Dynamic changes of rodent somatosensory barrel cortex are correlated with learning a novel conditioned stimulus

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Submitted 26 June 2012; accepted in final form 27 February 2013

Long JD 2nd, Carmena JM. Dynamic changes of rodent somatosensory barrel cortex are correlated with learning a novel conditioned stimulus. J Neurophysiol 109: 2585–2595, 2013. First published March 6, 2013; doi:10.1152/jn.00553.2012. —The rodent somatosensory barrel cortex (S1bf) has proved a valuable model for studying neural plasticity in vivo. It has been observed that sensory deprivation or conditioning reorganizes sensory-driven activity within S1bf. These observations suggest a role for S1bf in somatosensory learning. This study evaluated the hypothesis that the response properties of extracellularly recorded neurons in S1bf would change as subjects learned to respond to stimulation of S1bf. Intracortical microstimulation (ICMS) of S1bf was used as a means for bypassing feedforward drive from the sensory periphery, midbrain, and thalamus while exciting local cortical networks. To separate the learning of this conditioned stimulus-conditioned response (CS-CR) from other elements of the task, we employed a cross-modal transfer schedule. Long-Evans rats were initially trained to respond to an auditory stimulus. All subjects were then implanted in both S1bf with chronic microwire arrays for recording neural activity and delivering ICMS. Next, this association was transferred to ICMS of one hemisphere’s S1bf. S1bf responded to ICMS with a brief increase in firing rate followed by a longer reduction in activity. We observed that the duration of reduced activity elicited by ICMS increased as the subjects began to respond correctly more often than expected by chance, and the magnitude of the initial positive response increased as they consolidated this CS-CR. Subsequent ICMS of the opposite S1bf revealed that this CS-CR did not generalize across hemispheres. These results suggest that a mechanism involving a single hemisphere’s S1bf tunes cortical responses in concert with changes in rodent behavior during somatosensory learning.

behavior; learning; microstimulation; rat; somatosensory learning

THE RODENT VIBRISSA SYSTEM has proved a promising model system for investigating how the brain adaptively integrates sensory and motor information to generate perception (Diamond et al. 2008; Kleinfeld et al. 1999; Petersen 2007). Previous work has shown how the stimulus-driven responses of the vibrissa system, from the trigeminal ganglion to the somatosensory barrel cortex (S1bf), depend on the behavioral state of the animal (Leiser and Moxon 2007; Wiest and Nicolelis 2003). Specifically, the responses of S1bf neurons to whisker stimulation have been shown to vary dependent on whether the animal is anesthetized or awake (Petersen et al. 2003) and attentive or inattentive (Ferezou et al. 2007). Moreover, the functional reorganization of S1bf induced by sensory deprivation (Allen et al. 2002; Fox 2002) or aversive conditioning (Bekisz et al. 2010; Urban-Cieko et al. 2010) has demonstrated that the barrel cortex is highly plastic. Whether and how these changes in S1bf activity relate to the learning process remains unclear.

The difficulty of studying individual neural structures in behaving rats is underscored by the description of the rodent vibrissa system as a set of nested loops (Diamond et al. 2008; Kleinfeld et al. 1999). Microstimulation offers a method for directly activating individual regions along the sensory pathway while bypassing neural structures upstream of the area of interest. Previous work has shown intracortical microstimulation (ICMS) to produce a stereotyped response across all layers of S1bf (Butovas and Schwarz 2003) characterized by a brief increase in firing rate followed by a prolonged decrease in activity, which is sufficient for driving behavior (Butovas and Schwarz 2007). In our study, these neural features were tracked over the course of learning as subjects formed a stimulus-response association [conditioned stimulus-conditioned response (CS-CR)] to ICMS of S1bf.

Our behavioral model involved a cross-modal transfer schedule (Over and Mackintosh 1969) to separate the learning of this CS-CR from the learning of other task components (Gallistel 2003; Rescorla and Wagner 1972) and for comparison against a similar behavioral state. We proceeded by first training all subjects to respond to an auditory stimulus. We then transferred this association to ICMS of one hemisphere’s S1bf. This procedure allowed us to observe changes in the rodents’ somatosensory learning behavior that were common to all subjects, while evolving over a distinct time course for each subject (Gallistel et al. 2004). We then estimated correlations between features of the neural response to ICMS and the rodents’ somatosensory learning behavior.

We observed a lengthening of the period of decreased neural activity induced by ICMS as the subjects began to respond correctly to ICMS (n = 5). Later, as the subjects approached peak performance on the task, the magnitude of the initial increase in firing rate grew and the duration of decreased activity returned to previous levels. The pattern of decreased firing rate did show a negative correlation with distance from the stimulating electrode, but, in agreement with others (Histed et al. 2009), the pattern of increased activity did not. We found that these changes were not due to anticipatory modulation of S1bf, reflecting the subjects’ expectation of reward. In addition, after forming the CS-CR, ICMS of the opposite S1bf (n = 2) demonstrated that this association did not generalize across hemispheres. These results suggest that a mechanism involving...
S1bf modulates the cortical response to inputs in concert with changes in rodent behavior during somatosensory learning.

MATERIALS AND METHODS

Surgery

Adult male Long-Evans (hooded) rats \( (n = 5, 250–350 \text{ g}) \) were used in these experiments. All subjects received microwire array implants in the S1bf of both hemispheres. These consisted of two rows of eight isonel-coated, 35-\( \mu \text{m} \)-diameter tungsten blunt-tip microwires (Innovative Neurophysiology, Durham, NC). Arrays were situated within an Omnetics connector (Minneapolis, MN) and bound together at an intrarow spacing of 250 \( \mu \text{m} \) and an interrow spacing of 250 \( \mu \text{m} \).

A pair of 50-\( \mu \text{m} \) platinum/iridium wires was mounted in line with the recording microwires, 300 \( \mu \text{m} \) from one end, for delivering ICMS. For all subjects, the stimulating electrodes were positioned rostral to the recording microwires. Subjects were first anesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (4.5 mg/kg) and then transferred to a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The injected anesthetics were supplemented by isoflurane gas (0.6–1.5\% as needed. A single subcutaneous injection of Baytril (5 mg/kg) was used as an antibiotic and a subcutaneous injection of dexamethasone (0.5 mg/kg) initiated an immunosuppressive taper series. Supplementary intraperitoneal injections of ketamine (70 mg/kg) were administered at 45- to 60-min intervals.

A midsagittal incision, ~3 mm rostral to bregma and ~5 mm caudal to lambda, was followed by removal of the soft tissue and periosteum laterally until the sutures of the parietal/temporal bones were exposed. Five small holes were drilled in an evenly spaced crown to allow for the threading of stainless steel screws into the skull. One of these was designated the ground screw and threaded caudal to lambda to make electrical contact with the dura. A craniotomy (~3 mm × ~1.5 mm) was then performed over both S1bf, the rostromedial corner of which was 1–2.0 mm caudal and 4–5.5 mm lateral relative to bregma, straddling the parietal/temporal bone suture (Paxinos and Watson 2006). A duroctomy was performed to facilitate insertion of the array. Continuous electrophysiological monitoring of neural activity was performed throughout the surgery to confirm the position of each microwire. Our criterion for placement within the infragranular layer of S1bf was activity on both rows of the array at a depth of \( \geq 1.4 \text{ mm} \). The cranietomy was then sealed with Gelfoam followed by cyanoacrylate, and the arrays were then firmly attached to the skull with dental acrylic. All subjects were given a subcutaneous injection of buprenorphine (0.5 mg/kg), and a topical antibiotic ointment was applied to the wound border daily. An immunosuppressive taper series was continued via the administration of dexamethasone orally over the course of 7 days (starting at 0.5 mg/kg daily). All subjects were allowed to recover for 7–10 days before recording began. This surgery and all subsequent animal procedures were approved by the University of California, Berkeley Animal Care and Use Committee.

Electrophysiology

Neural signals were recorded with a multichannel neural recording system (Plexon, Dallas, TX). Cables with 34-gauge wires attached to a multichannel motorized commutator (Plexon) were used to allow the animal free movement in the operant chamber. The band used for sorting unit data was 0.5–9 kHz. All neural recordings in this study are considered single unit. To differentiate single-unit activity from background noise, we set our sorting program to run the following procedure each day: Samples of each electrode’s background voltage fluctuations were taken at regular intervals. The mean and standard deviation of each of these distributions were then estimated and used to set a threshold at the mean minus 4 times that channel’s standard deviation. In the event of a threshold crossing, a waveform starting 200 \( \mu \text{s} \) before and ending 600 \( \mu \text{s} \) after was recorded. Next, for each channel 500 samples of these 800-\( \mu \text{s} \) waveforms were collected and projected into a two-dimensional principal component space derived from these waveforms. Clusters in this space were used to generate online unit templates (Plexon). Finally, to minimize the contamination of background noise, during off-line sorting only those templates with \(<0.5\%\) of the recorded waveforms presenting interspike intervals of \(<1\text{ ms}\) were designated as single units and included in the subsequent analysis.

Microstimulation

Constant-current stimulation was delivered with a single-channel isolated pulse stimulator (A-M Systems, Carlsborg, WA). Stimulation parameters were set once for each subject and were within the range used in previous studies: bipolar stimulation, 6–14 biphasic pulses, 250 \( \mu \text{s} \) per phase, cathode first, at 200 Hz and 60–90 \( \mu \text{A} \) (Butovas and Schwarz 2003; Fridman et al. 2010; Romo et al. 1998). In the session prior to beginning ICMS conditioning, a three-axis accelerometer located within a conical nose-poke response port (Lafayette Instruments) was used to set the ICMS parameters for each subject as it sat quietly. The current level used for ICMS conditioning was set at 5 \( \mu \text{A} \) below the smallest current level that evoked a twitch, observable in the accelerometer data. It has been shown that with these materials and stimulation parameters it is possible to record the initial excitatory neural response to ICMS (Butovas and Schwarz 2003; Venkatraman et al. 2009) as well as drive behavior (Butovas and Schwarz 2007).

Experimental Setup and Operant Conditioning

All subjects were housed in a vivarium with a standard light-dark cycle. Prior to conditioning, all subjects were habituated to the experimenter and then water restricted to no less than 90\% of their ad libitum weight. During the task the rats were placed within an operant chamber (dimensions: 59.7 × 34.3 × 26.35 cm; Lafayette Instruments, Lafayette, IN). The task schedules were programmed in and controlled by ABET II software (Lafayette Instruments).

Stage 0: learning the tone as conditioning stimulus. Subjects were first continuously reinforced with water for breaking an infrared beam located within a conical nose-poke response port (Lafayette Instruments). All subjects were then conditioned on a fixed-interval schedule, in which every 30 s a tone conditioning stimulus (CS) was played continuously for 5 s, signaling the availability of the reinforcer. Subjects had continuous access to the reinforcer during the presentation of the tone CS. Then a reset period was imposed that required subjects to remain outside the response port for 5 s before the next trial began. At this time, the beginning of a trial was signaled by the illumination of a light within the operant chamber and the end of a trial by its being extinguished. Next, the schedule was changed to a variable-interval schedule with a mean interval of reinforcement of 15 s and a minimum interval of 2 s. A minimum 2-s interval was used to ensure the that subjects did not form a CS-CR to the illumination of the light within the operant chamber at the start of each trial. Also, at this step of conditioning if the subject made a response prior to the tone CS the schedule was set into the reset period. We define any responses made prior to the presentation of the CS as false alarms. During the final step of auditory conditioning, the variable interval was set to be between 2 and 11 s, the period of CS presentation was reduced to 1 s, the response period was set to 2.5 s, and the amount of reinforcement was fixed. At this time, responses made during the response period were defined as correct responses and not responding to the CS was defined as a miss. Once this final schedule was in place, the three response classes (false alarms, correct responses, and misses) were recorded across each session to calculate each subject’s within-session performance. In summary, the subjects had access to the nose poke throughout the trial but were only allowed one response per trial.

J Neurophysiol • doi:10.1152/jn.00553.2012 • www.jn.org

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Within a trial, a single nose poke was marked as a false alarm or a correct response depending on whether it occurred before CS presentation or during the 2.5-s response interval, respectively. Any trials in which the subject did not respond to the CS were recorded as a miss.

Stages 1–3: learning ICMS as CS. The schedule of reinforcement was the same as the final version described for stage 0. Performance was likewise evaluated in terms of correct responses, false alarms, and misses. To perform the cross-modal transfer from the auditory stimulus to ICMS, subjects were trained in alternating blocks of 40 trials. During each training block ICMS preceded the tone stimulus by 500 ms, and for each test block ICMS was presented alone. This interleaved block structure allowed us to control for the behavior of the subjects by comparing their behavior when performing the learned auditory CS-CR in the presence of the unlearned ICMS CS-CR to subsequent performance of the learned ICMS CS-CR. These alternating blocks were continued until a subject began to respond at greater than chance performance to ICMS over the course of 100 consecutive test trials, i.e., over the course of 2.5 test blocks, indicating learning of the ICMS CS-CR. Thereafter, only ICMS was presented.

Chance Performance Calculation

We modeled the subject’s probability of receiving reinforcement in the absence of the information provided by the CS. The structure of the task was such that only a single response was allowed within a trial, and this was marked as a false alarm or a correct response depending on whether it occurred before CS presentation or during the 2.5-s response interval, respectively. Accordingly, we proceeded by considering the likelihood of making a correct response at random, given the response time (2.5 s) and variable interval (2–11 s) used. We modeled the subjects’ behavior under these circumstances as a binomial process, with an expected number of successes out of 100 trials for a P value of 0.05, as the expected number of successes out of 100 trials according to the null hypothesis plus 2 times the expected standard deviation \( N \times P + 2 \times \sqrt{N \times P \times (1 - P)} \), equaling 30 out of 100 trials. The schedule intervals, the response time, and the variable interval were chosen on the basis of preliminary work in our laboratory (data not shown) and designed to produce a low probability of making a correct response by chance, while being high enough to sustain motivated behavior.

Stages in Terms of Performance

Sufficient conditioning on the auditory task, during stage 0 conditioning, was achieved when a subject exceeded a performance criterion of >70% correct responses for three consecutive sessions (n = 5). Stage 1 of learning ICMS was defined ad hoc as those sessions beginning with the introduction of ICMS as CS until when the subject performed >30/100 correct responses on ICMS-only test trials (Fig. 1B), i.e., until their behavior rejected the null model for chance performance. Stage 2 of learning was similarly defined by the subject achieving >30/100 but <60/100 correct responses in a single session (Fig. 1C). The entrance into stage 3 was defined as the session in which a subject performed ≥60/100 correct responses (Fig. 1D). The ≥60/100 cutoff for dividing between stages 2 and 3 was post hoc and based upon the observation that the subjects’ performance began to plateau at this level of performance.

Sham ICMS

Upon achieving asymptotic performance on the ICMS task, each subject was presented with a block of 20 sham ICMS trials interleaved throughout an ICMS-only session. This was done to confirm that the subjects were responding to ICMS of S1bf and not some extraneous feature of the laboratory setup coincident with delivery of ICMS. This was accomplished by disconnecting the cable between the stimulator and the implanted stimulating wires.

ICMS Detection Threshold Calculation

Approximation of each subject’s ICMS detection threshold was conducted over the course of two sessions after the subject had
reached asymptotic performance on the ICMS task. This process was aided by the fact that on this task, even at asymptotic performance, all subjects made ~15% false alarms. This made the occurrence of false negatives (misses) highly informative about whether or not a subject could detect ICMS. During the first session, the number of pulses, the most robust ICMS parameter, was reduced manually to the minimum number that consistently evoked a nose-poke response at 80 μA and 250 μs per phase. During the second session, the current parameter was fit with the QUEST method (Watson and Pelli 1983) in conjunction with the minimum variance method (King-Smith et al. 1993) by fitting a psychometric function. This provided a maximum a posteriori estimate of each subject’s detection threshold. These Bayesian adaptive methods were chosen to estimate the subjects’ detection thresholds because of their demonstrated accuracy and efficiency. We found that to obtain reliable detection threshold estimates it was very important to minimize the number of subthreshold stimuli, because of the disruptive effects delivering multiple undetectable stimulis had on the subjects’ motivation and behavior.

Having fixed pulse width and number, we followed the approach of others (Butovas and Schwarz 2003, 2007) and converted our three-dimensional ICMS parameter space (pulse width, numbers of pulses, and current level) into a single dimension of effective charge transfer (nC). By effective charge transfer, we mean the cathodic phase of each pulse (Stoney 1968). For example, 8 pulses at 250 μs and 80 μA would result in 160 nC.

The psychometric function fit was the commonly used Weibull psychometric function:

\[ w_T(x) = 1 - \exp\left[ -10^{\frac{x - \theta}{\delta}} \right] \]

The parameter \( T \) is the threshold (in nC), and \( x \) is the effective charge transfer as a function of current (range 0 – 60 μA). The parameters \( \gamma \) and \( \delta \) specify the chance and asymptotic performance of each subject. The slope of the psychometric function \( \beta \) was set at 3.5 × 10^4 s^-1 for all subjects. The detection threshold, \( T \), was estimated three times for each subject over the course of a single session, and each estimate was interleaved with blocks of superthreshold ICMS to maintain stable behavior.

### Three-Axis Accelerometer

A miniature custom-printed circuit board was designed to hold the ADXL330 accelerometer (Analog Devices, Norwood, MA; sensitivity of 300 mV/g, where g = acceleration due to gravity, using power supply of +3 V, dynamic range ≥ 3 g). This board also contains a 3-V voltage regulator (MAX6030, Maxim, Sunnyvale, CA), three 0.1-mF surface-mount capacitors, and an 18-pin Omnetics connector. This system is small and light enough (9 mm × 9 mm in size, 350 mg in weight) that a rat can carry multichannel neural recording headstages (Plexon) as well as the accelerometer board (Venkatraman et al. 2010). The acceleration data were sampled at 1 kHz. To maintain a fixed relationship between the position of the accelerometer and each subject’s head, one side of the accelerometer board was glued to one of the headstages. Since the headstage was attached to an Omnetics connector, which was embedded in the dental acrylic cap that was threaded into the subjects’ skulls, the latency between head movement and change in accelerometer data was negligible.

### Data Analysis

**Processing accelerometer data.** Attaching the accelerometer to one of the subject’s headstages maximized the device’s sensitivity to head movements. By analyzing each subject’s head movements around the time of ICMS delivery we could determine when the subjects began to react to ICMS. All ICMS deliveries, rewarded and unrewarded, were included in this analysis. Each time ICMS was delivered a segment of data extending 1 s before and 1 s after ICMS was extracted from all accelerometer channels. For each session, this generated a matrix with dimensions \( [2 \times (1 \text{s}) \times 1,000 \text{samples/s} + 1] \times (\text{number of ICMS deliveries}) \) by 3 accelerometer channels. Each accelerometer channel was then low-pass filtered at 10 Hz (2-pole Butterworth filter, filtered forward and backward for zero linear phase distortion) and z-scored. To compare accelerometer data across sessions these data matrices were concatenated along the sample dimension. Subsequently, principal component analysis (PCA) was applied to this multisession accelerometer data matrix to generate a common orthogonal basis set optimized to capture as much variance as possible in fewer dimensions. This allowed us to collapse movements coordinated across all three dimensions down to a single, informative dimension. Using the multisession accelerometer data matrix to generate these principal components ensured that meaningful comparisons could be made between sessions. PCA has been used successfully by several other groups for summarizing motion sensor data (Krause et al. 2003; Mantyjarvi et al. 2001). For these data, the first principal component always accounted for >60% of the variance. For each subject, the three-dimensional accelerometer data were then projected onto the first principal component and again separated by session and trial. Finally, the average of the principal component scores was taken over ICMS deliveries for each session and subject (as shown for a subset of sessions and subjects in Fig. 2).

**ICMS features.** The post-ICMS initial increase in firing rate and subsequent decrease in firing rate are represented as features derived from each unit’s peristimulus time histogram (PSTH) centered on the time of ICMS delivery. Both rewarded and unrewarded ICMS presentations were included in the PSTH. These histograms extended from 500 ms before to 500 ms after ICMS onset in 10-ms bins. The feature for the increase in firing rate was derived from each unit’s PSTH as a z score relative to the mean and standard deviation of that unit’s activity 500 ms prior to ICMS. This feature was estimated as the maximal positive z score within the first 100 ms after ICMS onset (see Fig. 3A, left, for schematic).

The feature corresponding to the subsequent decrease in firing rate was also derived from each unit’s pre-ICMS activity. While our system allowed us to detect spikes between ICMS pulses (interpulse interval 5 ms), some spikes were missed because of the remaining artifact, which biased us toward underestimating spike counts. Therefore, we only considered post-ICMS bins when defining this feature. The mean firing rate of a post-ICMS bin was required to be less than the mean minus the standard deviation of the pre-ICMS activity for that unit (similar to Butovas and Schwarz 2003). This feature was then estimated as the duration of consecutive bins showing a decrease in firing rate. To correct for differences in the ICMS pulse train length between subjects (30–70 ms), only those bins within the first 430 ms after ICMS were considered for this analysis (see Fig. 3A, right, for schematic). Throughout results we refer to the features corresponding to the post-ICMS initial increase in firing rate and the subsequent decrease in firing rate as the “positive feature” and the “negative feature,” respectively. This was done to remain agnostic to their origin, given our use of extracellular electrodes.

**Calculation of group averages.** When calculating group averages for each stage of learning, a single session was chosen for each subject. The first session in which ICMS was introduced was used to represent stage 1 behavior, because at this time the subjects had the least exposure to ICMS. To capture that session in which the subjects began to associate ICMS with reward, the first session in which a subject’s performance was significantly greater than that expected by chance on ICMS-only trials was used to represent stage 2 behavior. To compare all subjects at peak performance upon the ICMS-only task, that session in which each subject obtained the highest percentage of correct responses was chosen to represent stage 3 behavior. Effect size and statistical significance were estimated for each neural
RESULTS

Learning a Novel Stimulus-Response Association

The study of learning requires accounting for the often large intersubject differences in behavior in a principled manner before making group comparisons. Simply averaging over subjects and across sessions eschews these issues at the cost of important information about the learning process. Put simply, group averages often do not provide a clear description of the experimental cohort’s learning behavior (see Gallistel et al. 2004 for review). Our approach was to retain information about the learning process of individual subjects by defining a series of behavioral stages that emphasized changes in the subjects’ behavior and performance on the task. These were based on the observation that, while each subject had a unique learning rate, all subjects exhibited a similar pattern of behaviors that evolved over the course of learning. These behaviors were distinguishable in terms of performance, response time, and reaction time, as measured by accelerometer data.

We utilized a cross-modal transfer schedule (Over and Mackintosh 1969) to transfer a CR from an auditory stimulus to a novel CS in the form of ICMS of a single hemisphere’s S1bf. This separated the learning of a new stimulus-response association (CS-CR) from other elements of the conditioning process. During the first stage of conditioning, stage 0, all subjects were trained to respond to an auditory stimulus (Fig. 1A). This ensured that all subjects were habituated to the environment and established a consistent behavioral sequence between the presentation of the CS and the nose-poke CR. Stage 0 encompassed the entire process of conditioning the subjects to respond to the tone, although for comparison against the subjects’ learning to respond to ICMS, we refer to each subject’s asymptotic performance on the auditory task as stage 0 behavior. Then, for the conditioning requiring the learning of the novel ICMS CS, we observed each subject’s behavior to evolve over three distinct stages, stages 1–3. The replacement of the auditory tone with ICMS of S1bf meant that the expected relationship between stimulus and reward was no longer available and new options had to be explored. At first, this resulted in chance performance relative to the underlying variable-interval schedule, which was defined as stage 1 behavior (Fig. 1B). The response bias of each subject was apparent during stage 1 as a tendency to emit either false alarms or misses in anticipation of the auditory stimulus (Fig. 1E). Stage 2 behavior was defined as the period when subjects made more correct responses than expected by chance. At this time the frequency of misses decreased, yet they continued to make false alarms often (Fig. 1, C and E). During stage 3 behavior, subjects consistently responded to ICMS to obtain reward. This was evident from the high probability of a correct response and a much lower probability of making false alarms or misses (Fig. 1, D and E). Finally, after achieving asymptotic performance on the ICMS task, each subject was presented with a block of sham ICMS to confirm that they were in fact responding to ICMS (see MATERIALS AND METHODS).

These stages were clearly discernible in terms of the subjects’ behaviors as measured by response times and across-session performance (Fig. 1, A–D, center and right, a single subject’s data across stages 0–3). When a subject began to CR to ICMS, the response time, defined as the time interval

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Fig. 2. Accelerometer data demonstrated the emergence of a response to ICMS. A: example traces of 3-axis accelerometer data detailing the delivery of ICMS, the associated artifact, the subsequent reaction, and the resulting attainment of reward. B: left: movement data for 3 subjects during stage 1 of learning ICMS. Movement traces are plotted as trial-averaged 1st principal component scores derived from 3-axis accelerometer data at 1-ms precision around the time of ICMS delivery. Center: movement data for the same 3 subjects during stage 2 of learning as each subject began to respond to ICMS. Right: movement data for the same 3 subjects during stage 3 of learning the ICMS conditioned stimulus (CS)-conditioned response (CR). Insets: $R_j$ values indicate linear correlation coefficients between stage 1 and stage $j$ accelerometer data.
between stimulus onset and registration of a nose-poke response, was similar to that observed during performance of the auditory tone version of the task [Fig. 1, A–D, right; stage 0: 1.20 ± 0.57 s (mean ± SD); stage 1: 2.32 ± 0.95 s; stage 2: 1.30 ± 0.78 s; stage 3: 1.13 ± 0.61]. This observation was confirmed with a Mann-Whitney U-test for difference in medians while accounting for unequal sample sizes (stage 0 vs. stage 1: P < 10^-5, stage 0 vs. stage 2: P > 0.76). For each subject the performance data across sessions were very similar to those used to fit sigmoid learning curves (Gallistel et al. 2004), with stage 1 indicating the low asymptote, stage 2 the rapid rise, and stage 3 the high asymptote. Figure 1E shows the partitioning of three subjects’ performance into stages 0–3 via the criteria described in MATERIALS AND METHODS.

Changes in each subject’s performance corresponded with distinct changes in their overt behavior as each subject began to respond to ICMS. Preliminary video analysis (data not shown) demonstrated that each subject’s response behavior to ICMS was idiosyncratic but qualitatively similar to that observed when they performed the auditory task, suggesting the transfer of the auditory CS-CR to ICMS. Since the response behavior in this task consisted of a nose poke, tracking the head movements of each subject, via a three-axis accelerometer attached to the recording headstage (Venkatraman et al. 2010), allowed us to closely monitor this behavior. Using the accelerometer data, we validated the observation that during stage 2 of learning each subject developed a unique nose-poke response to ICMS, which was conserved across the rest of their...
exposure to ICMS. This was detectable by application of PCA to the three-axis accelerometer data recorded during each session (Fig. 2A). Figure 2B shows the result of this analysis applied to the accelerometer data recorded from three subjects during stages 1–3. The flat time series of the trial-averaged principal component scores during stage 1, ICMS-only trials (Fig. 2B, left) supports the observation that at this time the subjects emitted movements unrelated to the presentation of ICMS. The movement data around the time of ICMS during stage 2 (Fig. 2B, center) and stage 3 (Fig. 2B, right) tell a different story. Upon entering stage 2, each subject began to emit a distinct but stereotyped pattern of head movements—indicative of that subject’s nose poking behavior—that was conserved throughout the rest of their exposure to the ICMS CS-CR. This was clear from the similarity in the trial-averaged time series of each subject’s first principal component scores between stages 2 and 3, compared with stages 1 and 2 or stages 1 and 3 (linear correlation coefficients; Fig. 2B, insets, R values). For example, subject 1 exhibited an initial motor response to ICMS that was very similar across trials, followed by more variable approach behavior to the nose poke. In contrast, other subjects emitted a sustained bout of stereotyped behavior when making a nose-poke response (subjects 4 and 5). The performance and accelerometer measures of behavior validated the division of each subject’s learning behavior into distinct behavior-based stages.

The Neural Response to ICMS

Neural ensembles of 10–16 units were typically recorded from each animal during a recording session. As observed by others (Butovas and Schwarz 2003), the response of S1bf units to ICMS consisted of an initial increase in firing rates, at a delay that varied between units (Fig. 3, A and B), followed by a longer period of decreased activity and then a return to baseline activity. The capacitive discharge of the platinum/iridium stimulating electrodes was fast enough (~1.2 ms) to allow us to record the majority of unit activity between the 200-Hz (5-ms interpulse) ICMS biphasic pulses. Throughout ICMS conditioning, we tracked post-ICMS positive (Fig. 3A, left) and negative (Fig. 3A, right) features of the neural response to ICMS (see MATERIALS AND METHODS for a quantitative definition of these neural features).

Neural Correlates of Stimulus Selection

To investigate whether the neural response of S1bf to ICMS changed in concert with learning the novel ICMS CS, we performed a group analysis correlating the positive and negative features of the neural response with the subjects’ behavior across stages, partitioned according to the stages of learning described above. To account for unequal samples across subjects and non-Gaussian distributions, when making comparisons between stages we adopted the method of bootstrap sampling (Efron and Tibshirani 1993; see MATERIALS AND METHODS). This analysis revealed that upon entering stage 2 there was an increase in the duration of the negative feature relative to stage 1 (Fig. 3B, bottom; stage 1:stage 2, bootstrap test for difference of means, resamples = 5,000, P < 0.05, effect size = 55 ms). It also supported the observation that there was an increase in the positive feature in stage 3 relative to stages 1 and 2 (Fig. 3B, top; bootstrap test for difference of means, resamples = 5,000, stage 1:stage 3, P < 0.05, effect size = 4.6 (Z score), stage 1:stage 2, P < 0.05, effect size = 4.5 (Z score)). Finally, we observed that the negative feature returned to levels observed during stage 1 when the subjects reached asymptotic performance in stage 3 (Fig. 3B: bottom; stage 1:stage 3).

Wherever possible, we controlled for the effect of behavioral state upon brain state. Our use of an alternating block schedule during stage 1, i.e., tone followed by ICMS (Tone/ICMS) and ICMS alone, allowed us to compare two epochs in which the behavior of each subject was quite similar, while the CS+ varied. The Tone/ICMS blocks captured each subject’s behavior during stable performance of the tone CS-CR coincident with exposure to the unlearned ICMS CS+. The fact that the ICMS CS+ was unlearned at this time was clear from each subject’s performance during the ICMS-alone blocks within the same session (Fig. 1E, stage 1). We compared each subject’s behavior during the Tone/ICMS blocks in stage 1 against its stable performance of the learned ICMS CS-CR in stage 3. We first checked that the increase in the positive feature we observed between stages 1 and 3 was unaffected by comparing Tone/ICMS blocks in stage 1, as opposed to ICMS-alone blocks in stage 1, to stage 3 ICMS-alone trials [Fig. 3C, top; stage 1 Tone/ICMS (1st stage 3 ICMS, bootstrap test for difference of means, resamples = 5,000, P < 0.05, effect size = 4.5 (Z score)]. Having shown that the neural response to ICMS was unaffected by the presence of the tone, we next compared the behavior of our subjects within these epochs in terms of group performance (Tone/ICMS: 73.4 ± 10.4%; ICMS: 71.2 ± 6.8%; paired t-test, 2-tailed, n = 5 subjects, P > 0.47) and response times [Tone/ICMS (stage 1): 1.18 ± 0.73 s; ICMS (stage 3): 1.13 ± 0.61 s] and demonstrated their similarity (Fig. 3C, bottom). Together, these data support the claim that the increase in the positive feature observed between stages 1 and 3 was not due to differences in behavior.

To further unpack these observations, for the sessions included in the group analysis we next examined whether these changes in the post-ICMS positive and negative features exhibited spatial structure as a function of distance from the stimulating electrode. Linear regression of the positive feature upon distance from the stimulating electrode was insignificant (R = 0.04, P = 0.65). Thus the observed increase in the positive feature during stage 3 was distributed randomly over the extent of our 2.05-mm array (Fig. 3D, top). In contrast, regression showed an inverse linear relationship between distance from the stimulating electrode and the negative feature (R = −0.51, P < 0.001). The negative slope of the regression line was steepest at the beginning of stage 2 (Fig. 3D, bottom), suggesting that the duration of the negative feature increased predominantly around the site of ICMS and fell off linearly with distance. Note that the change in y-intercept explains the increase in the positive feature within stage 3 and the increase in negative slope explains the increase in the negative feature within stage 2.

We next investigated whether these observed changes in the positive and negative features were associated with anticipatory modulation of S1bf. Our concern was that the changes we observed reflected a general change in the motivation of the subjects related to the structure of the task, specifically the subjects’ expectation of reward, and not a stimulus-driven mechanism. Moreover, the 500-ms intervals around the time of ICMS delivery used to calculate the neural features would miss
any anticipatory modulation of S1bf beginning at the start of a trial, when the house light was illuminated and developing throughout the trial. We reasoned that any anticipatory modulation of S1bf would be captured by comparing each unit’s firing rate around the start trial event against its firing rate just before ICMS. Therefore, for each unit, on each trial resulting in the delivery of ICMS, we calculated the difference in spike counts across two intervals. The intervals compared were 1) before and after the start trial event (Δbase) and 2) before the start trial event and just before delivery of ICMS (Δstim) (Fig. 4A). These paired differences preserved trial-to-trial variability in each unit’s firing rate while being sensitive to any trial-to-trial differences in firing rate between the trial start and ICMS delivery. By this estimator, any systematic modulation of S1bf unit activity across trials within a session, positive or negative, would present as a difference in the medians of the paired difference distributions. An example of these distributions aggregated across all units and trials for a single session from one subject is shown in Fig. 4B, left. This aggregate example is representative of what we observed at the single-unit level across all our subjects and sessions: there was no systematic difference in the medians of these distributions. We did note a covariance between the Δstim and Δbase distributions, suggesting that perhaps anticipatory modulation of S1bf presented as an increase in firing rate variability and not a systematic change in spike counts. Further analysis regressing Δstim onto Δbase (Fig. 4B, right) showed a linear correlation between these quantities (R = 0.53, P < 0.001).

Together, these data point to a rebalancing of inhibition and excitation during novel association learning. The behavioral stages utilized for our analysis were defined in terms of not only performance and response time but also the emergence of response behaviors to ICMS, measured by a three-axis accelerometer. While the negative feature did show a relationship with distance from the stimulating electrode, the positive feature did not. Finally, our data suggest that these changes reflect a mechanism related to the delivery of reinforced ICMS and not the task structure.

The ICMS CS-CR Did Not Generalize to the Opposite Hemisphere

We next examined whether these changes reflected a global learning mechanism involving the whole brain or whether it was local to the cortical areas conditioned. We tested two nested hypotheses to investigate the spatial extent of the ICMS CS-CS. Our first hypothesis was that the ICMS CS-CS and the subject’s detection threshold for ICMS would generalize to the opposite hemisphere’s S1bf, which had never received direct stimulation by ICMS. The second hypothesis was that learning would generalize but the detection threshold would not, requiring an increase in effective charge transfer to elicit a CR. A local learning mechanism would be apparent if a subject emitted correct responses at a rate expected by chance when ICMS was introduced into the opposite hemisphere using parameters known to be sufficient for detection. These hypotheses were tested by first estimating ICMS detection thresholds in the conditioned S1bf and then introducing ICMS to the opposite hemisphere’s S1bf.

ICMS detection thresholds were estimated for a subset of subjects (n = 4). In all cases, the detection threshold for ICMS of S1bf (Fig. 5A) was approximately an order of magnitude lower than the effective charge transfer used to train the subjects to initially learn the ICMS CS-CS (from 266 ± 74 nC to 26 ± 19 nC). The steepness of the psychometric function was validated by the observation of a dramatic increase in the probability of a subject emitting a miss within a narrow current range below the estimated detection threshold (within 2 μA). The observations of misses were highly informative because they were rarely seen when the rats learned the ICMS CS-CS and ICMS was detectable, because of the group bias toward emitting false alarms. In addition, setting the ICMS current at
the minimum level predicted by the psychometric function to produce asymptotic performance did generate robust response behavior in all the subjects evaluated.

Having determined ICMS detection thresholds in the conditioned hemisphere, we then conditioned a subset of these subjects to respond to ICMS of the opposite S1bf (n = 2). Upon introduction of ICMS into the opposite S1bf, using parameters determined when approximating that subject’s detection threshold, both subjects exhibited behavior well described as stage 1: chance performance in response to ICMS (Fig. 5B; ≤30/100 correct trials). Even when the ICMS parameters were set to levels predicted by the psychometric function to produce asymptotic performance, chance performance persisted. When the ICMS parameters were then set with the three-axis accelerometer, just as they had been in the initially conditioned S1bf (see MATERIALS AND METHODS), chance performance persisted. It took three or four sessions using these ICMS parameters before these subjects again met the criterion for stage 2 learning behavior (Fig. 5B). It was striking to observe that the subjects’ previous learning did not influence their learning to respond to ICMS of the opposite S1bf. Taken together, our data suggest that the neural correlates of learning within the initially conditioned S1bf involve brain areas excluding the opposite hemisphere’s S1bf and reflect a coordinated modulation of local cortical responses to reinforced inputs.

DISCUSSION

A major function of the brain is to generate inferences about the world based on sensory information, conditional upon the needs of the organism, yet how the physiology of the brain endows the organism with the ability to learn novel associations remains largely unknown (Dayan et al. 2000; Gallistel 2003; John 1972; Olds et al. 1972; Rescorla and Wagner 1972). In this study we asked whether S1bf is involved in the brain dynamics of learning a novel association. We addressed this question by testing whether the response properties of S1bf would change in a manner correlated with the learning behavior of the rat. Our results suggest the existence of a dynamic, stimulus-driven mechanism involving S1bf, which operates in coordination with changes in behavior during somatosensory learning.

The use of a cross-modal transfer conditioning schedule (Over and Mackintosh 1969) provided a method for separating distinct stages of the rats’ somatosensory learning behavior from habituation to the experimental environment and learning the basic task structure. The appeal of classical conditioning paradigms is apparent if they are thought of as controlled processes for presenting organisms with uncertainty, requiring the modification of action policies to distinguish relevant from irrelevant information (Gallistel 2003). Following Gallistel et al. (2004), our approach to the behavioral data focused upon shared behaviors observed across subjects, which developed at unique rates for each subject. This is in contrast to examining a blind average over subjects and across sessions. We demonstrate that by analyzing the subjects’ behavior in this manner a consistent pattern emerged between the learning behavior of the subjects and the neural response to ICMS of S1bf.

A significant challenge for this work was discriminating between those changes in the neural features that were induced by a change in neural state from those caused by a change in behavioral state. This is of particular concern as modulation of the whisker sensory system by motor behavior has been observed by a number of researchers (Castro-Alamancos and Oldford 2002; Fanselow and Nicolelis 1999). Furthermore, it is known that the behavioral modification of S1bf to sensory afferents persists even after transection of the infraorbital nerve (Hentschke et al. 2006), suggesting that this modulation is generated by the brain and not induced by stimuli. Therefore, the use of ICMS to bypass the sensory periphery was insufficient to address this issue, although, while it has been found that ICMS-evoked oscillations in S1bf vary as a function of behavior (Venkatraman and Carmena 2009), this modulation was observed to occur after the positive and negative features described in this work, during the return to baseline excitation. Notably, we did not observe any anticipatory increase in firing rate over the course of a trial (Fig. 4), indicative of a behavioral state change during active whisking (Hentschke et al. 2006). Even still, our concern was that changes in behavioral state modulated the neural response to ICMS and influenced the features we tracked over the course of learning. For example, while the use of the three-axis accelerometer enabled us to observe at high temporal resolution the emergence of a response to ICMS, the behavior during this initial learning was highly variable, and therefore a direct comparison against some control behavioral state was untenable. So to go beyond observing just neural correlates of learning behavior, we adopted an alternating block schedule during ICMS conditioning, Tone/ICMS and just ICMS, to control for differences in response behavior across a change in CS+. The alternating block schedule allowed us to compare two epochs in which the behavioral states of the subjects were very similar while the neural states were quite different: S1bf in the presence of the unlearned and, later, learned ICMS CS+ (Fig. 3C). The observed differences in the neural response to ICMS between these conditions suggest that the change in neural state was not simply induced by a change in the subjects’ moment-to-moment behaviors.

The progression of neural changes we observed in response to ICMS of S1bf as our subjects learned this new CS-CR sheds new light on a possible functional role for these coordinated changes in the positive and negative modulation of sensory responses during learning. Significantly, psychophysical work in the rat utilizing ICMS as the CS+ has provided evidence that the initial post-ICMS excitatory response carries the relevant sensory information integrated by the subject to form a CR (Butovas and Schwarz 2007). The results from Butovas and Schwarz (2007) involving ICMS in the rat motivated us to examine the functional role of these features of the neural response to ICMS during learning. Our results led us to conceptualize the post-ICMS response as a signal-to-noise ratio (SNR), with the initial positive feature as the excitatory ICMS signal and any irregular activity thereafter being noise unrelated to, and possibly interfering with, the processing of this stimulus. This SNR may be increased by either increasing the post-ICMS excitatory response or increasing the negative feature (which we hereafter take to be an inhibitory response). Increasing the excitatory neural response would increase the fidelity of the ICMS stimulus, and increasing the inhibitory response would decrease the amount of unrelated, or interfering, neural activity. We suggest that the SNR of S1bf to incoming inputs may be modified in two ways dependent upon
the subject’s certainty about whether the resulting pattern of somatosensory activity is predictive of reinforcement. The first general mechanism for increasing the SNR of S1bf would involve decreasing the amount of ongoing activity within S1bf conditional upon input to S1bf (the inhibitory response). The second mechanism would involve amplifying the neural response to just those inputs associated with reinforcement (the excitatory response). By thinking of the post-ICMS neural response as a SNR, these learning-induced changes in excitatory and inhibitory responses may be synthesized into a behaviorally relevant context, implying that they serve distinct functional roles.

Physiological evidence for such a mechanism involving S1bf comes from recent ex vivo work in the mouse demonstrating increased tonic GABAergic currents in layer 4 excitatory neurons within the barrel associated with a whisker that is subjected to aversive conditioning (Urban-Ciecko et al. 2010). These data also indicate the origin of these increased inhibitory currents to be fast-spiking (FS) inhibitory neurons. In addition, among layer 4 excitatory cells a greater frequency of spikes at threshold has been observed in the conditioned barrel, indicative of an increase in intrinsic excitability (Bekisz et al. 2010). Notably, electrophysiological work in anesthetized rats involving ICMS has documented a prominent excitatory and inhibitory response to ICMS across all cortical layers (Butovas and Schwarz 2003), and work in the mouse has indicated the mechanism of the inhibitory response to be GABA$_B$ receptors and electrical synapses (Butovas et al. 2006). It is worth noting that, whereas Butovas and Schwarz (2007) estimated detection thresholds for single ICMS pulses of ~2 nC in head-fixed animals, the thresholds we estimated from our unrestrained animals, for pairs of pulses, were an order of magnitude larger. This is likely due to the increased level of background input to S1bf in unrestrained animals as well as the longer temporal intervals used in our task, resulting in a higher cost for a false alarm. With these differences in mind, our work in the infragranular layers of the rat S1bf partially fills the gap between the literature described above on learning behavior and neural data obtained ex vivo in S1bf after conditioning. We suggest that the conspicuous timing of these changes in inhibition and excitation may actively facilitate the rodents’ somatosensory learning.

The subset of subjects subsequently conditioned to respond to ICMS of the opposite S1bf demonstrated that this CS-CR did not generalize across hemispheres (Fig. 5). This striking lack of generalization occurred despite the intact corpus callosum and any reafference caused by ICMS that went detected by our methods. While evidence of increased communication between hemispheres has been found in mice during early learning, this effect has also been observed to disappear later in the learning process (Cybulski-Klosowicz and Kossut 2006). If these early learning changes hold in the rat, they do not seem to have aided our subjects in learning to respond to ICMS of the opposite hemisphere.

It is worth noting a few of the obvious differences between ICMS and natural stimulation. Unlike Bekisz et al. (2010), who observed a specific increase in the intrinsic excitability of excitatory cells just within the trained barrel in response to whisker stimulation, we observed an increase in the excitatory response that was uniformly distributed across our 2.05-mm array (~4 barrels at 500 μm per barrel). Our findings may be attributed to the fact that ICMS stimulates the fibers of passage around an ICMS site, resulting in a diffuse, sparse pattern of excitation (Histed et al. 2009). Nevertheless, our observation of an inverse relationship between the size of the inhibitory response and distance from the stimulating electrode, also found by Butovas and Schwarz (2003), suggests that some spatial information about the location of the ICMS site is retained by the inhibitory response, perhaps due to the anatomical distribution of inhibitory cells within S1bf.

Coordinated and asynchronous changes in the balance between excitation and inhibition have been observed by several other investigators in multiple experimental contexts and termed homeostatic plasticity (see Turrigiano and Nelson 2004 for review). Our results resemble these effects and may reflect a similar neurophysiological origin. Notably, in vivo work pairing nucleus basalis stimulation with the presentation of a tone induces changes in the primary auditory cortex of rats resembling those elicited by conditioning (Weinberger et al. 2006). Moreover, work by Froemke et al. (2007), recording from the primary auditory cortex of anesthetized rats, demonstrated a possible functional role for an asynchronous perturbation and rebalancing of inhibition and excitation within the primary auditory cortex. In contrast to the Froemke et al. (2007) results, wherein a decrease in inhibition is followed by an increase in excitation and then a rebalancing of inhibition, we observed an increase in inhibition followed by an increase in excitation concurrent with a renormalizing of inhibition within S1bf. Our recordings were extracellular, and therefore ignorant of the cell types of the recorded neurons, and our data are derived from behaving as opposed to anesthetized rats, making comparison difficult. Yet despite these experimental differences, both studies provide evidence for a sequential modulation of inhibition and excitation, and our work additionally demonstrates how these are coordinated during somatosensory learning.

The further study and interpretation of these neurophysiological properties within behaving animals holds the promise of providing a more direct link between plasticity and learning. Understanding these phenomena is fundamental for determining how the functions of distinct brain regions enable organisms to adapt their behavioral strategies in response to changing environmental demands.
REFERENCES


