Cerebral Cortex December 2013;23:2976–2986
doi:10.1093/cercor/bhs288
Advance Access publication September 17, 2012

Synchronization Across Sensory Cortical Areas by Electrical Microstimulation is Sufficient for Behavioral Discrimination

Hachi E. Manzur[1,2], Joel Alvarez[1], Cecilia Babul[1] and Pedro E. Maldonado[1,3]

[1]Biomedical Neuroscience Institute, Universidad de Chile, Santiago, Chile [2]Neural Circuits and Cognition Unit, Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, 21224, USA and [3]Faculty of Medicine, Program of Physiology and Biophysics, Universidad de Chile, Casilla Santiago, Chile

Address correspondence to Pedro E. Maldonado, PhD, Program of Physiology and Biophysics, Faculty of Medicine, Universidad de Chile, Independencia 1027, Santiago, Chile, 8380453. Email: pedro@neuro.med.uchile.cl

The temporal correlation hypothesis proposes that cortical neurons engage in synchronized activity, thus configuring a general mechanism to account for a range of cognitive processes from perceptual binding to consciousness. However, most studies supporting this hypothesis have only provided correlational, but not causal evidence. Here, we used electrical microstimulation of the visual and somatosensory cortices of the rat in both hemispheres, to test whether rats could discriminate synchronous versus asynchronous patterns of stimulation applied to the same cortical sites. To disambiguate synchrony from other related parameters, our experiments independently manipulated the rate and intensity of stimulation, the spatial locations of stimulation, the exact temporal sequence of stimulation patterns, and the degree of synchrony across stimulation sites. We found that rats reliably distinguished between 2 microstimulation patterns, differing in the spatial arrangement of cortical sites stimulated synchronously. Also, their performance was proportional to the level of synchrony in the microstimulation patterns. We demonstrated that rats can recognize artificial current patterns containing precise synchronization features, thus providing the first direct evidence that artificial synchronous activity can guide behavior. Such precise temporal information can be used as feedback signals in machine interface arrangements.

Keywords: coding, cortex, microstimulation, sensory, synchronization

Introduction

From the early experiments of Fritsch and Hitzig (1870), who microstimulated the brain of dogs to examine localized electrical excitability, direct electrical microstimulation has been extensively used in a wide range of experimental approaches, from functional anatomy to its use in the treatment of neurological and psychiatric illness (Young 1970; Bierer and Middelbrooks 2002; Bartlett et al. 2005; Tehovnik et al. 2006; Mohr et al. 2011). Many early studies used surface stimulation to create a map of the human motor cortex (Penfield and Boldrey 1937; Rasmussen and Penfield 1947; Woolsey et al. 1979). Further, deep microstimulation with microelectrodes was used initially to stimulate the hypothalamus (Hess 1957; Graziano 2008), but was later applied to explore eye movements (Robinson 1972; Tehovnik and Sommer 1997), motor mapping (Asanuma et al. 1976; Sessle and Wiesendanger 1982; Cooke et al. 2003), conditioning learning (Doty 1965), cortical plasticity (Nudo et al. 1992; Maldonado and Gerstein 1996), behavioral control (Talwar et al. 2002), and memory prosthesis (Berger et al. 2011).

In addition, microstimulation is widely used to explore perceptual processes. Besides evoking specific percepts such as phosphenes, by stimulation of early sensory areas (Nashold 1970; Tehovnik et al. 2005), microstimulation can bias (Salzman et al. 1990) or disrupt (Slocum and Tehovnik 2004) visual movement perception. In addition to these studies, microstimulation in multiple electrodes has been used to explore sensory coding mechanisms. Mouly et al. (1985) and Mouly and Holley (1986) explored the ability of rats to discriminate spatial microstimulation patterns in the olfactory bulb. Animals with permanently implanted electrodes were trained to use single or multisite sinusoidal microstimulation as the discriminative stimuli for selecting a palatable solution in a 2-choice test. In monkeys, Romo et al. (1998, 2000) found that 2 flutter stimuli delivered sequentially to the fingertips can still be discriminated if the second stimulus is replaced by direct microstimulation of the somatosensory areas. Recently, Fitzsimmons et al. (2007) found that monkeys are able to obtain a reward, guided by spatio-temporal patterns of cortical microstimulation delivered to the primary somatosensory cortex through implanted multielectrode arrays. In their study, the spatio-temporal patterns were composed of 4 trains of biphasic pulses, individually delivered through independent electrodes in a specific sequence. Monkeys were able to discriminate different sequences of activation to choose the site of the reward.

In the studies described above, microstimulation was typically delivered to one or several electrodes at a time, thus effectively producing an increase in the firing rate of the local neurons. In addition to the firing rate increment, the activation of these neuronal populations seems to be constrained to a window of time, producing a synchronous discharge of neurons and fibers surrounding the tip of the electrode (Butovas and Schwarz 2003; Tehovnik et al. 2006; Histed et al. 2009; Logothethis et al. 2010). Thus, the increase in firing rate at a given location cannot be disambiguated from the synchronous activation. This is important because a standing question regarding the mechanisms underlying microstimulation is whether these effects are the product of the changes in firing rate, neuronal synchrony or both. In contrast to population coding, (Sakurai 1996; Kristan and Shaw 1997; Averbeck et al. 2006) or hierarchical convergence (Barlow 1972), neuronal synchrony or temporal correlation (Milner 1974; von der Malsburg 1981 and Singer 1999), propose that neuronal ensembles representing perceptual objects are achieved by synchronizing the activity of neurons that are evoked by the same object. This activity, often associated with oscillatory patterns (Ahissar and Vaadia 1990; Gray 1999; Fries et al. 2002), has been found in many cerebral loci and, related to different sensory and motor tasks (Riehle et al. 1997; Colgin et al. 2009).

By using electrical microstimulation, we examined whether artificially induced synchronization at several cortical sites (dissociated from changes in activation frequency) is sufficient to produce a neuronal activation that evokes an artificial perception that rats can signal behaviorally. By delivering different patterns of artificial synchronization to the visual and somatosensory cortices in both hemispheres of the rat, we tested whether rats were consistently able to obtain a reward by selecting the lever associated with each pattern. We found that rats reliably distinguished between patterns differing only in the distribution of electrodes containing synchronized microstimulation. Moreover, their performance was a function of the degree of synchronization contained in the microstimulation pattern. These results provide strong evidence that the degree of synchrony in artificial spatiotemporal inputs to the brain can be causally correlated with behavior.

Materials and Methods

Subjects and Surgery

All experiments followed institutional (CBA0215 FMUCH) and NIH guidelines for the care and use of laboratory animals. Twelve male Long Evans and 4 male Sprague Dawley rats, weighing 250–450 g served as subjects for this study. Rats were maintained in a room with controlled temperature and inverted light cycle (12 h light, 12 h dark), and were fed daily with 3 to 4 dry pellets and 15 min/day of water to motivate them to obtain juice rewards during training. For electrode implantation surgery, rats were anesthetized with an intramuscular injection of ketamine [12 mg/kg. intraperitoneal (i.p.)], acepromazine (1 mg/kg. i.p.), and a dose of atropine (0.05 mg/kg. subcutaneous) to reduce salivation. Electrocardiogram and rectal temperature were continuously monitored. Rats were allowed to recover for at least 7 days during which ad libitum food and water were available, and analgesics (ketoprofen 5 mg/kg i.m. daily for 3 days) and antibiotics (enrofloxacine 5 mg/kg i.m. daily for 5 days) were provided.

During surgery, the rats were mounted in a stereotaxic device and 8 craniotomies (1–2 mm wide, each) were performed: 2 in sensory area V1, and 2 in sensory area S1B bilaterally (Fig. 1*A*). In each craniotomy, we inserted 1 electrode, independently placed. The distance between craniotomies located in the same cortex was 1 mm. The stereotaxic coordinates (relative to the bregma) used for implantation were: Visual cortex, −7.00 to −4.80 mm AP and 2.00–4.50 mm ML; somatosensory cortex, −3.60 to −0.30 mm AP and 2.00–5.00 mm ML (Fig. 1*A*). To ensure correct positioning, the electrodes were lowered until spikes were found (usually between 500 and 800 µm depth). Following positioning, rats received light or tactile stimulation of the whiskers to corroborate corresponding changes in neuronal activity. After stabilization of recordings, the electrodes were cemented to the skull using dental acrylic. A central screw (0–80), implanted in the mid line of the skull over the occipital region, −12 mm relative to the bregma, served as the ground, as all current were injected against this ground screw.

Behavioral Training

Animals were trained in an acrylic Skinner box. The front panel contained a central warning light, and 2 cue lights at each side above the response levers. A central stainless-steel tube, located below the warning light, delivered a drop of apple juice as a reward on successful trials. The Skinner box was controlled by a custom-made computer program written in LabWindows/CVI (National Instruments, Austin, TX, United States of America). A daily training session comprised 200 trials. Each trial start was signaled by the illumination of a central warning light, which lasted for 1 s (Fig. 1*B*). Following this, 1 of 2, left or right, cue lights was randomly illuminated, and instructed the animal to choose the corresponding lever. The light cue turned off after a 5-s period, or if a response is made within those 5 s. Responses made after 5 s did not result in reward delivery and were scored as omissions. After a correct choice, the light was extinguished and, the animal received a reward in the form of a drop of juice. Upon an incorrect response, the animal received neither punishment nor reward. The intertrial interval following omissions, correct responses and incorrect responses was fixed at 2 s.

After learning to distinguish natural light stimuli, we performed the surgery to implant the microstimulating electrodes. Following recovery, the rats were trained to discriminate microstimulation patterns paired with light stimuli (Fig. 1*B*). The other task parameters remained identical to the first discrimination phase. In the final, or test stage, training proceeded without the left or right cue lights, and discrimination was based on microstimulation alone (Fig. 1*C*). All the results reported here are from the final stage. The aim of this stage was to discern whether rats were able to discriminate between 2 different current patterns applied directly to the brain. In this case, learning was defined as an average of 65% or more correct responses [100 × (corrects)/(corrects + incorrects)] during 4 consecutive sessions, with 30% or less, omissions. Mean and 95% confidence intervals (CIs) for the mean were calculated within and across rats using a bootstrap of 1000 resamples.

Electrodes and Electrical Microstimulation

Stereotrodes were built using 25-µm diameter wires, (nichrome or platinum + 10% iridium) (Gray et al. 1995). Prior to training, to ensure that normal neuronal activity was present, multiunit and local field potential signals were recorded from the microstimulation sites. Robust spike and oscillatory activity around 7 Hz ("theta" band) in both visual and somatosensory cortices and between 40 and 60 Hz ("gamma" band) in the visual cortex after the onset of the visual cues and lever pressing (data not shown) was found.

Each pattern of microstimulation was created by a custom-made Matlab routine (The MathWorks, Natick, MA, USA) that constructed files containing the sequence of stimulation. These files were serially uploaded from the control computer to an 8-channel microstimulator (STG4008, Multichannel Systems, Reutlingen, Germany), and then digitally triggered with the same control computer. All the microstimulation pulses were delivered through both wires of each stereotrode to the ground screw. The current source for each stereotrode was independent and connected to a different channel of the microstimulator. Patterns of controlled current were designed as a sequence of biphasic square wave pulses (negative first) or sinusoidal waveforms. Synchronization was arbitrarily defined as the precise temporal co-occurrence of 2 or more pulses for square waveforms, or as zero-phase lag between 2 or more sinusoidal current waveforms. The details of each microsimulation pattern were different for each experiment, and thus they are described in the Results section.

Results

We implanted 8 stereotrodes in the visual and somatosensory cortices of 12 rats (Fig. 1*A*). In a Skinner box, we first trained rats to discriminate a random right or left light cue by pressing the corresponding lever located below each light. After reaching a correct response rate of 80%, we trained them to discriminate different patterns of microstimulation without cue lights (Fig. 1*C*). Figure 1*B* and *C* shows the timing schemes for training and testing respectively. All the results reported here are from this stage of training.

Left Versus Right Hemisphere Microstimulation

Since the microstimulation parameters needed to elicit putative behavioral responses are rather wide, we explored different combinations of frequency and current intensity. First, we trained 8 rats to discriminate between left and right hemispheric stimulation (Fig. 2*A*), and found that using 40 µA to 50 µA, and 2 ms biphasic square wave pulses at 40 Hz for 500 ms, yielded reliable training performance. Group statistics

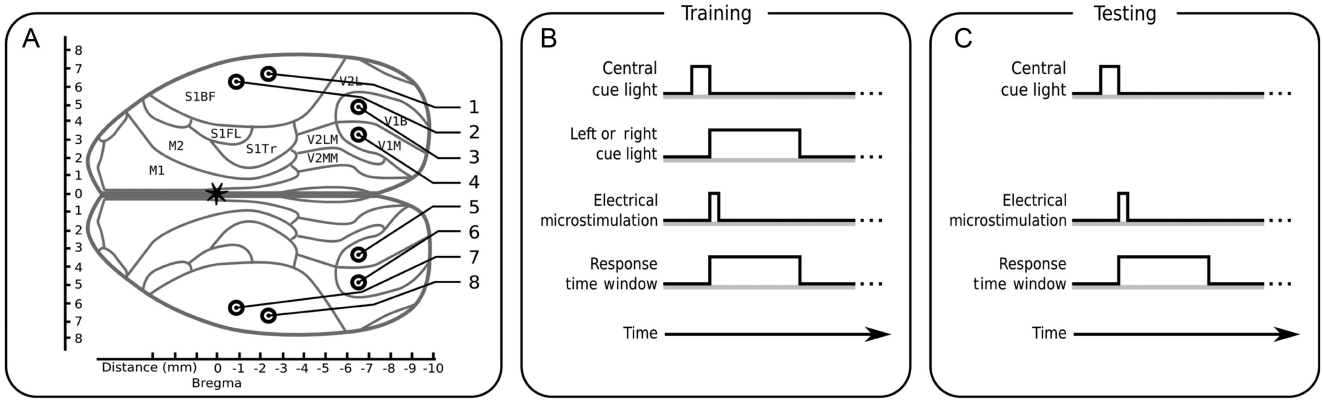

Figure 1. Electrode placement and training scheme. (*A*) Placement location of the array of electrodes used for electrical microstimulation is indicated by the black circles. Separation between electrodes in the visual and somatosensory cortex was at least 1 mm. The cortical regions were drawn based on Paxinos and Watson (1998). S1BF, somatosensory area barrel field; V1B, primary visual cortex, binocular; V1M, primary visual cortex, monocular. Asterisk indicates the bregma. Numbers will be used as reference for the rest of the figures. (*B*) Timing scheme for training. During training, rats had to press 1 of 2 levers, cued by the onset of a light located above the levers, in combination with the application of one microstimulation pattern. (*C*) Timing scheme for testing. Here, rats were forced to choose between left or right levers based only on the consequence of electric microsimulation applied through the implanted electrodes.

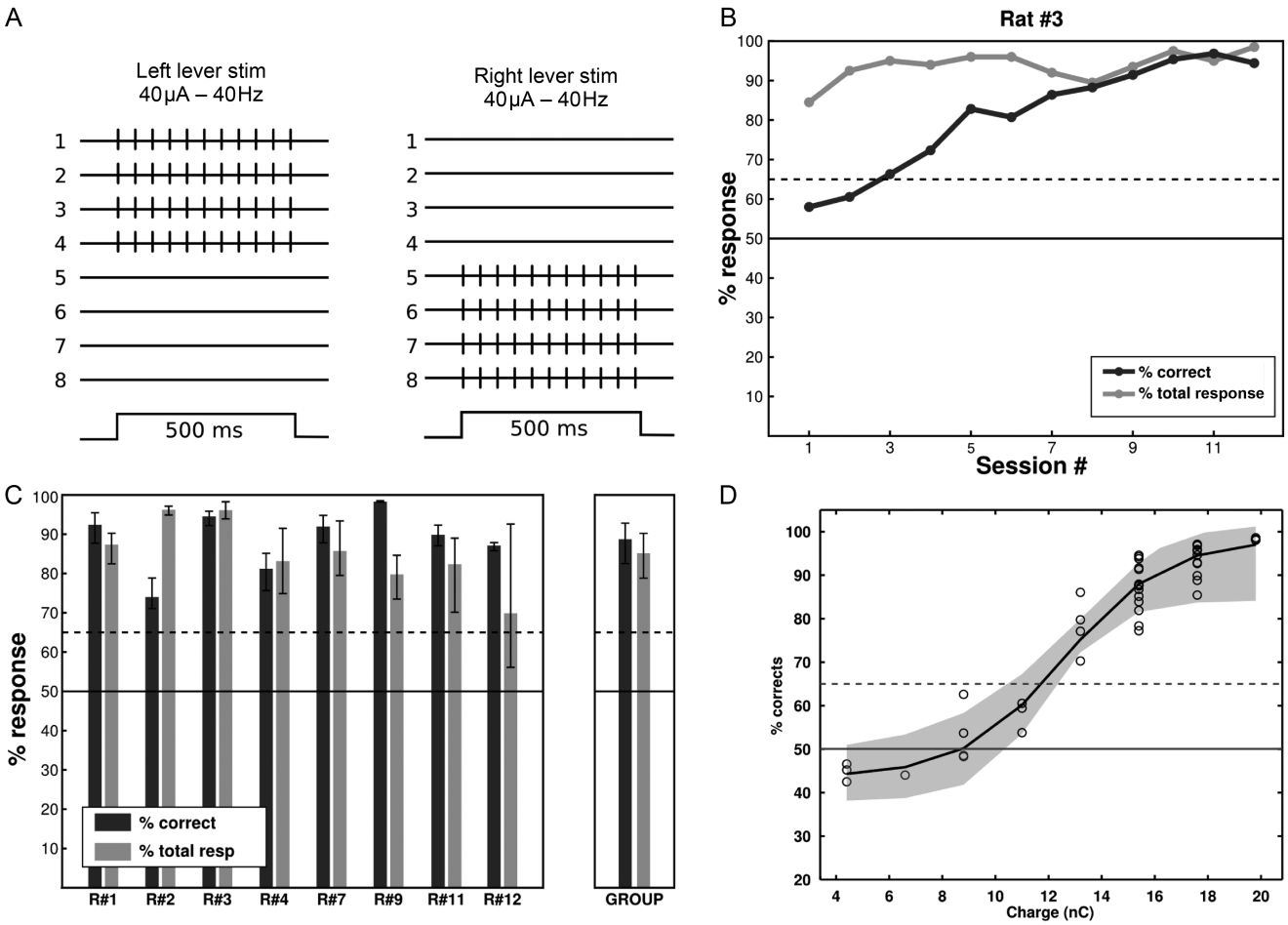

Figure 2. Performance of rats trained to discriminate between electrical microsimulation applied to electrodes inserted to each cerebral hemisphere. (*A*) Stimulus configuration. Electrodes 1–4 were implanted in the right hemisphere and electrodes 5–8 in the left hemisphere. In each trial, 20 synchronous square pulses (40 μA, 40 Hz, 500 ms) were applied to either one of the hemispheres at a time, and instructed the animal to choose one of the levers (right or left). (*B*) Time course of performance of one rat to the discrimination of microstimulation to different hemispheres. For each session of 200 trials, the percentage of total (correct + incorrect) responses is shown in gray and of those; the percentage of correct responses is shown in black. This animal and the other quickly and consistently showed performances above the learning criteria (65%, dashed line). (*C*) Average performance for 4 consecutive sessions—fter reaching learning criteria—for the 8 rats trained in this task, and for the group. Correct responses shown in black exceeded 65% in all cases. The % of total responses is shown in gray. Error bars depict 95% CI. (*D*) Psychophysical curve of detection of current. Gray area shows the 95% prediction bounds.

($n = 8$): % of correct, mean = 86.71, 95% CI = 91.98–78.89; % total response, mean = 81.21, 95% CI = 86.86–75.13; Binomial Test P-value < 0.005 (Fig. 2*B*,*C*). We repeated this experiment using different current levels to estimate the amount of electrical charge needed to elicit correct responses. We found that above 14 nC all rats quickly exceeded the 65% performance threshold (Fig. 2*D*). It is important to note that during all the microstimulation experiments reported here, we did not observe side effects of the stimulation such as seizures or muscle twitches, which rats could use as a discrimination cue. As an additional control, to test whether any other potential cue was being used, we measured the performance when the microstimulation cable was disconnected from the animal. In all cases, we observed a drop in performance to 50% or less, which demonstrates that cortical microstimulation was the sole basis of their discrimination.

Synchronization Discrimination Task

The main purpose of our study was to examine whether rats can discriminate 2 different microstimulation patterns that were similar in the number and amplitude of pulses, but different in the pattern of synchronous stimuli. We examined if rats could be trained to perform a 2-choice discrimination task. We constructed 2 sets of patterns that contained the same amount of synchronous pulses, but differed on which electrodes of the set contained synchronous pulses. This scheme is depicted in Figure 3. In this configuration, one of the pulse patterns (associated with the left lever) contained zero-time-lag synchronous pulses between the electrodes in right somatosensory and left visual cortices (see Fig. 3: Sequence A1). The remaining electrodes contained pulses that were asynchronous with all other pulses in the 8-electrode set. A second microstimulation pattern (associated with the right lever) had zero-time-lag synchronous pulses in the complementary configuration and asynchronous square pulses in the remaining electrodes (Fig. 3; sequence B1). To avoid the possibility that rats learned to distinguish these 2 patterns on the basis of a repetitive timing of pulses, using the temporal information contained in them, 5 different sequences of each pattern (5 for the right lever and 5 for the left lever) were constructed for each training session (Fig. 3). In each trial, one of the 10 versions was randomly chosen with a 10% probability.

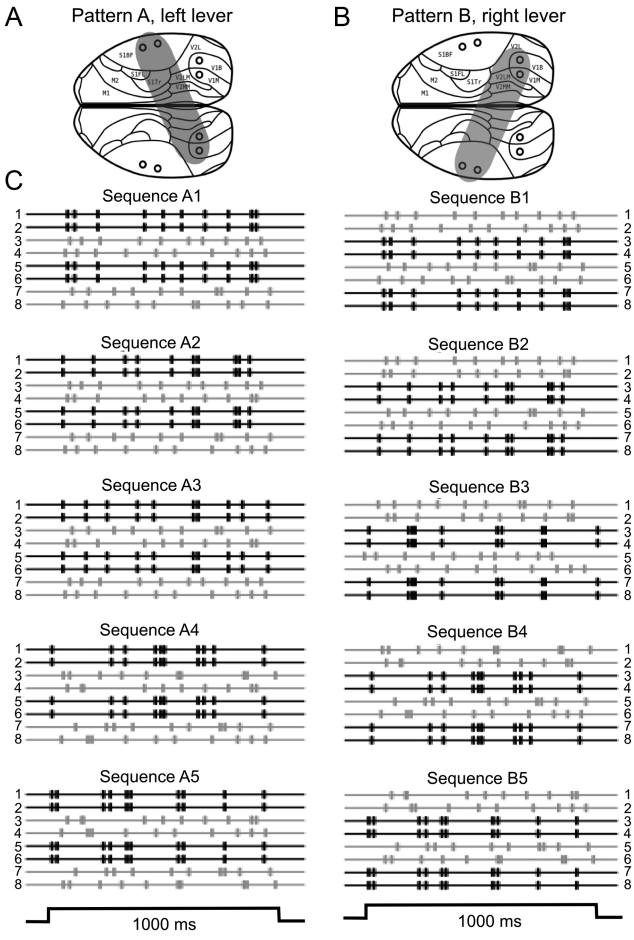

Figure 3. Discrimination between 2 synchronization configurations. (*A*) Artificial synchronization between right somatosensory cortex and left visual cortex (gray patch) instructed the rats to press the left lever. (*B*) The opposite pattern (left somatosensory cortex and right visual cortex), instructed rats to choose the right lever. (*C*) For each session, a new set of 5 versions of each pattern were constructed to ensure that discriminations relied only on the pattern and not in the specific temporal sequence. Parameters: Square pulses, 150 μA, 0.5 ms pulse width, negative first, 1000 ms train duration.

Five rats were trained in this task. Figure 4*A* and *B* shows the learning curve for 2 of rats and Figure 4*C* the average plateau performance. Group statistics ($n = 5$): % correct responses, mean = 84.49, 95% CI = 85.70–82.75; % total response, mean = 84.32, 95% CI = 89.76–77.55; Binomial Test P value < 0.05. These results show that rats reliably distinguish between 2 microstimulation patterns based on the distribution of synchronization and not in the specific temporal sequence contained in the train. This experiment demonstrates that artificial synchronized current pulses in the cortex of rats convey sufficient information to perform behavioral discriminations.

In the experiment described above, rats discriminated pulse sequences that contained synchronous pulses in 4 electrodes, from sequences of synchronous pulses in the complementary set of electrodes. However, there are many possible combinations to setting up spatial arrangements of microsimulation patterns among the 8 electrode set. In order to determine whether rats can perform this discrimination and then learn a different contingency, we first trained 3 rats to discriminate a contingency where all 4 electrodes in the somatosensory cortices received synchronous pulses. These pulses were associated with the left lever. Alternatively, we stimulated with synchronous pulses in all visual electrodes that were associated with the right lever. After the rats had reached a stable performance on one contingency, we switched to a new contingency using the same configuration as in the previous experiment (Fig. 5*A*). We found that all rats successfully learned the new contingency. The time course of the performance of one of the rats is shown in Figure 5*B*. After reaching the learning criteria for several sessions, the contingency was switched. In the first session (indicated by an arrow), the performance dropped to chance and then progressively increased to learning criteria. The performance during the sessions around the contingency change was not consistent between rats. Nonetheless, this was a feature in all experiments where the time for reaching learning criteria varied between rats. Interestingly, the average performance for the contingency that mixed electrodes from the visual and somatosensory cortices was higher than the performance for contingency that paired visual versus somatosensory electrodes (Fig. 5*C*,*D*).

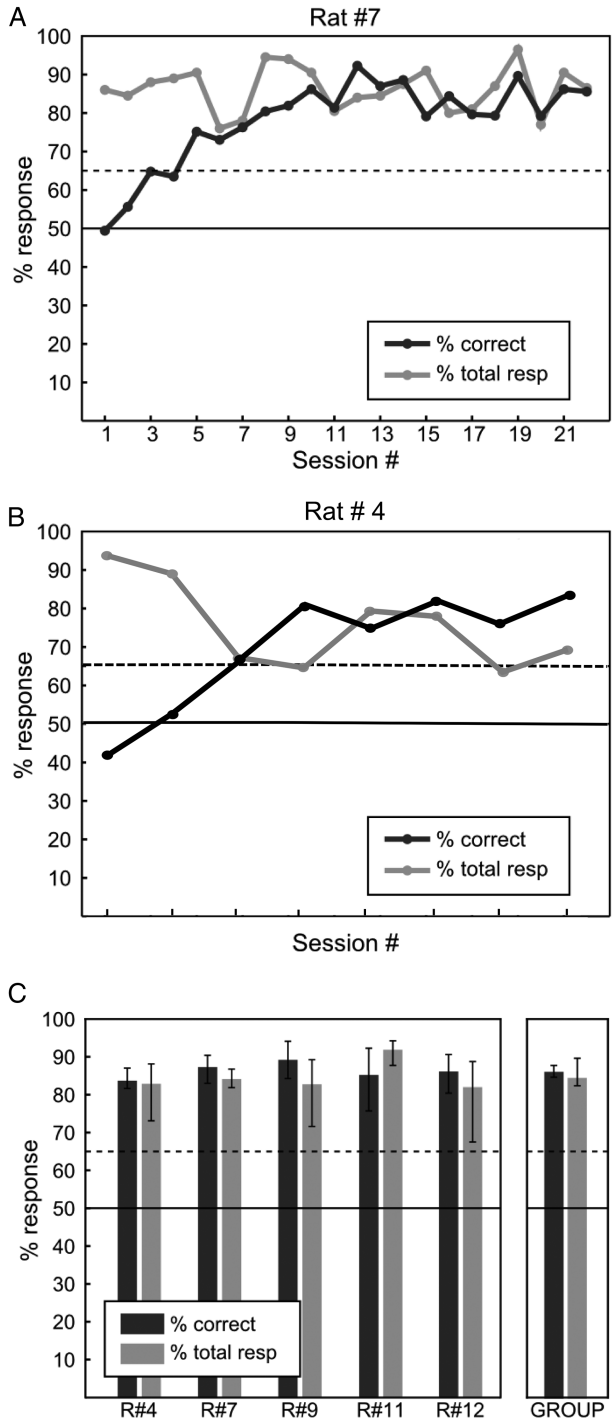

Figure 4. Rats reliably discriminate between different synchronization configurations. Rats had to discriminate between artificial synchronization applied in different configurations (see Fig. 3). (*A* and *B*) Example learning curves for 2 rats. In every session, a completely new set of stimuli was constructed. (*C*) Average performance for 5 rats trained in this task. All of them showed reliable performance levels according to defined learning criteria. The group statistics (mean and 95% CIs) are shown in the graph on the right. Continuous line: 50% chance level. Dashed line: 65% learning criteria.

Sinusoidal Current Discrimination Task

We demonstrated that rats can discriminate synchronous patterns of biphasic square pulses. In these cases, neuronal activity was directly evoked with biphasic pulse patterns. Yet, it is proposed that low-amplitude modulation of the field potential can also modify spike timing without altering the firing rate (Volgushev et al. 1998; Ito et al. 2011). Therefore, we tested whether rats could distinguish a pattern of rhythmic synchronization from a pattern of rhythmic asynchronization. For this experiment, we designed 2 sinusoidal current patterns with the same frequency (40 Hz), low-amplitude current amplitude (50 μA), and duration (500 ms) (Fig. 6*A*). In one of the patterns, associated with the right lever, all the sinusoids waveforms had the same phase through all the electrodes (synchronous). In the other pattern, associated with the left lever, the frequency and amplitudes remained unchanged; nevertheless, the phases of sinusoids applied to each electrode were randomized (asynchronous). Moreover, to avoid phase synchronization due to constant phase difference between stimulation sites (Lachaux et al. 1999), on every new training session, we constructed 10 versions of the asynchronous pattern by randomly jittering the phases of all the waveforms. In this experiment, we found that 3 rats trained on this discrimination task showed performance levels that exceeded learning criteria. Group statistics ($n = 3$): % of correct, mean = 76.00, 95% CI = 87.39–68.43; % total response, mean = 85.23, mean 95% CI = 92.81–75.39. These results demonstrate that synchronous oscillatory microstimulation in these cortical loci, evokes brain activation that is sufficiently distinctive from that produced by random phase oscillations.

Discrimination of Asynchronous Pulse Patterns

In the experiments described above, we compared the ability of rats to distinguish synchronous from asynchronous microstimulation patterns. In all cases, we constructed a variety of asynchronous patterns under the assumption that presenting the same timing sequence of asynchronous pulses may evoke a recognizable sensory activity, such that the rats do not need to distinguish the alternate synchronized pattern to perform the 2-choice discrimination task. To test this assumption, we trained 3 rats to associate 2 different asynchronous stimuli with each lever (Fig. 7*A*). In this experiment, the microstimulation patterns were not changed and were repeated on every training session. The results in Figure 7*B–D* show that the 3 rats trained in this task typically showed performance levels below the learning criteria. Two rats never reached performance above criteria, and a third animal showed occasional performance above the learning threshold (Fig. 7*C*). Group statistics: % correct responses, mean = 59.95, 95% CI = 70.15–54.09; % total response, mean = 72.81%, 95% CI = 81.20–67.30. This finding further supports the hypothesis that rats' discrimination can be based on the patterns of synchronized pulses applied in the electrode set, and this configuration of pulses appears to be easier to distinguish than an asynchronous, repetitive and, specific temporal sequences.

Parametrical Dependence on Artificial Synchronization

We showed that synchronous activity elicited by microstimulation in the cortex enabled rats to perform behavioral discriminations. We conjecture that as information contained in the synchronous pulses enabled rats to perform these discriminations, the amount of synchronous pulses in the microstimulation patterns should be reflected in their performance. To test this hypothesis, we trained 4 rats in a modified version of the 2-pattern discrimination task shown in Figure 3.

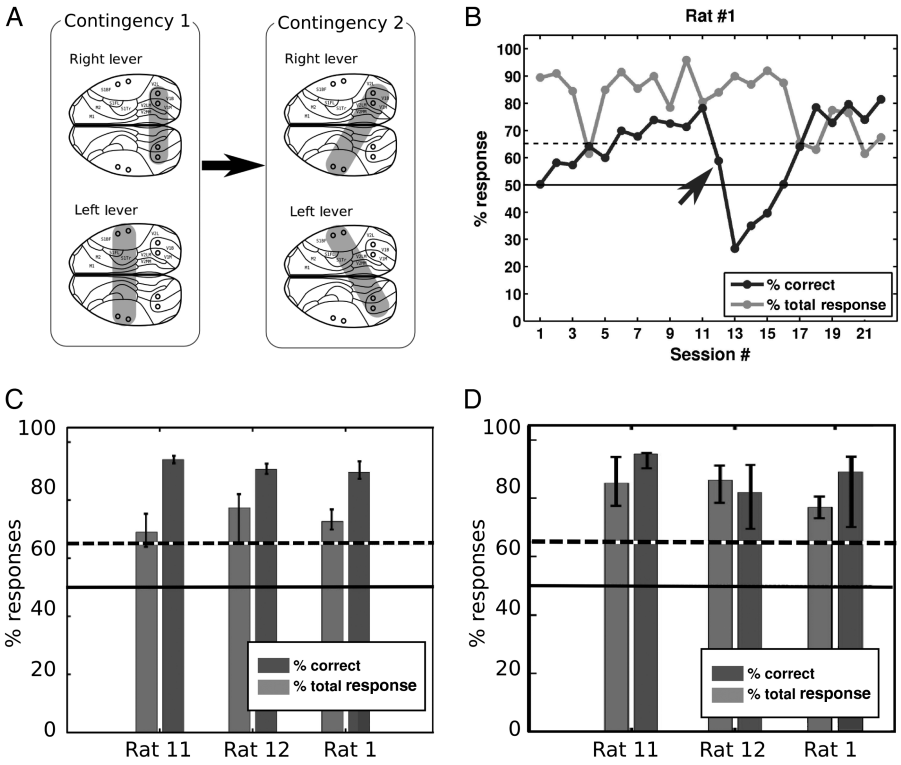

Figure 5. Rats can discriminate different microstimulation patterns after a change in contingency (*A*) The rats were first trained in a right visual + left visual, versus right somatosensory + left somatosensory discrimination (Contingency 1). After reaching the learning criteria for 4 sessions, the pattern was switched to a right visual + left somatosensory synchronization, versus the complementary pattern (Contingency 2). Parameters: Square pulses, 150 μA, 0.5 ms pulse width, negative first, 1000 ms train duration. (*B*) Learning curve for one animal trained in this task. The arrow indicates the first session in which pattern 2 was applied. Continuous line: 50% chance level. Dashed line: 65% arbitrary learning criteria. (*C*) Average performance for 3 rats trained on contingency 1. (*D*) Average performance for the same rats trained to switch between contingency 1 to contingency 2.

In each trial, a random percentage of the synchronous pulses belonging to each pattern was jittered to vary the level of synchronization from 0 to 100% (in 20% steps). Figure 8 shows the psychophysical curve constructed from the 4 rats tested in this task. In all subjects, we found that the level of synchronization to successfully discriminate the 2 ensembles, needed to be 60% or higher. This result demonstrates a parametrical dependence of the behavioral discrimination on the percentage of pulse synchronization.

Discussion

We found that rats reliably distinguished between 2 microstimulation patterns that differ in the presence of synchronized current pulses among the electrodes. Also, their performance was proportional to the level of synchronization in the microstimulation patterns. These results demonstrate that rats recognize artificial current patterns containing precise synchronization features, thus, providing the first direct evidence that artificial synchronous activity can guide behavior.

To determine the source of information that rats were using to perform in these tasks, we used several controls. Depending on intensity (current/frequency), microstimulation can produce muscle twitches that can be exploited by the animal as a source of information to predict reward. In our experiments, the magnitude of the current utilized here likely spread only through distances shorter than <400 μm (Tehovnik et al. 2006), and are similar to several other studies that have used microstimulation to explore perception (Salzman et al. 1990; Romo et al. 2000; Bartlett et al. 2005; de Lafuente and Romo 2005; Tehovnik and Slocum 2003). Consistently, we did not observe any muscle twitches or movements, indicative of a motor spread of microstimulation, suggesting that activation of sensory cortices was the only source of information for the animal. Also, by equating the stimulus frequency and duration, current intensity, pulse waveform, and by using several realizations of each pattern (Fig. 3) for every session, we were able to disambiguate synchronization from other potential sources of information, such as repetitive presentation of the same stimuli. Furthermore, it is unlikely that rats in our study were performing by monitoring a single cortical area (i.e., 2 electrodes located in left visual cortex), since the experiments with different contingencies (Fig. 5) demonstrate a disruption of performance around the contingency switch. This result suggests that rats were using information provided by the microstimulation through the entire set of electrodes in order to discriminate. Lastly, the performance of rats was proportional to the level of synchronization present in the microstimulation pattern. Given this experimental design, it seems unlikely that rats were using sources of information other than the presence or absence of synchronization, in the whole set of electrodes to perform in these tasks. These findings strongly suggest that artificial synchronization by itself was the main basis of their discrimination.

Interestingly, rats could distinguish synchronous from asynchronous patterns even when using sinusoidal current

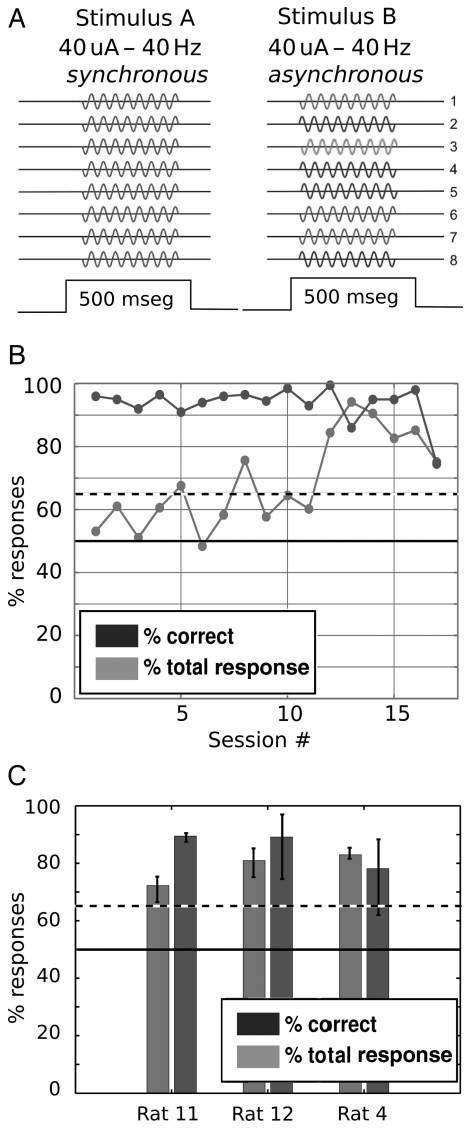

Figure 6. Rats can be trained to discriminate between sinusoidal synchronous versus asynchronous current patterns. (*A*) Sinusoidal stimulus configuration. Two current patterns were applied to all 8 electrodes. Stimulus A (synchronous) featured 0-phase lag sinusoidal waveforms, while stimulus B also contained sinusoidal waveforms, but with random phase lag, to avoid any pair of electrodes with the same oscillatory phase. Ten versions of stimulus B were built in each training session, to avoid phase locking (see text). (*B*) Learning curve for one rat showing a performance over 80% after the 11th training session. Dark gray: % of correct response, light gray: % of total response (see text). (*C*) Average performance for 3 rats trained in this task. All of them showed reliable performance levels according to defined learning criteria. Error bars represent the 95% CI. Continuous line: 50% chance level. Dashed line: 65% learning criteria.

patterns. It has been demonstrated that fluctuations of the local field potential, or the injection of artificial oscillatory currents, can entrain the firing of neurons producing an increment in phase locking of neuronal spikes to the current signal (Volgushev et al. 1998; Sirota et al. 2008; David et al. 2009; Ozen et al. 2010; Tchumatchenko et al. 2011). These experiments suggest that the modulation of the local field potential signal with sinusoidal currents may bias the activity of near-threshold neurons, causing an increase in synchronized firing (Volgushev et al. 1998; Ito et al. 2011), similar to what occurs with the precisely synchronized biphasic pulses.

It is unlikely that the patterns of microstimulation used here evoked neuronal activity similar to what occurs as a response to a visual or somatosensory stimulus. Feeding back the signals recorded during a sensory response into the same electrodes would not mimic this natural activation, which also would be even more difficult to reproduce with only 8 electrodes. Contrary to neural recordings, in which closely located neurons are captured, microstimulation mainly depolarizes fibers around the electrode tip, activating not only the local neurons, but also many others. Histed et al. (2009) found that the effective electrical field generated around the tip of the electrode was about 5 µm of diameter using 10 µA. They showed that the mechanism of activation is the direct depolarization of the neuropile (axons and dendrites) passing near the tip of the electrode. These finding accounts for their observed activation of some neurons located at long distances (millimeters) instead of others located in proximity (less than 10 µm) to the tip of the electrode. Moreover, they found that increments in current produced a higher density of activated neuronal bodies without increasing the distance at which neurons were activated. Additionally, direct microstimulation has a delayed inhibitory effect in the same locus that follows the excitatory activity and lasts for at least 100 ms, which significantly differs from what is observed during natural stimulation (Butovas and Schwarz 2003; Butovas et al. 2006). These cited experiments suggest that neuronal activity elicited by electrical microstimulation is not comparable with what is recorded in the same areas during natural stimulus presentations. Thus, it is very unlikely that microstimulation recreates the same brain activation as natural stimulation (e.g., light or sound). In addition, repetitive artificial microstimulation and training to detect electrical activation might cause a retinotopical impairment of thresholds for detecting stimuli (Ni and Maunsell 2010). In preliminary experiments, we also noted a reduced performance when we utilized repetitive stimulus sequence; therefore, we utilized different versions of microstimulation patterns in each trial. Nonetheless, spiking activity is likely occurring in our experiments, in a consistent pattern, as a consequence of the biphasic current pulses. Also, it is apparent these spiking patterns differ between the synchronous and asynchronous paradigms. Therefore, the microstimulation patterns utilized here created a sufficiently distinctive brain activation that allowed rats to make behavioral discrimination.

It could be argued that a synchronized sequence of microstimulation pulses delivered at defined positions in the brain will not necessarily translate into synchronized neuronal activity in those areas. Nevertheless, Butovas and Schwarz (2003), recorded cortical neurons in the rat as near as 450 µm from the microstimulation point, showing that a single stimulation pulse produces an increase in firing rate that begins 2.5 ms after the stimulation and lasts for approximately 2.5 ms. When they stimulated using trains of pulses in the range of frequencies used in our study (5–40 Hz), every pulse in the train was translated into an increase in firing rate that lasted 2.5 ms. This evidence strongly suggests that the temporal sequence contained in the microstimulation trains of our experiments was accordingly transformed (with a delay of ~2.5 ms) into the same temporal sequence of firing rate increases in the surrounding neurons and that synchronous microstimulation of different parts of the cortex was equally transformed into synchronous activity of the stimulated neurons. It seems that neurons "consider" events as synchronous when they

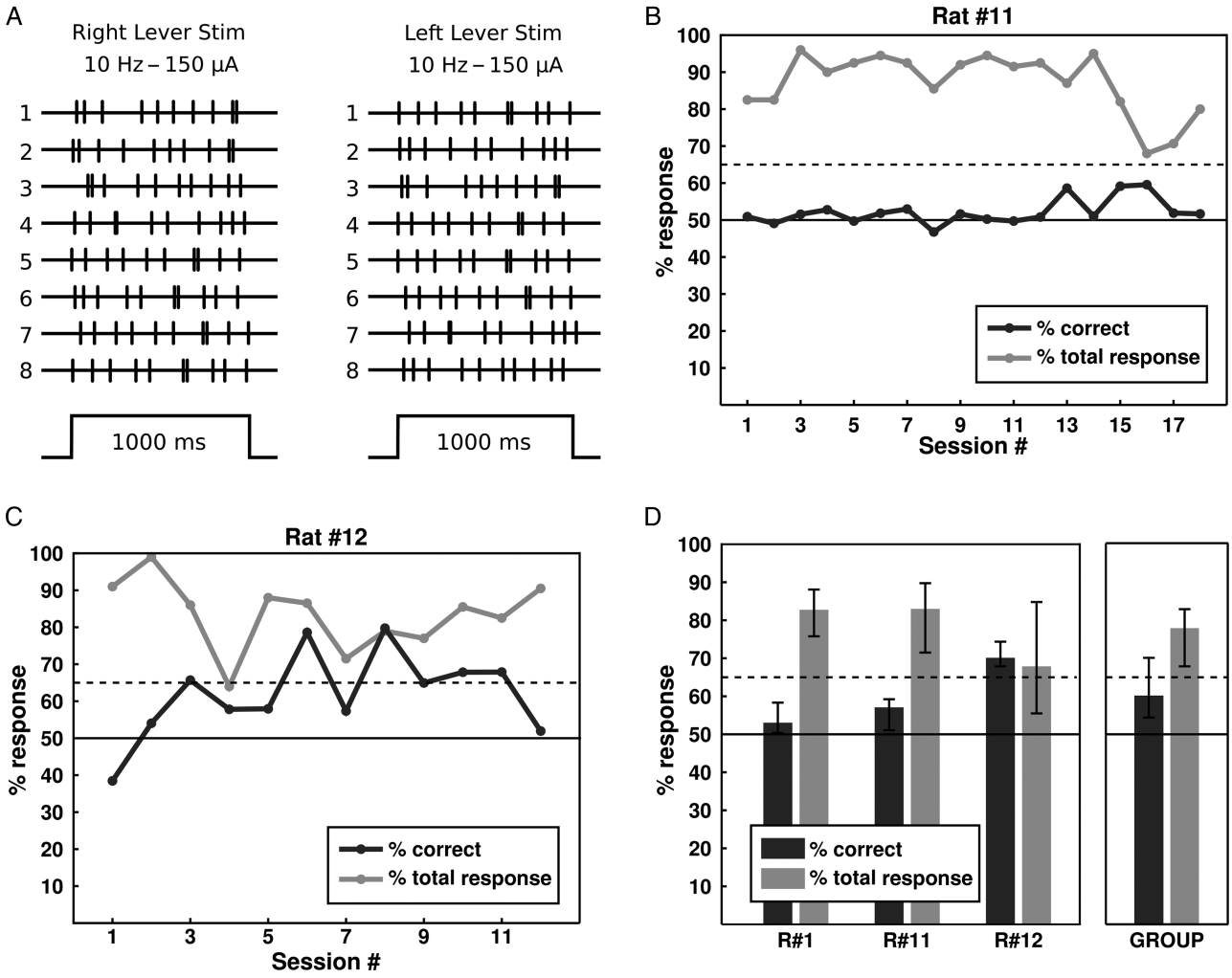

Figure 7. Rats have difficulties in distinguishing between 2 asynchronous pulse trains, even when they are stimulated with the same temporal sequence in each trial. (*A*) Each stimulus (150 μA, 1000 ms) was constructed with pulses at a random sequence, with an average firing rate of 10 Hz. Each sequence was the same for every training session. (*B* and *C*) Example learning curve for 2 of rats (#1 and #12) trained in this task. (*D*) Average performance for each of the 3 rats trained in this task, and for the group. (*B–D*) Error bars show the 95% CI. Continuous line: 50% chance level. Dashed line: 65% arbitrarily established learning criteria.

occur together in a time window (~10 ms for cortical neurons) (Buzsaki 2011). Thus, an increase in firing rate lasting for approximately 2.5 ms of 2 or more neurons might be considered as synchronous by a postsynaptic element in which they eventually converge.

Since we did not perform simultaneous recordings along with microstimulation, we cannot determine whether microstimulation in one point affected other cortical sites in the same or in the opposite hemisphere. It is known, for example, that a limited number of axons originating from one isocortical microcolumn project to distant cortices in the same or in the opposite hemisphere (Molnár and Cheung 2006; Tamamaki and Tomioka 2010); and that cortical neurons project massively to the thalamus and striatum. In particular, the striatum is a structure that receives a high number of corticofugal axons originating from layer V pyramidal cells across the entire isocortical mantle (Heimer 2003). Given the high levels of cortico-cortical, as well as cortico-subcortical connectivity, it seems likely that the microstimulation in our case might have resulted in a complex pattern of distributed activity in the brain that may not be completely captured by recording at a few specific locations. It seems clear, however, that small timing differences clearly impact on the detectability of electrical patterns as those occur in the saccadic system, where eye movements are sensitive to the temporal pattern of microstimulation independent of rate (Kimmel and Moore 2007). Future microstimulation, along with simultaneous recording studies of several structures, will be necessary to elucidate these questions.

This study is the first, to our knowledge, to manipulate the synchronous activation of distant neural populations and to correlate it with the performance of the animal (Fig. 8). It has been proposed for many years that naturally occurring oscillations and synchronization between distant brain areas play a role in attention, signal transmission, or consciousness (von der Malsburg and Schneider 1986; Gray et al. 1989; Singer 1999). Nearby groups of cells discharge in synchrony during stimulation (Ts'o y and Gilbert 1988; Engel et al. 1990; Gochin et al. 1991; Kreiter and Singer 1992; Livingstone 1996), and synchronized discharge among cells occur even when located in different cerebral hemispheres (Engel et al. 1991; Nelson et al. 1992). Neuronal synchrony is often

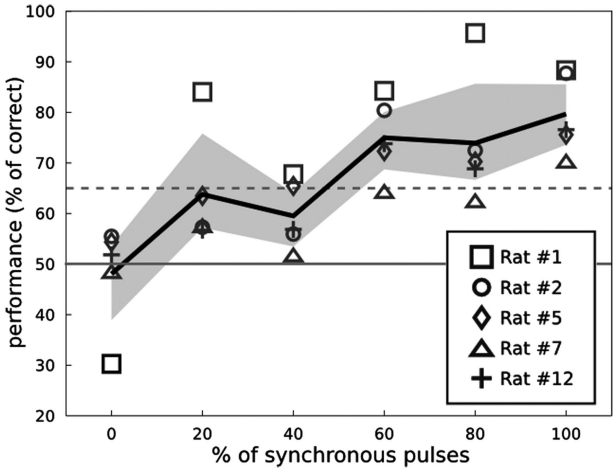

Figure 8. The performance of rats is directly proportional to the percentage of synchronous pulses contained in the stimulus patterns. The graph depicts the performance of 5 rats in the discrimination of different levels of synchronization. Continuous black line shows the group average, and the shaded area depicts the 95% CI for the mean. Continuous gray line, 50% chance level; dashed line, 65% arbitrary learning criteria.

associated with neuronal oscillations and, it is thought to contribute instrumentally to synchronization (Varela et al. 1999; Fries et al. 2007; Palva and Palva 2011; Buzaki and Wang 2012). In this manner, the measurement and observation of oscillatory activity in various types of signals appear correlated with synchronous activity. In our study, whether the differential activation caused by synchronized activity in the cortex is sufficient to enable perceptual responses, or a higher order integration of these cortices needs to occur in other cortical loci, cannot be directly deducted from this data. However, our results directly demonstrate that rats can use artificial synchronous activity as a cue for behavioral discrimination. Studies favoring temporal correlation coding have so far presented correlational evidence, where oscillatory or synchronous activity appears associated with behavioral performance (Riehle et al. 1997; Gray 1999; Fries et al. 2002; Colgin et al. 2009). We showed that this coding model can be directly tested by artificially activating specific populations of neurons in the rat's brain by using electrical microstimulation, and thus manipulate the temporal properties of electrical activity among the electrodes.

Finally, the results and experimental approach used in this study have important implications for brain–machine interfaces. Several studies demonstrate that artificial prosthesis can be manipulated directly by the brain by reading neuronal activity from the motor cortex (Chapin et al. 1999; O'Doherty et al. 2009, 2011,2012). However, to attain proper control, a closed loop system is required with sensory feedback signals (Miller and Weber 2011). These signals need to convey the consequence of motor actions providing sufficient tactile and proprioceptive information. We demonstrate that rats can easily differentiate synchronous versus asynchronous oscillatory current waveforms. One experimental approach can exploit phase comparison of oscillatory signals (Ahissar and Vaadia 1990; Ahissar et al. 1997). Therefore, artificial oscillations in a sensory system could be compared with artificial inputs from the periphery. In addition, our results show that correlated signals across electrodes can be effectively used as feedback information in machine interface arrangements. The precise timing that rats were able to distinguish in our experiments may translate into a diverse collection of microstimulation patterns that can provide more degrees of freedom which, are necessary to achieve a more efficient feedback control (Nicolelis 2003; Nicolelis and Lebedev 2010; O'Doherty et al. 2011).

Funding

This study was supported in part by Fondecyt 1090101 to P.M., Iniciativa Cientifica Milenio and a Conicyt Doctoral fellowship and Conciyt Anillo ACT 66 to H.M.

Notes

We thank Shih-Chieh Lin, Joseph O'Doherty, Jeff Mayse, Francisco Flores, Ruth Gesser, and the NIH Fellows Editorial Board for useful comments on the manuscript and for editorial assistance. *Conflict of Interest*: None declared.

References

Ahissar E, Haidarliu S, Zacksenhouse M. 1997. Decoding temporally encoded sensory input by cortical oscillations and thalamic phase comparators. Proc Natl Acad Sci USA. 94:11633–11638.

Ahissar E, Vaadia E. 1990. Oscillatory activity of single units in a somatosensory cortex of an awake monkey and their possible role in texture analysis. Proc Natl Acad Sci USA. 87:8935–8939.

Asanuma H, Arnold A, Zarzecki P. 1976. Further study on the excitation of pyramidal tract cells by intracortical microstimulation. Exp Brain Res. 26:443–461.

Averbeck BB, Latham PE, Pouget A. 2006. Neural correlations, population coding and computation. Nat Rev Neurosci. 7:358–366.

Barlow HB. 1972. Single units and sensation: A neuron doctrine for perceptual psychology? Perception. 1:371–394.

Bartlett JR, DeYoe EA, Doty RW, Lee BB, Lewine JD, Negrao N, Overman WH Jr. 2005. Psychophysics of electrical stimulation of striate cortex in macaques. J Neurophysiol. 94:3430–3442.

Berger TW, Hampson RE, Song D, Goonawardena A, Marmarelis VZ, Deadwyler SA. 2011. A cortical neural prosthesis for restoring and enhancing memory. J Neural Eng. 8:046017.

Bierer JA, Middlebrooks JC. 2002. Auditory cortical images of cochlear-implant stimuli: Dependence on electrode configuration. J Neurophysiol. 87:478–492.

Butovas S, Hormuzdi SG, Monyer H, Schwarz C. 2006. Effects of electrically coupled inhibitory networks on local neuronal responses to intracortical microstimulation. J Neurophysiol. 96:1227–1236.

Butovas S, Schwarz C. 2003. Spatiotemporal effects of microstimulation in rat neocortex: A parametric study using multielectrode recordings. J Neurophysiol. 90:3024–3039.

Buzsaki G. 2011. Rhythms of the brain. 1st ed. New York, USA: Oxford University Press.

Buzsáki G, Wang XJ. 2012. Mechanisms of gamma oscillations. Annu Rev Neurosci. 35:203–225.

Chapin JK, Moxon KA, Markowitz RS, Nicolelis ML. 1999. Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. Nat Neurosci. 2:664–670.

Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser M-B, Moser EI. 2009. Frequency of gamma oscillations routes flow of information in the hippocampus. Nature. 462:353–357.

Cooke DF, Taylor CSR, Moore T, Graziano MSA. 2003. Complex movements evoked by microstimulation of the ventral intraparietal area. Proc Natl Acad Sci USA. 100:6163–6168.

David FO, Hugues E, Cenier T, Fourcaud-Trocmé N, Buonviso N. 2009. Specific entrainment of mitral cells during gamma oscillation in the rat olfactory bulb. PLoS Comput Biol. 5:e1000551.

de Lafuente V, Romo R. 2005. Neuronal correlates of subjective sensory experience. Nat Neurosci. 8:1698–1703.

Doty RW. 1965. Conditioned reflexes elicited by electrical stimulation of the brain in macaques. J Neurophysiol. 28:623–640.
Engel AK, Koenig P, Gray CM, Singer W. 1990. Stimulus-dependent neuronal oscillations in cat visual cortex: Inter-columnar interaction as determined by cross-correlation Analysis. Eur J Neurosci. 2:588–606.
Engel AK, Koenig P, Kreiter AK, Singer W. 1991. Interhemispheric sychronization of oscillatory responses in cat visual cortex. Science. 252:1177–1179.
Fitzsimmons NA, Drake W, Hanson TL, Lebedev MA, Nicolelis MAL. 2007. Primate reaching cued by multichannel spatiotemporal cortical microstimulation. J Neurosci. 27:5593–5602.
Fries P, Nikolić D, Singer W. 2007. The gamma cycle. Trends Neurosci. 30:309–316.
Fries P, Schroder JH, Roelfsema PR, Singer W, Engel AK. 2002. Oscillatory neuronal synchronization in primary visual cortex as a correlate of stimulus selection. J Neurosci. 22:3739–3754.
Fritsch G, Hitzig E. 1870. Uber die elektrische Erregbarkeit des Grosshirns. Arch. f. Anat. 37:300–332.
Gochin PM, Miller EK, Cross CG, Gerstein GL. 1991. Functional interactions among neurons in inferior temporal cortex of the awake macaque. Exp Brain Res. 84:505–516.
Gray C, Konig P., Engel A.K., Singer W. 1989. Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties. Nature. 338:334–337.
Gray CM. 1999. The temporal correlation hypothesis of visual feature integration: Still alive and well. Neuron. 24:31–47.
Gray CM, Maldonado PE, Wilson M, McNaughton B. 1995. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. J Neurosci Methods. 63:43–54.
Graziano MS. 2008. The intelligent movement machine: An ethological perspective on the primate motor system. Oxford, UK: Oxford University Press.
Heimer L. 2003. A new anatomical framework for neuropsychiatric disorders and drug abuse. Am J Psychiatry. 160:1726–1739.
Hess WR. 1957. Functional organization of the diencephalons. New York: Grune and Stratton.
Histed MH, Bonin V, Reid RC. 2009. Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation. Neuron. 63:508–522.
Ito J, Maldonado P, Singer W, Grün S. 2011. Saccade-related modulations of neuronal excitability support synchronization of visually elicited spikes. Cerebral Cortex. 21:2482–2497.
Kimmel DL, Moore T. 2007. Temporal patterning of saccadic eye movement signals. J Neurosci. 27:7619–7630.
Kreiter AK, Singer W. 1992. Oscillatory neuronal responses in the visual cortex of the awake macaque monkey. Eur J Neurosci. 4:369–375.
Kristan WB, Shaw BK. 1997. Population coding and behavioral choice. Curr Opin Neurobiol. 7:826–831.
Lachaux JP, Rodriguez E, Martinerie J, Varela FJ. 1999. Measuring phase synchronization in brain signals. Hum Brain Mapp. 8:194–208.
Livingstone MS. 1996. Oscillatory firing and interneuronal correlations in squirrel monkey striate cortex. J Neurophysiology. 75 (6):2467–2485.
Logothetis NK, Augath M, Murayama Y, Rauch A, Sultan F, Goense J, Oeltermann A, Merkle H. 2010. The effects of electrical microstimulation on cortical signal propagation. Nat Neurosci. 13:1283–1291.
Maldonado PE, Gerstein GL. 1996. Neuronal assembly dynamics in the rat auditory cortex during reorganization induced by intracortical microstimulation. Exp Brain Res. 112:431–441.
Miller LE, Weber DJ. 2011. Brain training: Cortical plasticity and afferent feedback in brain-machine interface systems. IEEE Trans Neural Syst Rehabil Eng. 19:465–467.
Milner PM. 1974. A model for visual shape recognition. Psychol Rev. 81:521–535.
Mohr P, Rodriguez M, Slavíčková A, Hanka J. 2011. The application of vagus nerve stimulation and deep brain stimulation in depression. Neuropsychobiology. 64:170–181.
Molnár Z, Cheung AFP. 2006. Towards the classification of subpopulations of layer V pyramidal projection neurons. Neurosci Res. 55:105–115.
Mouly AM, Holley A. 1986. Perceptive properties of the multi-site electrical microstimulation of the olfactory bulb in the rat. Behav Brain Res. 21:1–12.
Mouly AM, Vigouroux M, Holley A. 1985. On the ability of rats to discriminate between microstimulations of the olfactory bulb in different locations. Behav Brain Res. 17:45–58.
Nashold B.S. Jr. 1970. Phosphenes resulting from stimulation of the midbrain in man. Arch Ophthal. 84:433–435.
Nelson JI, Salin PA, Munk M, Arzi M, Bullier J. 1992. Spatial and temporal coherence in cortico-cortical connections: A cross-correlation study in areas 17 and 18 in the cat. Visual Neurosci. 9:001–017.
Ni AM, Maunsell JH. 2010. Microstimulation reveals limits in detecting different signals from a local cortical region. Curr Biol. 20:824–828.
Nicolelis MA. 2003. Brain–machine interfaces to restore motor function and probe neural circuits. Nat Rev Neurosci. 4:417–422.
Nicolelis MAL, Lebedev MA. 2010. Principles of neural ensemble physiology underlying the operation of brain–machine interfaces. Nat Rev Neurosci. 10:530.
Nudo RJ, Jenkins WM, Merzenich MM, Prejean T, Grenda RJ. 1992. Neurophysiological correlates of hand preference in primary motor cortex of adult squirrel monkeys. Neurosci. 12:2918–2947.
O'Doherty JE, Lebedev MA, Hanson TL, Fitzsimmons NA, Nicolelis MA. 2009. A brain-machine interface instructed by direct intracortical microstimulation. Front Integr Neurosci. 3:20.
O'Doherty JE, Lebedev MA, Ifft PJ, Zhuang KZ, Shokur S, Bleuler H, Nicolelis MAL. 2011. Active tactile exploration using a brain-machine-brain interface. Nature. 479:228–231.
O'Doherty JE, Lebedev MA, Li Z, Nicolelis MA. 2012. Virtual active touch using randomly patterned intracortical microstimulation. IEEE Trans Neural Syst Rehabil Eng. 20:85–93.
Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, Buzsáki G. 2010. Transcranial electric stimulation entrains cortical neuronal populations in rats. J Neurosci. 30:11476–11485.
Palva S, Palva M. 2011. Functional roles of alpha-band phase synchronization in local and large-scale cortical networks. Front Neurosci. 2:1–15.
Paxinos G, Watson C. 1998. The rat brain in stereotaxic coordinates. San Diego, USA: Academic Press.
Penfield W, Boldrey E. 1937. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain. 60:389–443.
Rasmussen T, Penfield W. 1947. The human sensorimotor cortex as studied by electrical stimulation. Fed Proc. 6:184.
Riehle A, Grun S, Diesmann M, Aertsen A. 1997. Spike synchronization and rate modulation differentially involved in motor cortical function. Science. 278:1950–1953.
Robinson DA. 1972. Eye movements evoked by collicular stimulation in the alert monkey. Vision Res. 12:1795–1808.
Romo R, Hernández A, Zainos A, Brody CD, Lemus L. 2000. Sensing without touching: Psychophysical performance based on cortical microstimulation. Neuron. 26:273–278.
Romo R, Hernández A, Zainos A, Salinas E. 1998. Somatosensory discrimination based on cortical microstimulation. Nature. 392:387–390.
Sakurai Y. 1996. Population coding by cell assemblies—what it really is in the brain. Neurosci Res. 26:1–16.
Salzman CD, Britten KH, Newsome WT. 1990. Cortical microstimulation influences perceptual judgements of motion direction. Nature. 346:174–177.
Sessle BJ, Wiesendanger M. 1982. Structural and functional definition of the motor cortex in the monkey (*Macaca fascicularis*). J Physiol. 323:245–265.
Singer W. 1999. Neuronal synchrony: A versatile code for the definition of relations? Neuron. 24:49–65.
Sirota A, Montgomery S, Fujisawa S, Isomura Y, Zugaro M, Buzsáki G. 2008. Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm. Neuron. 60:683–697.
Slocum WM, Tehovnik EJ. 2004. Microstimulation of V1 input layers disrupts the selection and detection of visual targets by monkeys. Eur J Neurosci. 20:1674–1680.

Talwar SK, Xu S, Hawley ES, Weiss SA, Moxon KA, Chapin JK. 2002. Rat navigation guided by remote control. Nature. 417:37–38.
Tamamaki N, Tomioka R. 2010. Long-range GABAergic connections distributed throughout the neocortex and their possible function. Front Neurosci. 4:202.
Tchumatchenko T, Malyshev A, Wolf F, Volgushev M. 2011. Ultrafast population encoding by cortical neurons. J Neurosci. 31: 12171–9.
Tehovnik EJ, Slocum WM. 2003. Microstimulation of macaque v1 disrupts target selection: effects of stimulation polarity. Exp Brain Res. 148:233–237.
Tehovnik EJ, Slocum WM, Carvey CE, Schiller PH. 2005. Phosphene induction and the generation of saccadic eye movements by striate cortex. J Neurophysiol. 93:1–19.
Tehovnik EJ, Sommer MA. 1997. Electrically evoked saccades from the dorsomedial frontal cortex and frontal eye fields: A parametric evaluation reveals differences between areas. Exp Brain Res. 117:369–378.
Tehovnik EJ, Tolias AS, Sultan F, Slocum WM, Logothetis NK. 2006. Electrical microstimulation direct and indirect. J Neurophysiol. 96:512–521.
Ts'o DY, Gilbert CG. 1988. The organization of chromatic and spatial interactions in the primate striate cortex. J Neuroscience. 8 (5):1712–1727.
Varela F, Lachaux JP, Rodriguez E, Martinerie J. 1999. The brainweb: Phase synchronization and large-scale integration. Nat Rev Neurosci. 2:229–239 (April 2001).
Volgushev M, Chistiakova M, Singer W. 1998. Modification of discharge patterns of neocortical neurons by induced oscillations of the membrane potential. Neuroscience. 83:15–25.
von der Malsburg C. 1981. The correlation theory of the brain. Internal Report. Göttingen, West Germany: Max-Planck-Institute for Biophysical Chemistry.
von der Malsburg C, Schneider W. 1986. A neural cocktail-party processor. Biol Cybern. 54:29–40.
Woolsey CN, Erickson TC, Gilson WE. 1979. Localization in somatic sensory and motor areas of human cerebral cortex as determined by direct recording of evoked potentials and electrical stimulation. J Neurosurg. 51:476–506.
Young R. 1970. Mind, brain and adaptation in the nineteenth century: Cerebral localization and its biological context from gall to ferrier. New York, USA: Oxford University Press.