Infragranular barrel cortex activity is enhanced with learning

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Ward RL, Flores LC, Disterhoft JF. Infragranular barrel cortex activity is enhanced with learning. J Neurophysiol 108: 1278–1287, 2012. First published June 13, 2012; doi:10.1152/jn.00305.2012.—The barrel cortex (BC) is essential for the acquisition of whisker-signaled trace eyeblink conditioning and shows learning-related expansion of the trained barrels after the acquisition of a whisker-signaled task. Most previous research examining the role of the BC in learning has focused on anatomic changes in the layer IV representation of the cortical barrels. We studied single-unit extracellular recordings from individual neurons in layers V and VI of the BC as rabbits acquired the whisker-signaled trace eyeblink conditioning task. Neurons in layers V and VI in both conditioned and pseudoconditioned animals robustly responded to whisker stimulation, but neurons in conditioned animals showed a significant enhancement in responsiveness in concert with learning. Learning-related changes in firing rate occurred as early as the day of learning criterion within the infragranular layers of the primary sensory cortex.

The barrel cortex (BC) is widely appreciated for its well-defined and distinct barrel representation of individual vibrissae, facial whiskers, in layer IV of the primary somatosensory cortex. Originally examined in mice and described as barrels by Woolsey and Van Der Loos in 1970, barrel regions of the cortex have been reported in many species ranging from the rat (Killackey 1973) to the rabbit to the rhesus monkey (Woolsey and Van Der Loos 1970), barrel regions of the cortex have been reported in many species ranging from the rat (Killackey 1973) to the rabbit to the rhesus monkey (Woolsey and Van Der Loos 1970), although the presence of barrel organization is only easily visualized in layer IV of the cortex, this organization is preserved within the cortical column as layer IV sends projections both above to layers II/III and below to layer V (Armstrong-James et al. 1992; for a review, see Fox 2008).

The distinct delineation of individual barrels has made the BC a very popular region for examination. Studies have shown that altering input from the periphery can shape the receptive fields in the adult cortex. For example, whisker trimming leads to a remapping of the receptive fields of individual whiskers in the barrel field (Diamond et al. 1993; Lebedev et al. 2000), and removal of peripheral sensory input also leads to a reorganization of the cortical representation (Welker et al. 1989; Kossut et al. 1988). Alterations in the BC brought about by experience-dependent plasticity have also been visualized at the level of individual cells through examination of spine morphology (Holtmaat et al. 2006). Additionally, it has been demonstrated that learning can affect the barrel representation in the cortex. Siusinska and Kossut (1996, 2004) established that three sessions of conditioning (pairing whisker stimulation with tail shock or sweet water presentation) are sufficient to create an expansion of the functional representation of the trained whiskers compared with controls. The above studies provide direct evidence that the BC is capable of plasticity based on altered input from the periphery or whisker-signaled learning.

Work from our laboratory has demonstrated the importance and involvement of the BC of rabbits in the acquisition of the whisker-signaled trace eyeblink conditioning (tEBC) task. Galvez et al. (2006) demonstrated that there is a learning-specific expansion of the trained barrels after animals acquire tEBC. Similar to the work by Siusinska and Kossut (1996, 2004), this expansion was only seen in the trained whisker row and not in the surrounding rows or in animals undergoing unpaired stimulus presentations during pseudoconditioning, suggesting that the changes are dependent on learning the task rather than merely experiencing whisker vibration. Additionally, the primary somatosensory cortex region was shown to be essential for the acquisition of tEBC; animals who received pretraining lesions of the BC were unable to learn the task (Galvez et al. 2007). These data clearly indicate that important changes occur in the BC as animals learn whisker-signaled tEBC.

Previous work on the BC has focused on gross changes in the cortex and barrel representation rather than on how these changes manifest in individual cells as an animal learns. There is some evidence for training-associated differences in layer IV BC neurons between conditioned and pseudoconditioned animals during tEBC (Galvez et al. 2007), and work in the auditory cortex has demonstrated interesting learning-associated changes in individual cells in the deep cortical layers (Weinberger et al. 1984). We wanted to examine the infragranular layers, layers V and VI, of the BC to better understand information processing within the BC and to identify whether changes in the infragranular layers are associated with training or learning. We achieved this through single-unit extracellular recordings during the acquisition of whisker-signaled tEBC.

METHODS

Animals

Eleven 3-mo-old female New Zealand White albino rabbits were used for this study. All animals were housed individually, given ad libitum access to food and water, and kept on a 12:12-h light-dark cycle. All procedures described were in accordance with guidelines of, submitted to, and approved by the Animal Care and Use Committee of Northwestern University.

Recording Electrode Design and Construction

Each tetrode consisted of four formvar-coated nichrome wires (25-μm diameter bare, 38-μm diameter coated) bound tightly together at one end by twisting and heating. The free end of each wire was soldered into a gold-plated Amphenol pin. The tetrodes of each array...
were passed through a single section of 20- or 23-gauge hypodermic tubing. Each electrode array was composed of either 6 or 12 tetrodes. Two 1-77 ¾-in. peripherally mounted machine drive screws allowed the entire array to be adjusted vertically after implantation.

Surgical Procedures

All surgeries were performed in a sterile manner in a dedicated surgical facility. Animals were anesthetized with intramuscular injections of ketamine (60 mg/kg) and xylazine (10 mg/kg). The tops of their heads were shaved, and their eyes were coated with a thin layer of ophthalmic ointment to keep them moist during the procedure. Animals were placed in a stereotaxic holder, and a midline incision was made along the scalp to expose the skull. The head was aligned with lambda 1.5 mm below the bregma. Six small burr holes were made to allow for the placement of self-tapping stainless steel anchor screws (no. 2x ¼ in.). A larger hole was made for the placement of an electrode array. Electrodes were placed in the BC (anterior-posterior: 2.5 mm, medial-lateral: 7 mm, and dorsal-ventral: 1 mm, relative to the bregma) contralateral to the trained side of the face. The electrode and a headbolt assembly were affixed to the skull using dental acrylic. Rabbits were given Buprenex (0.03 mg/kg im) immediately before the surgery and given injections of Metacam (0.2 mg/kg sc) immediately after and 24 h after surgery. All animals were given at least 7 days to recover before habituation began.

Behavioral Training

After recovery, animals received 1 day of habituation followed by behavioral training. During these sessions, animals were restrained in a cloth bag with an opening allowing for the extension of the head and then placed in a Plexiglas restrainer, again allowing for the head to protrude. The right eye was held open using two dress hooks attached to Velcro straps, and an infrared sensor and air-puff delivery system was placed in front of the animal’s open eye. A single row of whiskers was grasped using a 7.5 × 1-cm paper strip (the sticky portion of a Post-It Note) and affixed to a piezoceramic strip (T220-A4-303, Piezo Systems) ~1 cm from the animal’s face. The whisker row used for training was determined by deflecting individual whiskers and listen-
ing to the neural response via a Cheetah data-acquisition system; when robust firing to whisker deflection was identified, that row of whiskers was chosen as the trained row for the duration of training. Animals also received earplugs, and white noise (70 dB) was generated through two speakers on either side of the animal’s head to prevent any auditory interference with conditioning.

**tEBC.** A total of 6 rabbits underwent tEBC. Animals received 80 paired trials of the whisker stimulation and the air puff during each training session. These stimuli were given via a computer running custom LabView software. There was a pseudorandom intertrial interval ranging from 30 to 60 s that averaged 45 s. Each trial consisted of a 500-ms preconditioned stimulus (pre-CS) baseline, a 250-ms CS followed by a 500-ms stimulus-free trace period, and then a 150-ms unconditioned stimulus (US) presentation. The CS was a whisker stimulation presented at 60 Hz. The US, an air puff, was generated at 3 psi to the eye on the same side to which the whisker vibration CS was presented. The infrared sensor measured the movement of the nictitating membrane via reflectance of the infrared beam; as the eye closed, the measurement (in V) increased (Thompson et al. 1994).

**Pseudoconditioning.** A total of 5 rabbits underwent pseudoconditioning. The setup for these rabbits was exactly the same as the conditioned rabbits, including the habituation session. During pseudoconditioning, rabbits received daily sessions consisting of 160 unpaired CS alone or US alone trials, which varied randomly with an intertrial interval that ranged from 15 to 30 s with an average of 22.5 s. These rabbits provided measurements of potential sensitized behavioral responses to unpredictable whisker stimulation and corneal air puffs in an unpaired nonlearning situation.

All animals were trained until they attained learning criterion (average: 10 sessions) and were then trained for at least three additional sessions; criterion is defined as the day in which an animal exhibited 8 conditioned responses (CRs) within a block of 10 consecutive trials. Pseudoconditioned control animals were yoked to conditioned animals.

**Behavioral Analysis**

For each trial, the baseline average voltage was recorded for 250 ms before the CS onset. The movement of the nictitating membrane during the CS-US interval resulting in a change >4 SD over the pre-CS baseline was considered a CR. CRs that resulted in a 4-SD change within 20 ms of the US onset were defined as appropriately timed, adaptive CRs. The day in which a rabbit displayed 8 adaptive CRs within 10 consecutive trials was considered the day of acquisition or criterion for that rabbit.

**Electrophysiological Analysis**

Single neuron signals were amplified (10,000×), filtered between 600 Hz and 6 kHz, and collected on a personal computer that sampled each channel at 32 kHz. Data were collected continuously during daily training sessions and were analyzed from 1 s before to 2 s after the CS onset using a Cheetah data-acquisition system (Neuralynx, Bozeman, MT). Single action potentials were isolated using measurements of spike height and width using the Spike Sort Cluster Cutting Tool from Neuralynx, generating two and three-dimensional scatterplots allowing the activity of a neuron to be tracked throughout a single session. Cells recorded from each day were treated as individual neurons because of the possibility of electrode drift between recording sessions. A total of 1,335 neurons were recorded from over the 8 days of examination (n = 684 conditioned and 648 pseudoconditioned). Averages of 85 cells in conditioned animals and 82 cells in pseudoconditioned animals were recorded daily from layers V and VI of the BC. Raster plots were generated using Neuroexplorer (Nex Technologies, Littleton, MA). To be included in this study, only tetrodes that were histologically verified in layers V or VI of the BC were used, and individual neurons had to have passed a 2.5:1 signal-to-noise ratio. Cells with a baseline firing rate of >16 Hz were considered interneurons (Swadlow 1989) and were not included in further analysis. Mann-Whitney analysis was used to determine whether there was a significant increase or decrease in firing rate during the CS period compared with baseline. Neurons that demonstrated a significant (P < 0.05) alteration in firing rate compared with baseline were selected for further analysis. Populations of neurons with similar characteristics were grouped and analyzed together to build group histograms of neural activity. There were three different response types found in the cortex: cells that responded to the CS with a significant increase in firing rate, with a significant decrease in firing rate, or with no significant change in firing rate. Regression analysis was used to determine if there was any significant (P < 0.05) difference in the percentage of responsive cells across days within each group. z-Scores for each bin were calculated using the following formula: 

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    z = \frac{(x - \mu)}{\sigma}
\]

where \(x\) is the value of an individual bin, \(\mu\) is the average of all bins of the baseline, and \(\sigma\) is the SD of all bins of the baseline. A z-score value above 1.96 corresponds to a confidence value of 0.05. To account for the neuronal response to the onset, duration, and offset of the CS period, an examination was taken of the 250-ms CS period and the 50-ms period immediately after; this 300-ms period will be

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**Fig. 3.** Cells recorded from per day as a percentage of the response profile to conditioned stimulus (CS) presentation. Data from conditioned and pseudoconditioned animals were collected, and cells with a significant change (P < 0.05) in firing rate during the CS period, as determined by a Mann-Whitney analysis, were grouped together by training group and by day. A: graph showing the percentage of cells in conditioned animals that significantly increased, decreased, or did not change their firing rate to CS presentation during each day of training. As animals progressed through training and acquired the task, they began to devote a lower percentage of cells toward the task. There was a significant decrease in the percentage of both CS-increasing and CS-decreasing cells across training days (CS increase: \(P = 0.0097\) and CS decrease: \(P = 0.0012\)) in conditioned animals. B: graph showing the percentage of cells in pseudoconditioned animals that significantly increased, decreased, or did not change their firing rate to CS presentation during each day of training. Pseudoconditioned animals showed a steady percentage of CS-responsive cells across training days.
referred to as the CS response period. Repeated-measures ANOVA was performed for the CS response period, and those days with significant differences were then subjected to post hoc Fisher protected least-significant-difference (PLSD) tests for each of the six 50-ms periods within the CS response period. Similarly, to examine the US period, Mann-Whitney analysis was used to determine whether there was a significant increase in firing rate during the US period compared with baseline. For each responsive cell, z-scores for each bin were calculated. Those responsive cells were grouped together, and ANOVA was performed for the US period.

Histology

Marking lesions were made by passing direct current (50 μA) for 5 s through each tetrode on the day before perfusion. Animals were euthanized via an intravenous administration of a pentobarbital sodium overdose. They were then perfused through the heart first with 1 liter of a saline solution and then 1 liter of a 4% paraformaldehyde solution. The brain was then postfixed overnight in 4% paraformaldehyde and then cryoprotected in 30% sucrose in PBS. All brains were blocked and embedded in Tissue-Tek OTC compound and sectioned at 75 μm using a freezing stage microtome. Every other section was stained for cytochrome oxidase (CO) for barrel visualization. For CO staining, free-floating sections were placed in the staining solution (0.05% diaminobenzidine, 0.03% cytochrome c, and 4% sucrose in PBS) at 37°C for 5 h to ensure consistent staining. CO staining was terminated via a series of PBS washes. Sections were then mounted on gelatin-coated slides. The CO lab protocol has been completely described by Galvez et al. (2006). For Nissl staining, every other section was mounted and stained according to a standard cresyl violet staining protocol.

RESULTS

Rabbits were trained on the tEBC task until they reached learning criterion and were then trained for an additional three sessions; pseudoconditioned animals were yoked to conditioned animals. Using a combination of CO and cresyl violet staining, all electrodes were histologically verified to be in layers V or VI of the BC (Fig. 1). All data were normalized to the day of learning criterion (see Fig. 2 for learning curves). A total of 1,335 neurons were recorded from over the 8 days of examination (n = 684 conditioned and 648 pseudoconditioned). Averages of 85 cells in conditioned animals and 82 cells in pseudoconditioned animals were recorded daily from layers V and VI of the BC. Each cell was examined via a Mann-Whitney U-test and grouped according to the response during the CS period: significantly increase, significantly decrease, or no significant change. Figure 3 shows the percentage of cells in each CS period group recorded from each day.

![Fig. 4. A: average firing rate (in Hz) of CS-responsive neurons in conditioned and pseudoconditioned animals across the 7 training days. Pseudoconditioned data are displayed only for CS presentation trials. B: baseline firing rates of neurons with a significant increase in firing rate during the CS period from conditioned and pseudoconditioned animals were compared across training days. There was no significant modulation of baseline firing rate across or within training groups, as demonstrated by the bar graphs [C-3: F(1,54) = 0.206, P = 0.651; C-2: F(1,27) = 0.900, P = 0.765; C-1: F(1,25) = 2.574, P = 0.121; C (Crit): F(1,23) = 0.466, P = 0.501; C +1: F(1,21) = 3.277, P = 0.084; C +2: F(1,17) = 2.029, P = 0.172; and C +3: F(1,17) = 0.212, P = 0.651].](http://jn.physiology.org/)

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Cells That Increased Their Firing Rate to the CS Show Enhanced Signaling with Learning

Data from conditioned and pseudoconditioned animals were collected, and cells with a significant increase in firing rate during the CS period were analyzed together by training group and by day. To ensure that there were no training-related differences in baseline firing rate, cells were examined first by layer using the characterization of Swadlow (1989). Repeated-measures ANOVA demonstrated that there were no significant differences in baseline firing between the training groups within layers [layer V: \( F(1,64) = 0.694, P = 0.41 \); layer VI: \( F(1,131) = 0.086, P = 0.77 \)]. Additionally, CS responsive cells were examined on each training day for baseline firing rate differences, and there was no significant difference in baseline firing rate on any training day.

Figure 4 shows the average firing rate of all cells that increased CS on the 3 training days before and after as well as on the day of learning criterion. To adjust for baseline firing rate and normalize the data for an examination of the overall infragranular activity change, \( z \)-scores (calculated for each 10-ms bin by subtracting from each bin the average firing rate of the baseline period and then dividing that number by the SD of the baseline) for each cell were calculated (Fig. 5). An examination of the results shown in Fig. 5 demonstrates strong responsivity during the CS response period and minimal responsivity during the trace period.

On day 1 of training (T01), there was no significant difference in the magnitude of the response to CS in cells from conditioned (36 neurons) or pseudoconditioned (17 neurons) animals (Fig. 6). On the 3 days preceding learning criterion (C-3 to C-1), cells in conditioned and pseudoconditioned animals also showed no significant difference in the magnitude of the CS response. However, on the day of criterion, on C+2, and on C+3, cells in conditioned animals showed significantly higher responses in the first 50-ms period \( (period\ 1) \) of CS presentation \( [criterion\ period\ 1:\ F(1,23) = 7.38, P = 0.01;\ C+2\ period\ 1:\ F(1,17) = 15.646, P = 0.001;\ and\ C+3\ period\ 1:\ F(1,17) = 10.025, P = 0.006] \). Additionally, on C+2, animals showed significantly higher responses during \( period\ 2\) \( [F(1,17) = 6.929, P = 0.02],\ period\ 3\) \( [F(1,17) = 5.063, P = 0.04],\ and\ period\ 4\) \( [F(1,17) = 4.932, P = 0.04;\ Fig.\ 7] \). On the day of criterion, there were also significant CS offset responses during the initial 50-ms of the trace period \( [period\ 6:\ F(1,23) = 4.887, P = 0.04] \).

Figure 8 shows examples of individual cells

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**Fig. 5.** Average \( z \)-scores for neurons in conditioned or pseudoconditioned animals that showed a significant increase to CS in the 3 days before through the 3 days after learning criterion demonstrate the learning-associated enhancement in CS response magnitude from the day of criterion onward. During the 3 days preceding criterion, conditioned and pseudoconditioned animals showed similar response magnitudes during the CS period. Pseudoconditioned data are presented only for CS presentation trials. Also shown is an overlay of the average blink for each 80-trial training session for the conditioned group of animals. On the days leading up to criterion, there was not a robust CR, blink, during the trace period, but on the day of criterion onward, animals displayed robust CRs during the trace period. US, unconditioned stimulus.
from conditioned and pseudoconditioned animals selected to show the median response size at select time points during training that demonstrate the changes that occurred in conditioned versus pseudoconditioned animals.

**Cells That Decreased Their Firing Rate to the CS Showed No Difference Between Conditioning and Pseudoconditioning**

Data from conditioned and pseudoconditioned animals were collected, and cells with a significant decrease in firing rate during the CS period were assembled together by training group and by day. z-Scores for each cell were taken to normalize responses to baseline. Repeated-measures ANOVA was then performed for the overall CS period, and those days with significant differences were then subjected to post hoc Fisher PLSD tests for each of the five 50-ms periods within the CS presentation.

On C-3 to C+3, there were no significant differences between cells in the conditioned or pseudoconditioned groups in terms of the magnitude of the CS response, with one exception. On C+1, there was a significant difference between cells in the conditioned and pseudoconditioned groups during the first 50-ms of CS presentation [period 1: $F_{(1,10)} = 5.18, P = 0.044$].

**Conditioned Animals Devoted Fewer Cells to the Task After Reaching Behavioral Criterion**

The percentage of cells with a significant increase or decrease to the CS presentation were assembled by group by day and examined using a regression analysis. For pseudoconditioned animals, there was a steady percentage of CS-increasing and CS-decreasing cells across training days, and this percentage did not change significantly. For conditioned animals, there was a significant decline in the percentage of cells that increased ($r^2 = 0.76, P = 0.0097$) or decreased ($r^2 = 0.89, P = 0.0012$) firing rate to the CS presentation over training days (Fig. 3).

**The US Response Is Not Modulated With Learning**

Data from conditioned and pseudoconditioned animals were collected, and cells with a significant increase in firing rate during the US period were assembled together by training group and by day. z-Scores for each cell were taken to normalize responses to baseline. ANOVA was the performed for the overall US period. There were no differences between groups in terms of the magnitude of the response to the US on any training day [conditioned average z-score: 2.3 and pseudoconditioned average z-score: 2.2; T01: $F_{(1,63)} = 1.338, P = 0.2518$; C-3: $F_{(1,58)} = 3.945, P = 0.0517$; C-2: $F_{(1,36)} = 0.888, P = 0.7678$; C-1: $F_{(1,48)} = 1.57, P = 0.2163$; C: $F_{(1,43)} = 2.606, P = 0.1138$; C+1: $F_{(1,40)} = 2.929, P = 0.0948$; C+2: $F_{(1,45)} = 0.42, P = 0.52$; and C+3: $F_{(1,37)} = 0.468, P = 0.4981$].

**DISCUSSION**

Much of the research examining the role of the BC in learning has focused on anatomic changes occurring in the layer IV representation of the cortical barrels (Siusinska and Kossut 1996, 2004; Galvez et al. 2006), but these studies have not shown how or when those changes occur in relation to behavioral learning. We extended this work by exploring the activity of individual cells in the infragranular layers of the BC as animals acquired the tBc task. Our research revealed that there are significant learning-related differences in responses to the CS in neurons in animals that are conditioned compared with pseudoconditioned. These changes occurred in concert with learning and manifested themselves from the day of learning criterion onward.

An assessment of CS response magnitude during the whisker vibration CS presentation revealed that on the training days preceding learning, neurons in both conditioned and pseudoconditioned animals showed similar and constant responses to CS. When conditioned animals learned the task, infragranular BC neurons demonstrated enhanced responses within the first 50 ms of CS presentation. An examination of neuronal firing on C+1 demonstrated that there was a decrease in the magnitude of the response to CS presentation compared with the day of criterion and on C+2 and C+3; this decrease occurred in tandem with a decrease in behavior. Together, these findings demonstrate the link between enhanced neuronal firing and improved performance on the behavioral task. The response to
the CS in both conditioned and pseudoconditioned animals
extended beyond the 250-ms CS period and advanced into the
beginning of the trace period. This response was accounted for
in statistical analyses; the day of learning criterion was the only
day to demonstrate a significant difference in the first 50 ms of
the trace period. This suggests that the learning-associated
changes observed are primarily in response to the CS onset.
There was no modulation of baseline firing rate during acqui-
sition, and the baseline firing rate between conditioned and
pseudoconditioned animals was not significantly different on
any training day. Additionally, there was no significant differ-
ence between groups in terms of the magnitude of the US
response. During training, neurons in conditioned animals
demonstrated a significant decrease in the number of signifi-
cantly responsive cells over the training sessions, whereas the
number of responsive neurons in pseudoconditioned rabbits
stayed constant. Animals that learned the task began devoting
fewer cells as they became more proficient. These findings
suggest that as an animal receives whisker stimulation, it
initially recruits many cells to attend to the sensory input, but
as it begins to learn a whisker-signal task there are fewer
cells involved that enhance the magnitude of their signal.
Neurons in conditioned animals showed significantly enhanced
responses to the CS coinciding with learning, while neurons in
pseudoconditioned animals showed a constant CS response
both in the number of neurons involved and the magnitude of
their response across days. This demonstrates that in the
primary somatosensory system, there are mechanisms that can
differentiate a constant and neutral sensory input from a mean-
ful input essential to learning. During learning, the infra-
granular layers not only amplify their output within some
neurons but also focus and delimit it.

Examinations of neuronal processing of whisker-signal-
ted tEBC in the ventral postero medial (VPM) barreloids (Ward et
al. 2010) and layer IV cortical barrels (Galvez et al. 2007) have
revealed training-associated changes much earlier in condition-
ing, but these changes do not correlate with learning as the
layer V and VI changes we observed. This suggests that
additional processing is necessary within the cortex between
the input layer IV and output layers V and VI before animals
begin to display learning-correlated activity. A recent exami-
nation of layer V processing during whisker-trimming experi-
ments revealed that plasticity in layer V occurs before changes
in any other cortical layer. These changes were attributed to
plasticity in the layer II/III to V pathway (Jacob et al. 2012).
Additionally, an examination of single units in the deep layers
of the primary auditory cortex during acquisition of the pupil-
lar conditioned response demonstrated that cells mainly in
layers V and VI developed discharge plasticity at the same rate
as behavioral learning compared with pseudoconditioned con-
trols (Weinberger et al. 1984). These studies support our
finding of learning-associated changes in deep cortical layers

Fig. 7. As animals acquired the tEBC task, they showed significantly enhanced responses to the CS presentation. Data from conditioned and pseudoconditioned
animals were collected, and cells with a significant increase in the firing rate during the CS period were analyzed together; data were normalized to baseline firing
rate via a z-score analysis. Repeated-measures ANOVA of the CS response period divided into 50-ms bins was then performed. On C-3 to C-1, there were no
significant differences between groups; neurons in both conditioned and pseudoconditioned animals showed similar levels of responding during the CS response
period. On C (Crit), C+2, and C+3, there were significant increases in CS magnitude in conditioned animals. During the first 50-ms period of the CS presentation
(period 1), conditioned animals displayed significantly enhanced responses. These enhanced responses coincided with improved behavioral performance on the
tEBC task.
and suggest that the changes summarized here may be attributed to cortical processing after input is received from layer IV.

The increase in response magnitude to the CS onset is what differentiates neurons in conditioned animals as they acquire the task. While the work presented here is among the first to examine the activity of individual BC neurons during acquisition of a whisker-signaled task, there is support for these findings in other sensory regions. In the primary auditory cortex, multiple-unit responses to CS+/H11001 significantly enhance their activity during conditioning and discrimination, whereas there were no changes in response to CS+/H11002 (Oleson et al. 1975). These changes became more pronounced with additional training sessions, demonstrating a correlation between behavior and enhanced neuronal response. During tone-signaled appetitive classical conditioning in rats, changes to the CS presentation occurred within the same block of trials as behavioral learning (Disterhoft and Stuart 1976). And an examination of the auditory association cortex during tone-signaled eyelink conditioning revealed that before acquisition of the task, neural responses were no different between conditioned and pseudoconditioned animals, but once trained animals began to show conditioned responses, there was pronounced response plasticity within the cortex (Kraus and Disterhoft 1982). These studies confirm that with behavioral learning, neurons in the sensory cortex amplify their signal in association with learning, whereas neurons in pseudoconditioned control animals show no such changes. We should stress that the present study demonstrates that these learning-associated neuronal changes are found only in layers V/VI of the BC. Neurons in layer IV just superficial to this population show training-related increases in firing that occur before those reported here but are not further enhanced when behavioral learning actually occurs (Galvez et al. 2007). Similarly, neurons in the VPM thalamus that project vibrissae sensory input to layer IV show training-related but not learning-related enhancements in firing during tEBC (Ward et al. 2010).

Additionally, work examining frequency tuning and learning show similar learning-associated enhancements in cortical activity. For example, Bao and colleagues (2004) demonstrated that after animals were trained in a sound maze, neurons in the primary auditory cortex showed enhanced responses to noise pulses compared with control animals and concluded that learning resulted in processing improvements in the cortex. Bakin and Weinberger (1990) demonstrated enhanced tuning and increased responsiveness to the training tone after animals acquired tone-shock learning. These studies indicate that the enhanced frequency tuning in auditory cells in concert with learning is much like the whisker-responsive cell’s increased response to whisker stimulation in association with learning and suggest the possibility of parallel mechanisms for sensory processing during learning.

By increasing the magnitude of the response to the CS onset, the cortex may be able to counteract noise from surrounding cells to ensure the perpetuation of the signal to other processing areas. There is strong evidence for lateral inhibition within the cortex (Kelly et al. 1999; Brumberg et al. 1996); therefore, any important somatosensory signal must be strong enough to overcome this inhibition and exert inhibition itself onto surrounding barrels. Neurons that decrease their firing rate during the CS presentation may be reflecting the lateral inhibition they...
are experiencing. An examination of the changes in response magnitude for cells that decreased firing during the CS presentation revealed that there was no difference across training days between conditioned or pseudoconditioned animals. This indicates that these neurons are not as involved in the learning process as those neurons that enhance their firing rate. It should be noted that we focused our examination on cells located within the vibrissae row that was processed the CS. It could well be that infragranular neurons in adjacent rows (not studied here) show marked inhibition during learning as a result of the center-surround inhibition.

Signals from the infragranular layers of the cortex are sent to many different brain regions, including the postero medial nucleus of the thalamus (Bourassa et al. 1995), reticular thalamic nucleus (Bourassa et al. 1995; Wright et al. 2000), VPM (Chmielowska et al. 1989; Zhang and Deschenes 1997), secondary somatosensory cortex (Carvell and Simons 1987; Chakrabarti and Alloway 2006), pons (Mercier et al. 1990), striatum (Mercier et al. 1990), and motor cortex (Chakrabarti and Alloway 2006). The enhanced learning-associated BC response to the CS may provide important signals to these regions compared with the relatively constant input being provided in pseudoconditioned animals. This enhanced signal may prime these regions for altered input from other essential learning-related regions such as the prefrontal cortex (Weible et al. 2000), hippocampus (Moyer et al. 1990, MeChron and Disterhoft 1997), or caudate nucleus (Flores and Disterhoft 2009).

Previous work from our laboratory and others has demonstrated changes in cellular excitability, specifically a reduction of the afterhyperpolarization (AHP), which occur in concert with learning (Disterhoft et al. 1986; Disterhoft and Oh 2006; Moyer et al. 1996; Saar et al. 1998). These changes are seen in well-trained rabbits (Disterhoft and Oh 2006) as well as in rabbits that are in the early stages of task acquisition (Disterhoft et al. 1988), similar to our defined day of learning criterion and the first day we observed CS activity enhancement. This work examining the AHP demonstrates that there are changes intrinsic to individual neurons that develop specifically in response to learning. A reduced AHP allows for a greater number of action potentials during a burst of activity and could mediate the increase in neuronal responsivity to the CS onset that we observed. Since acetylcholine increases neuronal excitability by reducing the Ca\(^{2+}\)-mediated K\(^+\) current underlying the slow AHP (Cole and Nicoll 1984), there is also the possibility that the changes we observed could be due to modulation by cholinergic input. The somatosensory cortex, specifically the infragranular layers, responds with excitability to the application of acetylcholine, and this input helps to enhance the stimulus response in individual cells (Lamour et al. 1983). It has generally been reported that in addition to facilitation of the stimulus response, cholinergic input also enhances baseline firing rates (Krnichevic et al. 1971; Donoghue and Carroll 1987), although in a small subset of cells, there is an enhancement of the stimulus response without the concurrent increase in baseline firing rate (Donoghue and Carroll 1987). We did not observe any significant changes in baseline firing rate in concert with learning (Fig. 4). However, activation of cholinergic input through synaptic release in vivo, as would have occurred during learning trials, is quite different from application of the compound iontophoretically, so the increases in firing rate we observed may well have been mediated by cholinergic neuromodulation. Alterations in the firing rate of auditory cortex neurons during associative learning and as a result of stimulation of nucleus basalis, attributed to cholinergic drive, have been repeatedly observed by Weinberger and Merzenich (1998, 2003). These studies suggest that cholinergic drive could have been an important factor in determining the firing rate changes we observed in the infragranular BC during learning.

Cells in layers V and VI in both conditioned and pseudoconditioned animals robustly respond to whisker stimulation. As behavioral learning occurs, cells in conditioned animals show enhanced and more spatially focused CS responsiveness. Significant learning-related changes in the functional response to the CS occur as early in learning as the day of learning criterion within the infragranular layers of the primary somatosensory cortex.

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