1.5% isoflurane, was provided by a tube placed close to the nose (the exact dose of isoflurane applied depended on the breathing rate in order to maintain a level of 55–65 breaths per minute). Body temperature was maintained at 37°C (Harvard Apparatus, Cambridge, UK) and the breathing rate was monitored by a tube placed close to the nose (Datex Capnomac Ultima, Finland). Subcutaneous injections were administered of atropine sulfate (to reduce mucous secretions and facilitate breathing, 50 µg/ml in saline, 1 µg/10 g body weight [bw]), dexamethasone (a corticosteroid medication to reduce cortical edema, for general support of the organism and to improve the recovery process, 0.2 mg/mouse), Tolfedine (tolfenamic acid, an anti-inflammatory, antipyretic and analgesic agent, 4 mg/kg bw), and the antibiotic Enroxil (enrofloxacin, 5 mg/kg bw). Lidocaine (a local anesthetic, lignocaine 2%, 0.05-0.10 ml) was applied subcutaneously before exposing the skull. The animal's head was positioned using non-rupture ear bars (Kopf, model 922) and the tips were covered with lidocaine ointment (5%). Bregma and lambda were

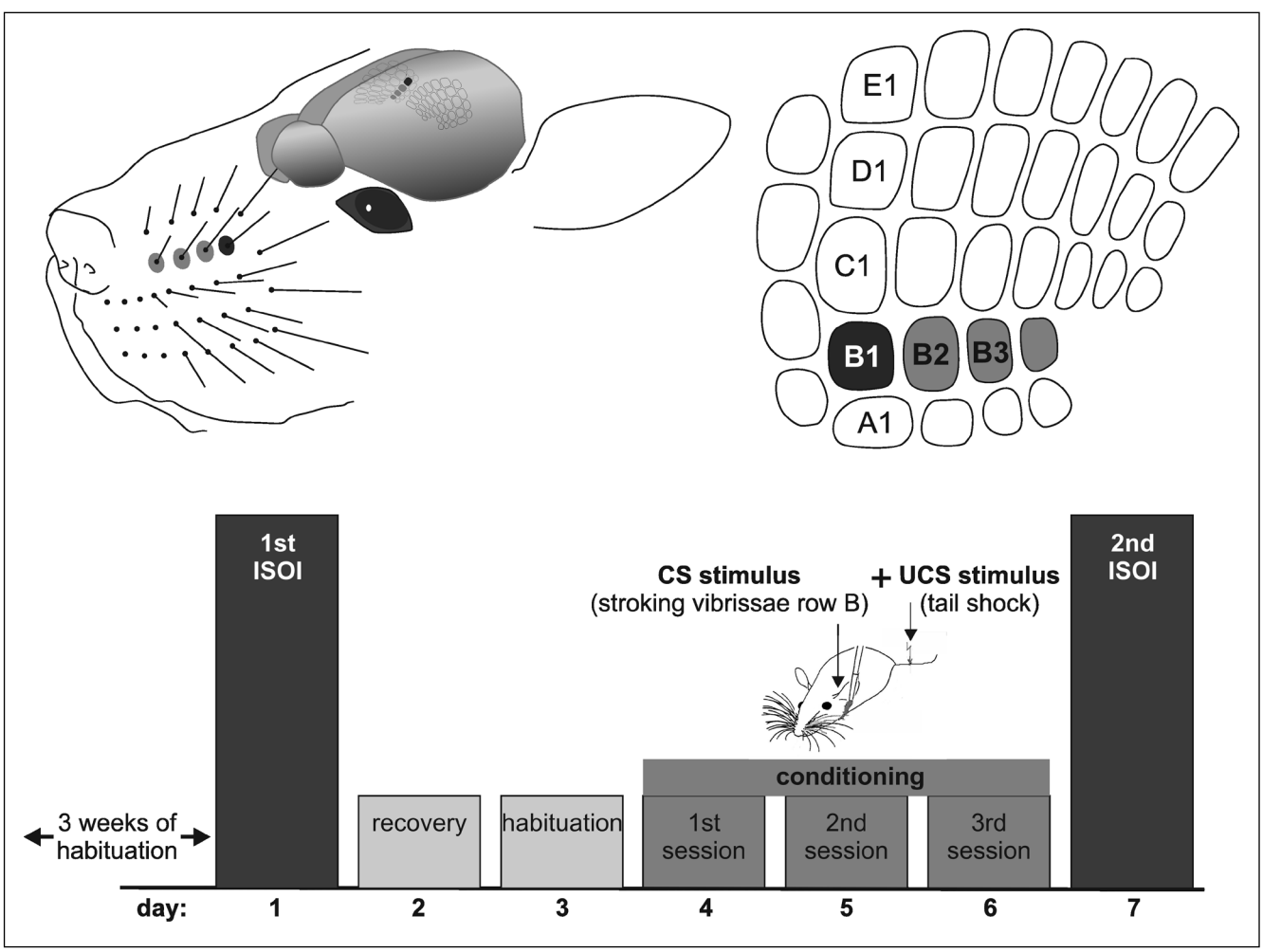

Fig. 1. (A) A schematic drawing of the vibrissae arrangement on the mouse snout and the map of vibrissae representation in layer IV of the primary somatosensory cortex in the contralateral hemisphere. Vibrissae row B (used during learning) and its cortical representations are marked with gray and vibrissa B1 and its cortical representation (chosen for ISOI imaging) are marked with black. (B) A diagram of the experimental design for the conditioned group. In the control group, no conditioning was performed and intrinsic signal optical imaging (ISOI) was carried out two times with the same interval as in the conditioned group.

positioned at the same horizontal level. The skull was covered with a solution of agarose in saline (2.5%) and sealed with a coverslip from the top and petroleum jelly from the side in order to make the skull above the barrel field transparent.

Intrinsic signals from the cortex were imaged using a pair of front-to-front camera lenses (Nikkor 50 mm f/1.2, Nikon) on a complementary metal oxide semiconductor (CMOS) camera (Photon Focus MV1-D1312-160-CL-12) with a maximum resolution of 1312 pixels × 1082 pixels and a pixel size of 8 µm × 8 µm. Synchronization of image acquisition with vibrissae stimulation was controlled with the imaging system, Imager 3001 (Optical Imaging, Rehovot, Israel). At the beginning of the recording session, the cortex was illuminated at 546 nm (green light) to capture the pattern of superficial blood vessels, these images were compared to blood vessel patterns prior to the second ISOI session to ensure that the camera was at the same position (Fig. 2). The camera was focused 400 µm beneath the pial surface and the functional imaging was performed with red light illumination (630 nm). Images (data frames) were acquired as averages over 0.1 s intervals.

Vibrissa B1, one of the vibrissae used for conditioning, was chosen for ISOI of its cortical functional representation (before and after conditioning). A thin bar attached to a piezoelectric element was glued with nail varnish to vibrissa B1 at 7 mm from the skin. To obtain a map of vibrissa B1 functional cortical representation, the sensory vibrissa-barrel system was activated by displacing the vibrissa 0.5 mm rostro-caudally with a frequency of 10 Hz for 6 s under the control of a Master-8 pulse stimulator triggered by the imaging system.

The ISOI trial consisted of frames acquired 1 s prior to vibrissa stimulation, at 6 s of vibrissa stimulation, and 8 s after the end of stimulus, over a total of 15 s. The intertrial interval lasted for 1 s. Trials with vibrissa stimulation alternated randomly with trials without stimulation (blank trials). The agarose window was removed from the skull after optical imaging, Lidocaine (lignocaine 2%, 0.05 – 0.1 ml) was applied to the cut, and the skin was sutured with a non-absorbable suture (Dafilon, Braun, Germany). Following the first imaging session, mice received subcutaneous injections of Tolfedine (tolfenamic acid, 4 mg/kg bw) for three days and antibiotics Enroxil (enrofloxacin, 5 mg/kg bw) for five days.

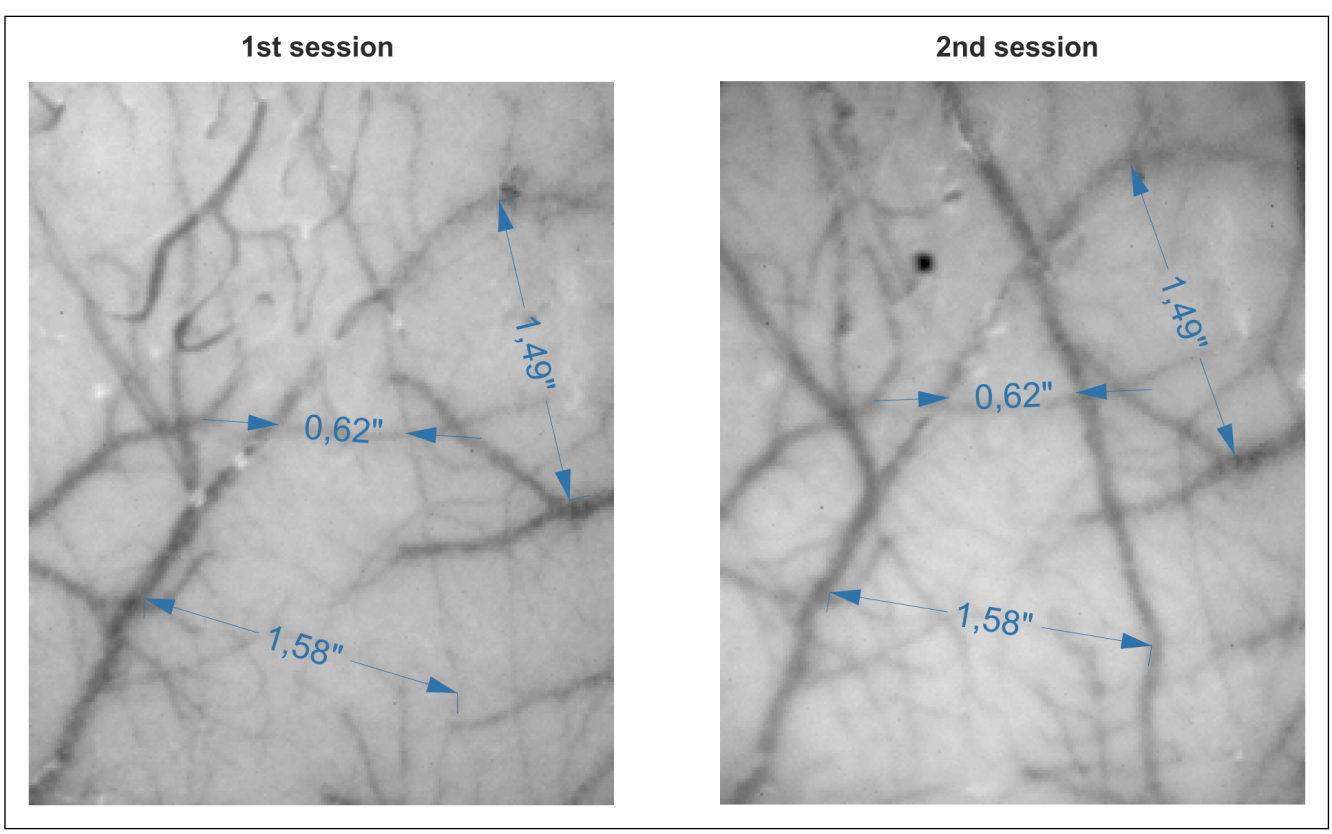

Fig. 2. An example of the images of blood vessel patterns obtained with green light at the beginning of the first and second intrinsic signal optical imaging (ISOI) session in one animal. In order to ensure the reliability of the repeated imaging prior to the 2nd ISOI session distances between different points were measured and compared to images obtained from the 1st ISOI session.

Data analysis of conditioned learning efficacy

Video files were used to assess the efficacy of conditioned learning (Fig. 3). As a measure of learning, we calculated the percentage of trials during which a mouse moved its head in response to the CS per minute of conditioning (each conditioning session comprised a total of 40 trials, i.e., 4 trials/min). Other head movements than those performed in response to the CS were not included in the analysis. Statistical comparisons between the first minute of conditioning and each of the subsequent minutes of conditioning were performed using one-way ANOVA with Tukey's multiple comparison post-test.

Data analysis of ISOI

The ISOI maps were analyzed using OptImage, MATLAB-based software for image processing (Optical Imaging Ltd., Rehovot, Israel), and custom-made scripts in MATLAB (MathWorks, Natick, MA). We chose data frames with maximum response to vibrissa stimulation for the analysis: these were frames obtained from 2 s to 8 s after the beginning of the stimulation. The averages of these selected frames comprise our post-stimulus data. Cortical activation is detected as a change in the light reflectance from the brain tissue; thus, in order to visualize the cortical vibrissa functional representation, the post-stimulus data was converted into a ratio value, relative to the data from pre-stimulus and blank trials, for each individual pixel. For the purpose of displaying the data, ratio value images of post-stimulus data have been adjusted according to the grayscale, where mid-gray represents the baseline and black and white are a decrease or increase of 2.5×10^{-4} from the baseline, respectively (Fig. 4A, Fig. 5A). The median ratio value from an area of non-activated cortex of a constant size in each mouse (0.4 mm × 0.3 mm) located outside the barrel field was measured for baseline cortical activity (precisely the same anatomical location, assessed by the blood vessel pattern, was used for analyzing the data from the first and second ISOI session in the same mice). For the purpose of normalizing the extent of cortical vibrissa representation, we set the activity threshold at ratio values of 3 standard deviations (SD) from the baseline. The area including pixels below threshold was measured using the open-source ImageJ software (Fig. 4B, 5B). Comparisons between the area of cortical vibrissa representation obtained from the first and second optical image acquisitions in conditioned and control groups were performed with repeated measures two-way ANOVA with Sidak's multiple comparison post-test.

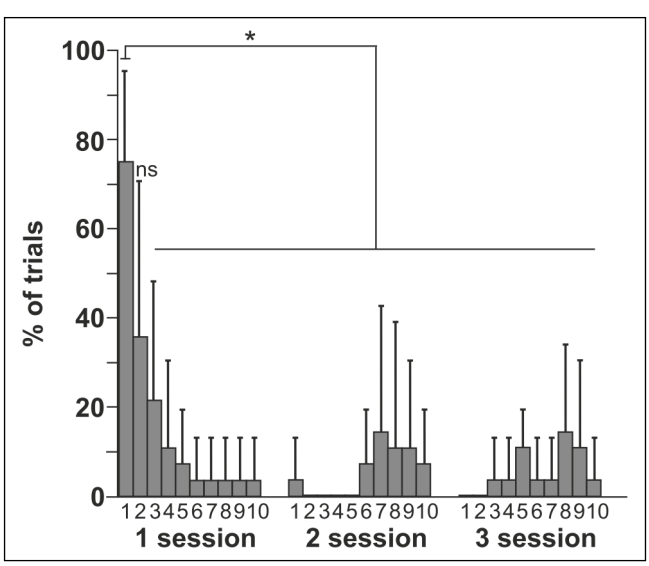

Fig. 3. The development of the conditioned response – mini-freezing, assessed as a decreasing number of head movements towards the stimulator touching the vibrissae. The percentage of trials in subsequent minutes of conditioning during which head turning in the direction of the stimulus was observed. Mean values ± standard deviation (SD) are shown. Comparisons were made between the 1st minute of conditioning and each subsequent minute in the 1st and the remaining two sessions; * $p<0.05$, ns – not significant, Tukey's multiple comparison post-test.

Analysis of the correlation between learning success and change in the area of vibrissa functional representation visualized with ISOI

To assess learning success, we recorded the number of trials per minute in which a mouse moved its head in response to the CS. The measures fell within a range of 0–4, as during a training session 4 trials were carried out per minute. We calculated the success rate of learning for each animal by taking the average of this measure over the total 10 min of the final (third) session. We calculated the change in the area of vibrissa functional representation visualized with ISOI as the ratio between the second (post-training) ISOI acquisitions and the first (pre-training) ISOI acquisitions. We analyzed the linear correlation between these two sets of data – the change in the area of vibrissa functional representation and the learning success rate.

Statistical analysis

All statistical comparisons were carried out using GraphPad Prism 5 software (GraphPad Software, Inc.). Results are presented as the mean value with the standard deviation (SD). The significance threshold was set at 0.05.

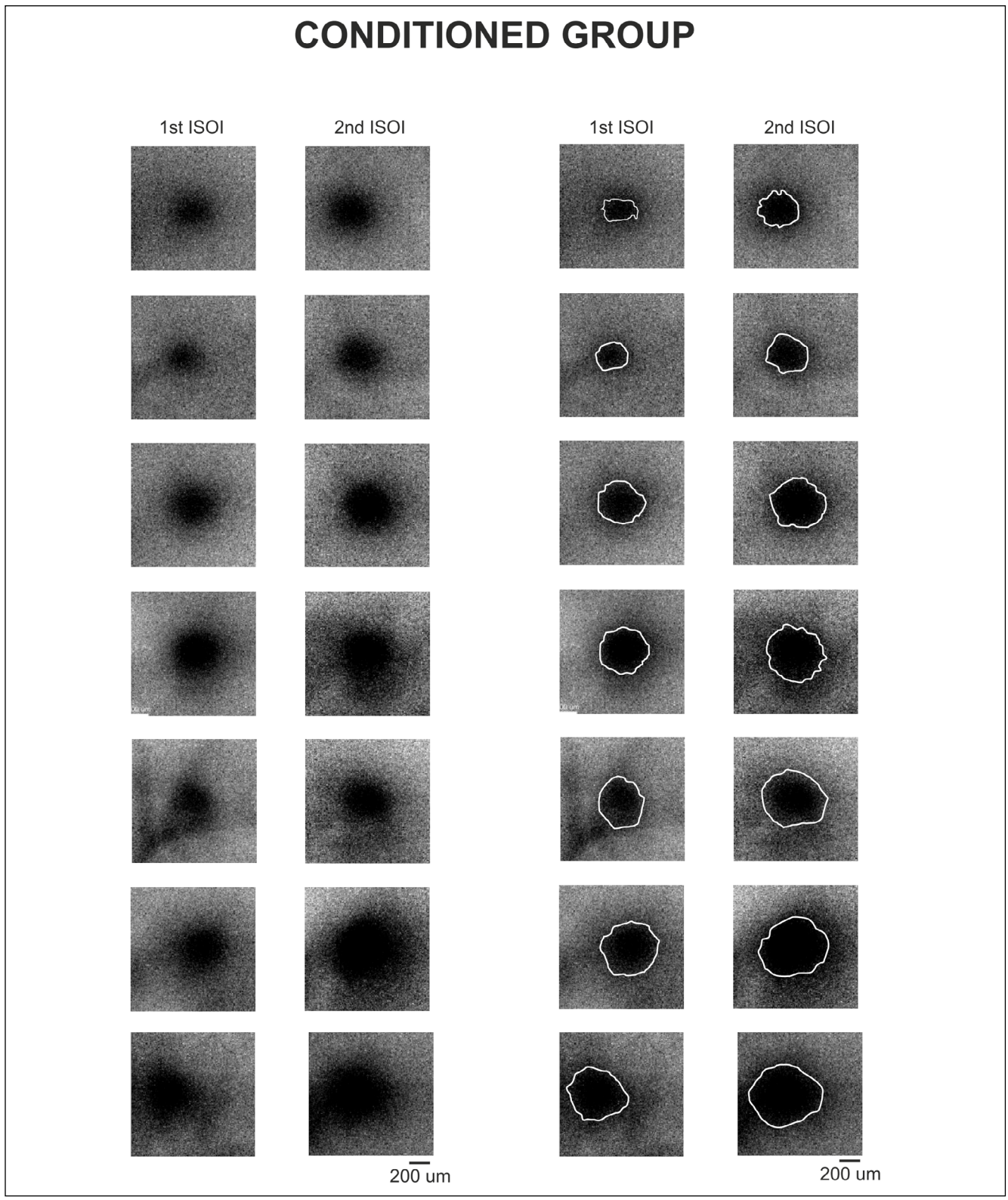

Fig. 4. The effect of conditioning on the areal extent of vibrissa B1 cortical representation visualized with intrinsic signal optical imaging (ISOI). Pairs of the images obtained from each mouse before conditioning (1st ISOI session) and after conditioning (2nd ISOI session) are shown. (A) and (B) represent the same images, but in (B) white lines delineate the area including pixels in which change in the light reflectance was equal to or below SD from the baseline cortical activity. Images are a result of the conversion of the ratio values to grayscale values where mid-gray represents the baseline, and black and white are a decrease or increase of 2.5×10^{-4} from the baseline.

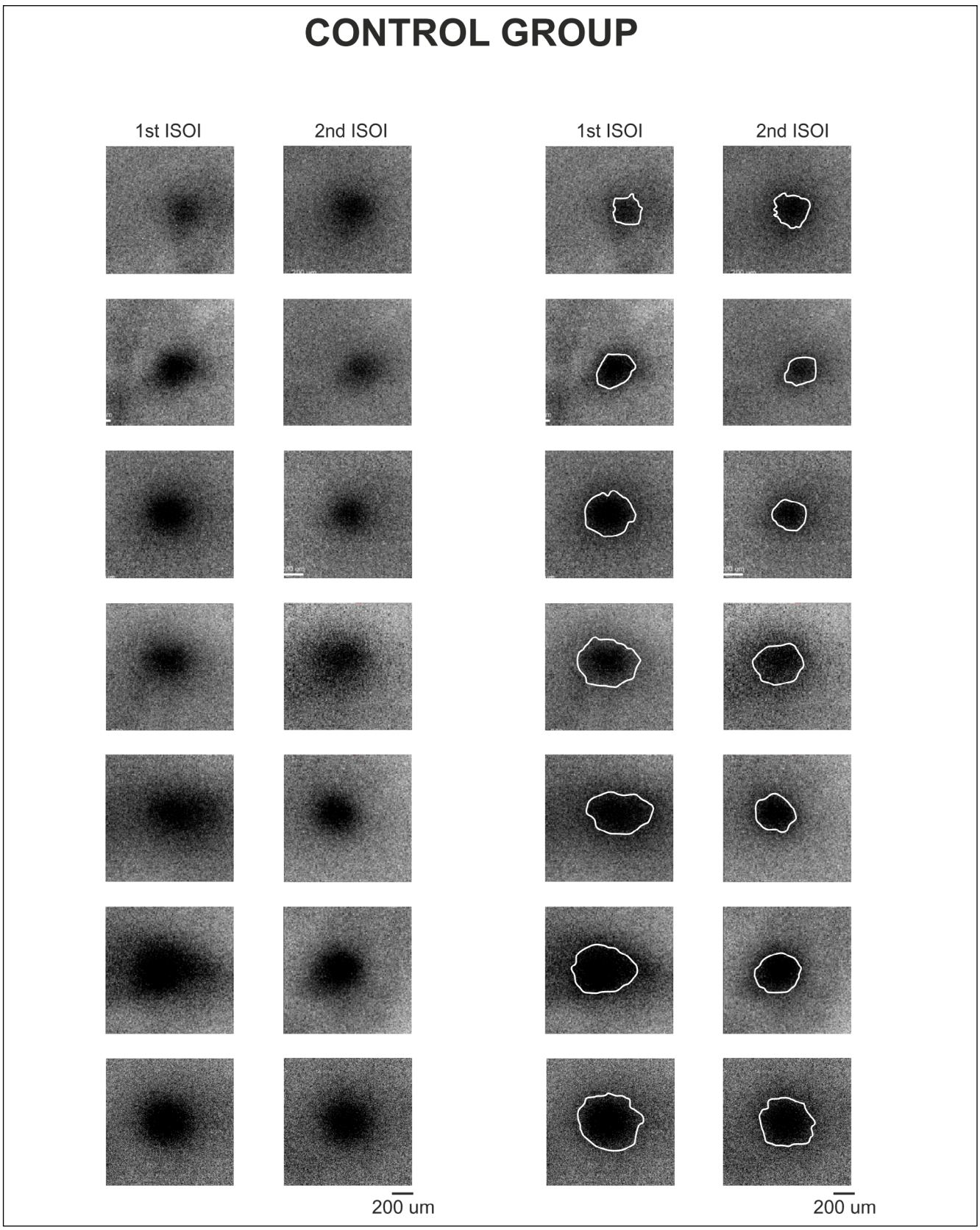

Fig. 5. The areal extent of vibrissa B1 cortical representation in the control group visualized with repeated intrinsic signal optical imaging (ISOI) performed with the same time interval, six days, as in the conditioned group. Other conventions are as in Fig. 4.

RESULTS

In order to visualize the effect of conditioning on the areal extent of the functional cortical representation of vibrissae *in vivo*, we used ISOI before and after conditioning in the experimental (conditioned) group (n=7). The control group (n=7) was imaged at the same time interval, with the omission of the behavioral training. In the conditioned group, behavioral performance during learning was recorded.

Acquisition of learning

To assess acquisition of learning during conditioning, we recorded the head movements of the mice in response to a CS. As described previously (Cybulska-Klosowicz et al., 2009), during the initial trials of whisker conditioning (CS-US), the mice often reacted to vibrissa stimulation by turning the head toward the stimulus, with the frequency of this reaction decreasing during subsequent trials. This reaction has been called "minifreezing" and can still be observed 24 h after the end of conditioning (Cybulska-Klosowicz et al., 2009); however, this phenomenon was not observed in pseudo-conditioned mice (Jasinska et al., 2010). The number of head movements during CS application (CS-head movements) possibly reflects the amount of learning by the mouse. In the present study, a significant change in the number of CS-head movements in the subsequent minutes over the entire training (total of 30 min) was revealed with one-way ANOVA ($F_{(2.519,15.11)}$=7, p=0.0036). During the first session of conditioning, the percentage of trials during which CS-head movements occurred per minute from the third minute to the end of the session was significantly lower compared to the first minute of the training session (Tukey's multiple comparisons test: p<0.05, Fig. 3), while the data from the second minute did not differ significantly from the first minute. This reduction persisted through the end of conditioning, as shown by comparisons between the first minute of the first session and each of the minutes from the second and the third sessions (Tukey's multiple comparisons test: p<0.05, Fig. 3). We observed this dramatic decrease in CS-head movements for each mouse; therefore, all mice that experienced conditioning were confirmed to have developed a conditioned response.

ISOI results

ISOI data are presented for each mouse from the conditioned and control group (Fig. 4, Fig. 5, and Fig. 6). In each animal from the conditioned group, an increase in the vibrissa B1 representational area was visible after learning (Fig. 4A). An analysis of the areas of activated cortex (activation threshold was set at 3 SD in change

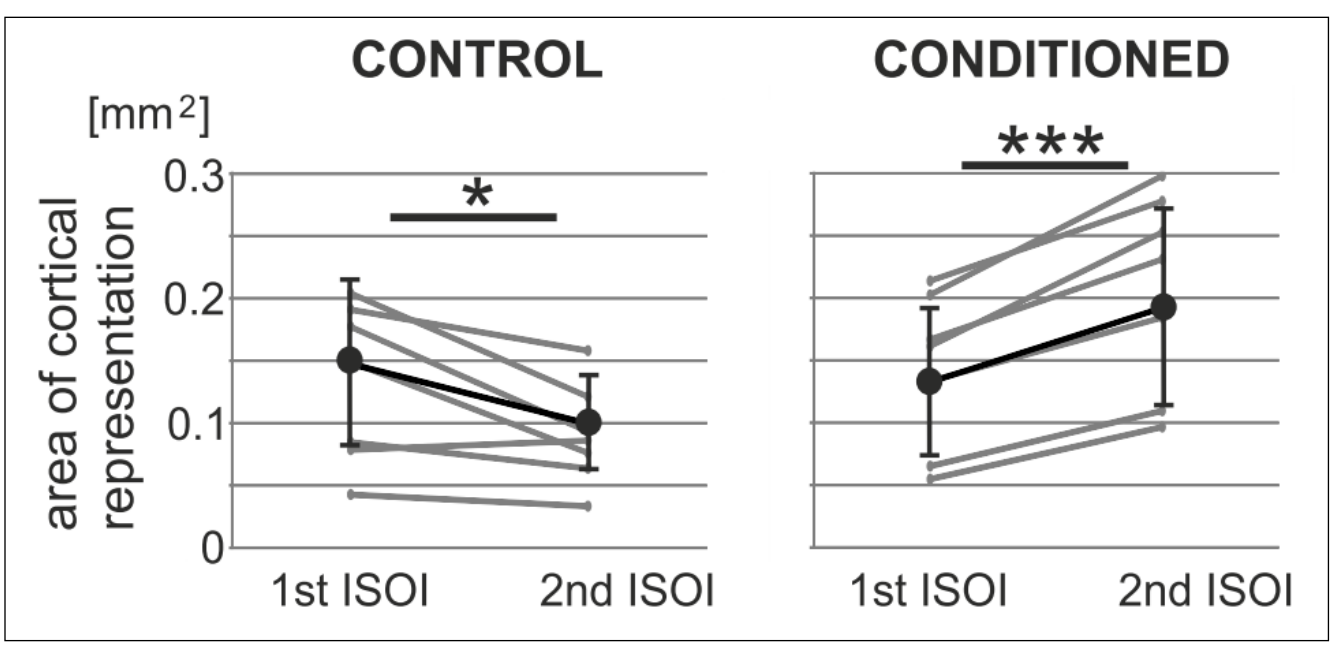

Fig. 6. The values of the area including pixels in which change in the light reflectance was equal to or greater than 3 SD from the baseline cortical activity from the 1st and the 2nd intrinsic signal optical imaging (ISOI) session linked together for each mouse from the control and experimental group. The black circles represent mean values (both, from the 1st and the 2nd ISOI session, linked together) and error bars represent SD. Sidak's multiple comparisons post-test: * p<0.05, *** p<0.001.

in light reflectance below the baseline, Fig. 4B) confirmed an average enlargement of the vibrissa B1 cortical representation after conditioning of 44 ± 17% (i.e., range of 30% to 76%), compared to before conditioning. In the control group, six of seven animals showed a visible decrease in the vibrissa representational area between the first and the second ISOI acquisition sessions (Fig. 5A). The decrease was determined to be 27 ± 20% (i.e., range of -9% to 49%) after setting the activation threshold (Fig. 5B). Individual values for the estimated area of the vibrissa B1 cortical representation, as well as the mean and SD, are presented in Fig. 6. Analysis of the changes in the area of the vibrissa B1 cortical functional representation with repeated measures two-way ANOVA showed a significant interaction between optical sessions, first *vs.* second, and conditioned *vs.* control group (ANOVA; $F_{(1,12)}$=31.69, p=0.0001).

In the conditioned group, the area of the vibrissa B1 cortical representation after conditioning increased from 0.142 ± 0.062 mm^2 (mean ± SD) to 0.207 ± 0.080 mm^2 (Sidak's multiple comparisons test: df=12, p<0.001), while in the control (non-conditioned) group, the B1 representation area decreased from 0.132 ± 0.063 mm^2 to 0.090 ± 0.040 mm^2 (Sidak's multiple comparisons test: df=12, p<0.05). In the conditioned group, the range of the vibrissa B1 cortical representation areas from the first ISOI session ranged from 0.055 mm^2 to 0.213 mm^2, and for the second ISOI session ranged from 0.096 mm^2 to 0.298 mm^2. In the control group, the vibrissa B1 cortical representation area from the first ISOI session included values that ranged from 0.043 mm^2 to 0.204 mm^2, and for the second ISOI session from 0.033 mm^2 to 0.158 mm^2. There was no statistical difference between the conditioned and the control group for vibrissa B1 cortical representation for the first ISOI session (Sidak's multiple comparisons test).

Learning success was not related to the change in the area of vibrissa functional representation visualized with ISOI

The analysis of the correlation between area of vibrissa functional representation visualized with ISOI and the learning success rate resulted in a Pearson's correlation coefficient of 0.08, indicating no correlation between the two sets of data (F=0.47; p>0.05).

DISCUSSION

In this study, we used a classical conditioning protocol pairing row B vibrissae stroking (CS) with an electric tail shock (US) to induce plastic changes in the barrel cortex of mice and to visualize them with ISOI. Analysis of the video files recorded during conditioning showed that all animals from the conditioned group developed a minifreezing response to the CS. In each mouse that underwent conditional learning, ISOI revealed an expansion of the functional representation of vibrissa B1 of, on average, 44%.

At the behavioral level, development of the conditioned response was observed during the first conditioning session, while we demonstrated the change in somatosensory cortex 24 h after the completion of the three conditioning sessions. The choice of the time point for the post-conditioning imaging of the somatosensory cortex was based on our previous data from 2DG mapping, indicating that changes of cortical representation of the vibrissae engaged in conditioning were not statistically significant after one training session (unpublished). A plausible reason for the delay in change in the development of behavior *vs.* the changes observable in the somatosensory cortex is that at the beginning of the conditioning the activity of subcortical structures, rather than the sensory cortex, triggers the appearance of the conditioned response (Boatman and Kim, 2006; Herry and Johansen, 2014).

The effect of behavioral training on the functional cortical representation of vibrissae row B was evaluated with ISOI performed under conditions of isoflurane anesthesia. Isoflurane, especially at the level of approximately 1.5% that was used in the present experiment, affects functional connectivity considerably as demonstrated with functional magnetic resonance imaging (Paasonen et al., 2018). It also reduces the amplitude and prolongs the latency of the response to stimulus in the cortex (Nivinsky-Margalit et al., 2022). While these effects of isoflurane can disturb information processing in the neuronal circuits, Frostig and Chen-Bee (2009) called attention to the importance of anesthesia level during ISOI, indicating that too light anesthesia results in an increased ratio of signal from surface vasculature in the imaged signal. We could not, therefore, decrease the level of anesthesia. On the other hand, while ISOI was used for testing, the behavioral training for inducing cortical plasticity was performed in the awake animals when functional properties of the neuronal circuits were preserved. The data indicate that learning introduces some, temporarily remaining, changes in the cortical neuronal network that can also be revealed in anesthetized animals.

A striking feature of the images of the vibrissa B1 cortical representations (Fig. 4, Fig. 5) obtained during the first ISOI session is the areal variability across mice in both experimental and control groups.

To what extent could this result from possible differences in the anesthetic state of the animal? We cannot completely rule out this possibility, but for each imaging session we took great care to provide the same experimental conditions for each imaging session and the state of the animals was very carefully controlled (see Methods). However, this result was not fully surprising, as the area of vibrissae cortical representation was also found to be variable between animals in other ISOI studies of the barrel cortex (Masino and Frostig, 1996; Polley et al., 1999; 2004; Drew and Feldman, 2009).

Another important issue directly related to the problem of individual variability in the cortical representational area that should be taken into consideration is the possible variability of ISOI responses within the same animal across time. This problem was addressed by Masino and Frostig (1996) in their long-term imaging study of the C2 vibrissa cortical representation in rats without any experimental manipulation between imaging sessions. Their experiments revealed unsystematic changes in the size of the activated cortical area in 50% of experimental animals. This was not the case in our experiment. In the control group, we found a systematic decrease in the areal extent of vibrissa B1 representation (in six of seven mice: $p<0.05$). While this effect was unexpected, we propose that it could result from the intense stimulation used for imaging. During each 40-minute imaging session, vibrissa B1 was deflected with a frequency of 10 Hz for 6 s within each of 80 trials. It is possible that such intense stimulation could influence cortical neuronal circuits and result in a kind of homeostatic downregulation (Peng et al., 2010). The vibrissae stimulation used in the present study, while quite intense, was of a similar magnitude to stimuli applied in other studies (Chung et al., 2002; Knutsen et al., 2016); in these studies, recording or imaging was not repeated. Conversely, in the experimental group, we observed a pronounced ($p<0.001$) expansion of the vibrissa B1 cortical representation in each mouse. On the basis of the above rationale, we assume that the results obtained in our experimental group are not the effect of random variability, but reflect the functional reorganization of the barrel cortex induced by conditioning.

Learning-dependent reorganization of cortical representations has been described in a number of experimental models based on different kinds of learning rules (Recanzone et al., 1992; 1993; Gilbert et al., 2001; Crist et al., 2001; Rutkowski and Weinberger, 2005; Blake et al., 2006; Polley et al., 2006; Bieszczad and Weinberger, 2010; Conner et al., 2010; Reed et al., 2011; Rosselet et al., 2011). Several reports showed that the magnitude of cortical map expansion was correlated to the amount of learning (Recanzone et al., 1993; Rutkowski and Weinberger, 2005; Polley et al., 2006; Bieszczad and Weinberger 2010). We also analyzed whether learning success was related to the area of vibrissa functional representation visualized with ISOI. In our data, however, no correlation between these two variables was found. It is difficult to determine the lack of correlation. It is possible that a correlation might materialize with the use of larger experimental groups.

The neuronal network mechanisms underlying the cortical plasticity induced by our learning protocol depend on increased excitability of excitatory neurons (Bekisz et al., 2010), but are concurrently accompanied by increased activation of the inhibitory (GABAergic) system (Gierdalski et al., 2001; Urban-Ciecko et al., 2010; Posluszny et al., 2015). Plastic changes in the inhibitory system are of dual significance at the functional level. On one hand, inhibition seems to have a stabilizing effect on the neuronal network under increased excitatory input (Tokarski et al., 2007; Urban-Ciecko et al., 2010). On the other hand, a circuit action of one type of interneuron via interneuron-interneuron connections results in a decreased activation of the other interneurons and eventually promotes activation of the neuronal network (Letzkus et al., 2011). We found in the barrel cortex a candidate for this kind of disinhibitory action – GABAergic somatostatin-immunoreactive cells in the interneuron-interneuron connections with parvalbumin-immunoreactive neurons (Cybulska-Klosowicz et al., 2013; Liguz-Lecznar et al., 2016).

The areas of the vibrissa cortical representation mapped by ISOI in a number of previous reports, while centered over the anatomical representation, extended beyond it (Petersen and Diamond, 2000; Johnson and Frostig, 2016; Knutsen et al., 2016; Jacobs and Frostig 2017). The reason for this extension is due to the nature of stimulus processing by the neuronal networks in the barrel cortex (Petersen and Diamond, 2000; Johnson and Frostig, 2016; Jacobs and Frostig, 2017). Initially, at the level of the primary thalamic input-recipient layer IV, inputs are processed within a cortical column (representing particular vibrissa), but as they reach layer II/III the signals are transmitted to the adjacent columns by horizontal axons (Petersen and Diamond, 2000; Johnson and Frostig, 2016; Jacobs and Frostig, 2017). Therefore, only during the first few milliseconds after the beginning of the vibrissa stimulation, is the response limited to the neurons assembled in the appropriate cortical column. This timescale is beyond the capability of ISOI. However, it was shown by ISOI, together with single-unit recordings, that images obtained with stimulation as long or longer than 2 s, with a frequency of 10 Hz, were better delineated

and resulted in better accuracy when compared to the actual anatomical localization of the vibrissa cortical column (Sheth et al., 1998; Knutsen et al., 2016). It was shown by electrophysiological recordings and pharmacological manipulation of GABAergic transmission that intrinsic inhibitory cortical mechanisms control the areal extent of the cortical response to the stimulus (Kyriazi et al., 1996).

We propose the following interpretation of our data. Repeated pairings of conditioned and unconditioned stimuli bring about lasting rewiring of connectivity within the cortical representation of the cognate whiskers, together with changes in neuronal excitability. The strong stimuli used for our ISOI launched intrinsic processes limiting the cortical activation and areal extent of the cortical representation of vibrissa B1 mapped in the control group. The conditional training, with repeated activation of neuromodulatory systems by the tail shock (Chiang and Aston-Jones, 1993; Letzkus et al., 2011), counterbalanced (neutralized) this homeostatic downregulation. In the training-induced reorganization of the excitatory and inhibitory neuronal networks, strong inputs from the vibrissa are released from powerful inhibition, possibly via disinhibition, with the engagement of somatostatin-immunoreactive neurons, which leads to enlargement of the vibrissa B1 functional representation compared to that observed before conditioning.

CONCLUSION

Intrinsic signal optical imaging was demonstrated to be an extremely useful tool for investigating learning-induced cortical plasticity in rodents, as the method is non-invasive for brain tissue, provides for quantitative assessment of the areal extent of functional cortical units and enables repeated imaging in the same animal, thereby allowing for any experimentally required time interval (days, months).

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