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Learned spatiotemporal sequence recognition and prediction in primary visual cortex

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Learning to recognize and predict temporal sequences is fundamental to sensory perception and is impaired in several neuropsychiatric disorders, but little is known about where and how this occurs in the brain. We discovered that repeated presentations of a visual sequence over a course of days resulted in evoked response potentiation in mouse V1 that was highly specific for stimulus order and timing. Notably, after V1 was trained to recognize a sequence, cortical activity regenerated the full sequence even when individual stimulus elements were omitted. Our results advance the understanding of how the brain makes 'intelligent guesses' on the basis of limited information to form visual percepts and suggest that it is possible to study the mechanistic basis of this high-level cognitive ability by studying low-level sensory systems.

The ability to recognize and generate serially ordered temporal sequences is a defining feature of the brain[1]. Although this capability contributes to almost every neural function, from recognizing speech to generating muscle movements, the underlying neurophysiology is poorly understood[2]. Much of our knowledge comes from human psychophysical, modeling and imaging studies that have implicated multiple cortical and subcortical regions in sequence learning[3–5]. The techniques used to study sequence learning in humans do not transfer easily to animal models[6], however, and provide limited mechanistic insight.

Mouse V1 is a readily accessible region that has been used for decades to study cortical development and experience dependent plasticity[7], with well-documented responses to stimulus orientation, size and motion, but not, notably, serial order. We found that repeated exposure to sequential visual stimuli over multiple days was sufficient to encode predictive representations in V1 of both the ordinal and temporal components of the stimulus patterns.

RESULTS

To test whether visual experience can evoke sequence representations in the visual cortex, we assigned mice to yoked experimental and control groups. On each of four training days, mice in the experimental group were shown 200 presentations of a single sequence of oriented sinusoidal gratings (termed ABCD, where each letter represents a unique orientation; **Fig. 1a**,**b**) and control animals were shown 200 random permutations of the same sequence elements (CBDA, DACB, etc.). On the fifth day, both groups were shown the trained sequence and a novel sequence constructed by reordering the same elements (DCBA). We measured visual evoked potentials (VEPs) in binocular layer 4 (see Online Methods) and found that ABCD elicited a markedly larger response after training than DCBA in the experimental group (**Fig. 1c** and **Supplementary Fig. 1**), but not in the control animals, which, as a result of the randomized nature of their training, had no reason to expect the sequence elements to appear in any particular order. Thus, repeated exposure to a visual sequence is sufficient to encode a neural representation of that sequence.

The same mice were also tested with the familiar sequence presented with novel timing ($ABCD_{300}$, where the subscript indicates that each stimulus element was held on the screen for twice the 150-ms duration used during training). The initial response to the first sequence element was very similar to that seen with the trained timing, but responses to subsequent sequence elements were clearly smaller (**Fig. 1d**). Comparing the average sequence evoked response magnitudes (**Fig. 1e**) confirmed what was qualitatively obvious from the VEP waveforms; in the experimental group, serial order and timing both strongly influenced evoked response magnitudes. The effects of reordering were not specific to sequence reversal; other tested sequence permutations also caused decreased response magnitudes similar to those shown in **Figure 1** for DCBA. These data suggest that any manipulation of sequence content after training disrupts the response magnitude. In contrast, there was no effect of sequence order or timing in the control group, although there was a magnitude increase relative to day 1 (**Fig. 1f**). Sequence-specific effects were also visible in cortical spiking activity, as demonstrated by the trained sequence driving higher multi-unit spike rates than a novel sequence (**Fig. 1g** and **Supplementary Fig. 2**).

To further investigate the temporal specificity with which sequences can be learned and to rule out the possibility that there is something inherently special about the 150-ms timing used in the previous experiments, we trained a cohort of mice using a protocol in which the four sequence elements were held on-screen with alternating short and long durations (**Fig. 2a**). After training, the mice were tested with the trained sequence presented with both familiar (short-long-short-long) and novel (long-short-long-short) timing. Although the difference between familiar and novel timing was subtle, the cortical response to the trained sequence presented with familiar timing was larger than the response to either a reordered or re-timed sequence (**Fig. 2b**,**c**). That this specificity was a consequence of training was

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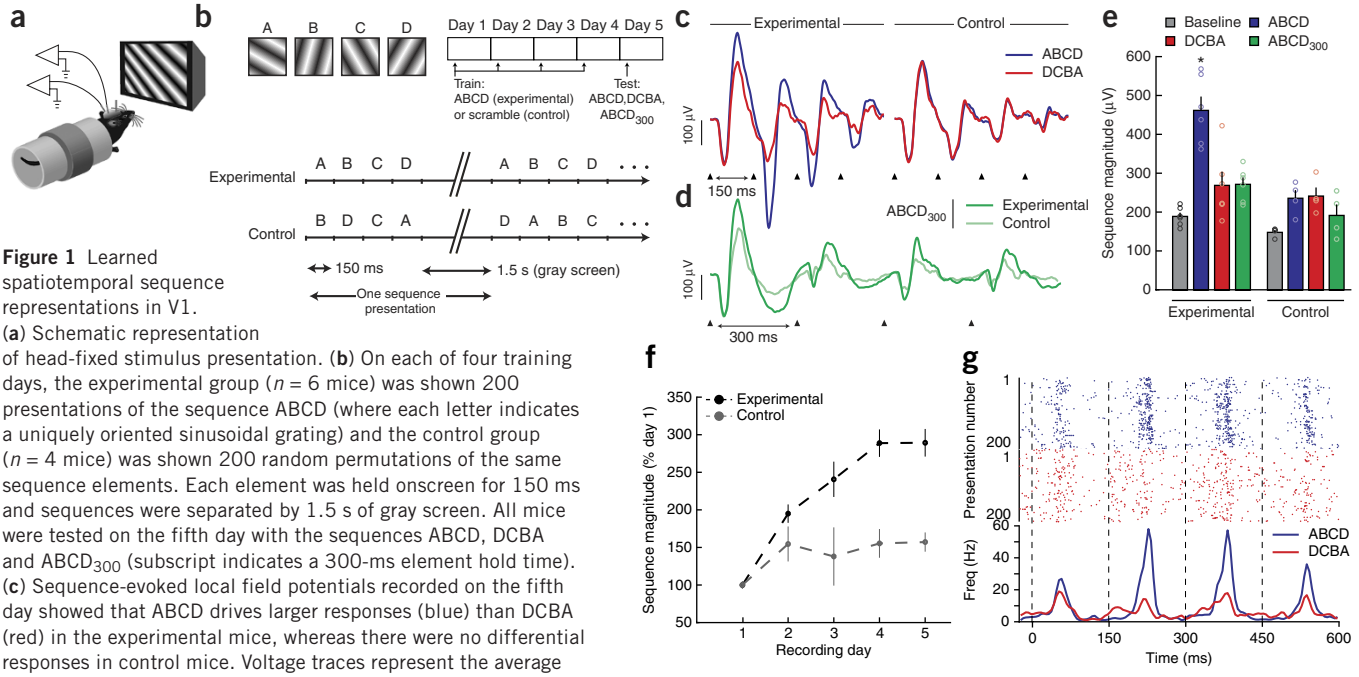

Figure 1 Learned spatiotemporal sequence representations in V1. (**a**) Schematic representation of head-fixed stimulus presentation. (**b**) On each of four training days, the experimental group ($n = 6$ mice) was shown 200 presentations of the sequence ABCD (where each letter indicates a uniquely oriented sinusoidal grating) and the control group ($n = 4$ mice) was shown 200 random permutations of the same sequence elements. Each element was held onscreen for 150 ms and sequences were separated by 1.5 s of gray screen. All mice were tested on the fifth day with the sequences ABCD, DCBA and $ABCD_{300}$ (subscript indicates a 300-ms element hold time). (**c**) Sequence-evoked local field potentials recorded on the fifth day showed that ABCD drives larger responses (blue) than DCBA (red) in the experimental mice, whereas there were no differential responses in control mice. Voltage traces represent the average response of all mice in each group and triangles mark the onset of each sequence element. (**d**) $ABCD_{300}$ drove relatively small responses in both groups. (**e**) Training regime had a significant effect on sequence response magnitude (quantified as the average peak-to-peak response to each of the four elements; **Supplementary Fig. 1**) potentiation (two-way RM-ANOVA, $F_{1,8} = 22.560$, $P = 0.001$). There was a significant interaction (two-way RM-ANOVA, $F_{1,8} = 6.638$, $P = 0.008$) between sequence and experimental group on day 5 and *post hoc* analysis revealed that the response to ABCD was significantly larger (indicated by asterisk) than either DCBA ($t_5 = 5.738$, $P = 0.002$) or $ABCD_{300}$ ($t_5 = 4.923$, $P = 0.005$). Sequence effects were not significant in the control group ($P > 0.5$ for all *post hoc* comparisons). Error bars show s.e.m. (**f**) Potentiation time course. (**g**) Sequence effects were evident in spiking neural activity. In this representative example, ABCD drove higher peak firing rates than DCBA (multi-unit spike rasters above peristimulus time histograms, dashed lines indicate element onset times).

clear from the minimal effect of timing evident in responses driven by a novel sequence (**Fig. 2c**,**d** and **Supplementary Fig. 3**).

One notable aspect of this plasticity is the small amount of sensory experience necessary to potentiate the cortical response. The largest increase in sequence magnitude occurs after the first training day (**Fig. 1f**), at which point each mouse had seen the sequence only 200 times (corresponding to 2 min of active visual stimulation). This rapid change is similar to a form of cortical plasticity called SRP (stimulus-selective response potentiation), which is characterized by a daily increase in VEP magnitude following repeated exposure to a

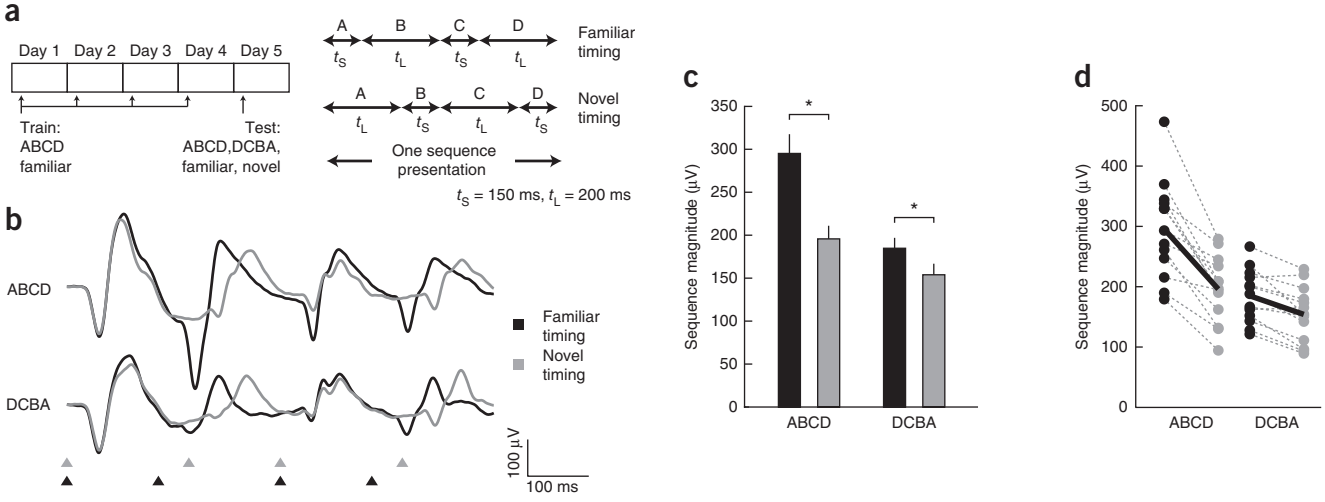

Figure 2 Sequence learning is temporally specific. (**a**) Mice ($n = 13$) were trained using ABCD presented with a short-long-short-long temporal profile. On the fifth day, the mice were tested with ABCD and DCBA presented with both familiar (black) and novel (long-short-long-short, gray) timing. (**b**) The largest responses occurred when the trained sequence was presented with the trained timing (top). Timing made little apparent difference when a novel sequence was shown (bottom). (**c**) There was a significant interaction between sequence order and timing (two-way RM-ANOVA, $F_{1,12} = 22.925$, $P < 0.001$). *Post hoc* analysis revealed the response to ABCD with trained timing was significantly larger than ABCD with novel timing ($t_{12} = 8.760$, $P < 0.001$). There was also a small effect of timing in DCBA ($t_{12} = 2.722$, $P = 0.012$). Error bars show s.e.m. $^*P < 0.05$. (**d**) The relative effect of timing as a function of sequence demonstrated by paired-response plots (dashed lines connect responses for single animals, black indicates the mean).

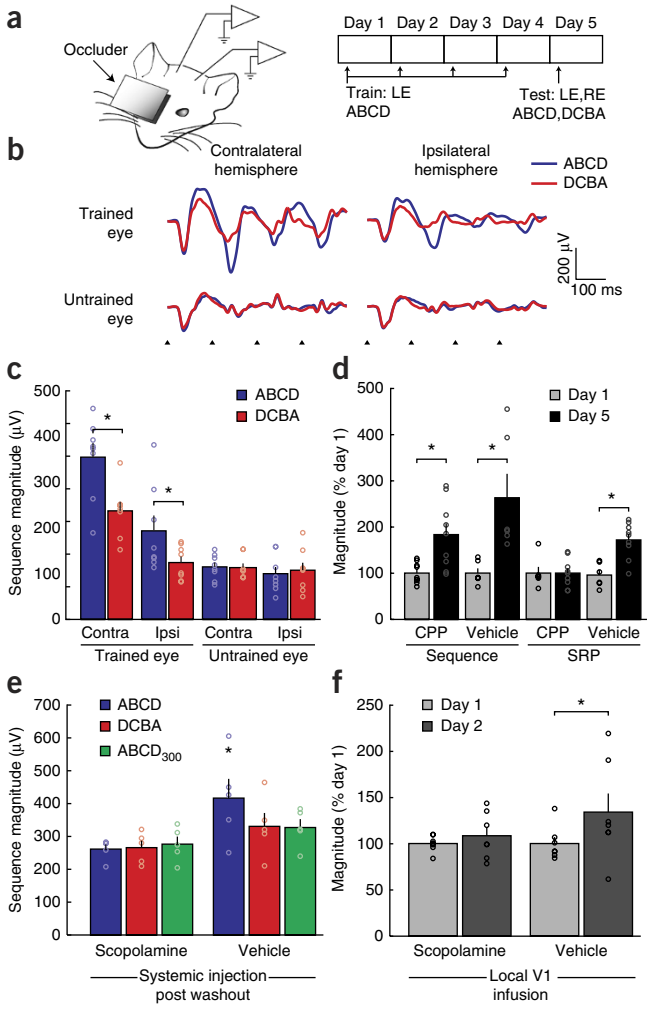

Figure 3 Learning does not transfer between eyes and requires muscarinic acetylcholine receptors in V1, but not NMDA receptors. (**a**) Mice ($n = 8$) were trained with an occluder restricting visual stimulation to the left eye (LE). Responses were recorded in the hemispheres contralateral and ipsilateral to the viewing eye. On the fifth day, sequences were presented to both eyes. (**b**) ABCD drives larger responses than DCBA in both hemispheres only when viewed through the trained eye. (**c**) There was a significant interaction between viewing eye and sequence in both hemispheres (two-way RM-ANOVA, contra: $F_{1,7} = 25.041$, $P < 0.001$; ipsi: $F_{1,7} = 10.426$, $P = 0.002$). The response to ABCD was significantly larger than DCBA in both hemispheres only when viewed through the trained eye (contra: $t_7 = 8.246$, $P < 0.001$; ipsi: $t_7 = 5.091$, $P < 0.001$). (**d**) Systemic CPP treatment (left, $n = 9$, 30–60 min before stimulus presentation during training) had no significant effect (two-way RM-ANOVA, $F_1 = 1.660$, $P = 0.220$) on sequence potentiation compared with vehicle ($n = 6$) and response potentiation was significant within both treatment groups (main effect: $F_{1,13} = 35.525$, $P < 0.001$; *post hoc* analysis: $t_8 = 3.186$, $P = 0.007$ and $t_5 = 5.093$, $P < 0.001$). The same CPP blocked subsequent SRP induction in the same mice (right, regrouped after washout, CPP $n = 6$, vehicle $n = 9$). There was a significant interaction between treatment and SRP recording session (two-way RM-ANOVA, $F_{1,13} = 42.210$, $P < 0.001$) and potentiation was significant only in the vehicle control group ($t_8 = 9.692$, $P < 0.001$). (**e**) Muscarinic receptor antagonism during training blocked sequence potentiation. There was a significant day 5 interaction between treatment and sequence in scopolamine-treated ($n = 5$) and vehicle-treated ($n = 5$) mice (two-way RM-ANOVA, $F_{1,8} = 5.827$, $P = 0.013$) and ABCD was significantly larger than DCBA ($t_4 = 3.661$, $P = 0.004$) or $ABCD_{300}$ ($t_4 = 3.813$, $P = 0.005$) only in vehicle-treated mice. (**f**) Local unilateral infusion of scopolamine in V1 ($n = 7$ mice) blocked potentiation relative to the opposite vehicle-treated hemisphere (two-way RM-ANOVA, $F_{1,6} = 30.189$, $P = 0.002$). Error bars show s.e.m. $^*P < 0.05$.

sinusoidal grating[8]. This increase is stimulus specific and involves local plasticity in V1 (refs. 8,9). Consistent with forms of learning that occur early in the visual processing hierarchy[10], SRP does not transfer between the eyes. To determine whether sequence learning shares this property, we trained mice with sequence presentation restricted to one eye and tested them with monocular presentation to both eyes (**Fig. 3a**). Although there was a clear effect of sequence on cortical responses driven by the trained eye, learning did not transfer to the untrained eye (**Fig. 3b**,**c**). These findings indicate that the modifications elicited by training occur at a site where information from the two eyes can be separated.

SRP is mechanistically similar to classical long-term synaptic potentiation, including the requirement for NMDA receptor activation[8]. To test whether sequence learning shares similar mechanisms and might represent a higher order expression of SRP, we systemically treated mice with either the NMDA receptor antagonist 3-(2-carboxypiperazin–4yl)propyl-1-phosphonic acid (CPP, 10 mg per kg of body weight, intraperitoneal) or saline before sequence presentation on each training day. Notably, expression of sequence learning was comparable between CPP-treated and control mice (**Fig. 3d**). To confirm the effectiveness of the CPP in blocking NMDA receptors under our experimental conditions, we subsequently reassigned the same mice after a 3-d washout period into new CPP and vehicle control groups and exposed them to the SRP induction protocol. We found that the same CPP prevented induction of SRP (**Fig. 3d** and **Supplementary Fig. 4**). Thus, sequence learning is a phenomenon distinct from SRP and does not require NMDA receptor activation.

Several forms of experience-dependent plasticity in V1 have been shown to require the cholinergic input arising from the basal forebrain[11,12]. To test whether sequence potentiation requires acetylcholine, we systemically treated mice with either the muscarinic receptor antagonist scopolamine or vehicle. Mice in the scopolamine-treated cohort showed no evidence of sequence potentiation over the training period or recognition of the trained sequence on day 5 (**Fig. 3e** and **Supplementary Fig. 5**). Likewise, local microinfusion of scopolamine into V1 of one hemisphere blocked potentiation in that hemisphere even as the vehicle-treated hemispheres of the same mice potentiated normally (**Fig. 3f** and **Supplementary Fig. 6**). These results demonstrate the involvement of the cortical cholinergic system in the mechanisms underlying sequence learning.

It is clear from these data that the mice learned neural representations of the familiar visual sequence, but it is not clear whether this representation was sufficient to reproduce the sequence absent external stimulation. To test this possibility, we trained a cohort of mice with the sequence ABCD and tested with two sequences where the second element was omitted and replaced by a gray screen (**Fig. 4a**). In the first test sequence (A_CD) the omitted element was preceded by A, established during training to predict element B, whereas the second test sequence (E_CD) was initiated by a novel element E that had not been established to predict anything. The cortical response to a gray screen preceded by E was small and consisted solely of a late positive-going bump (**Fig. 4b**). In contrast, the response following A shared a similar morphology and timing with the response actually evoked by the element B: the average latency to peak negativity during the second element was almost identical when the response was driven by element B (60.8 ± 2.6 ms) or anticipatory based on the presence of element A (60.3 ± 3.5 ms). There was no statistical difference in the average sequence magnitude between ABCD and A_CD ($t_6 = 0.964$, $P = 0.354$), but both were larger than E_CD (**Fig. 4c**).

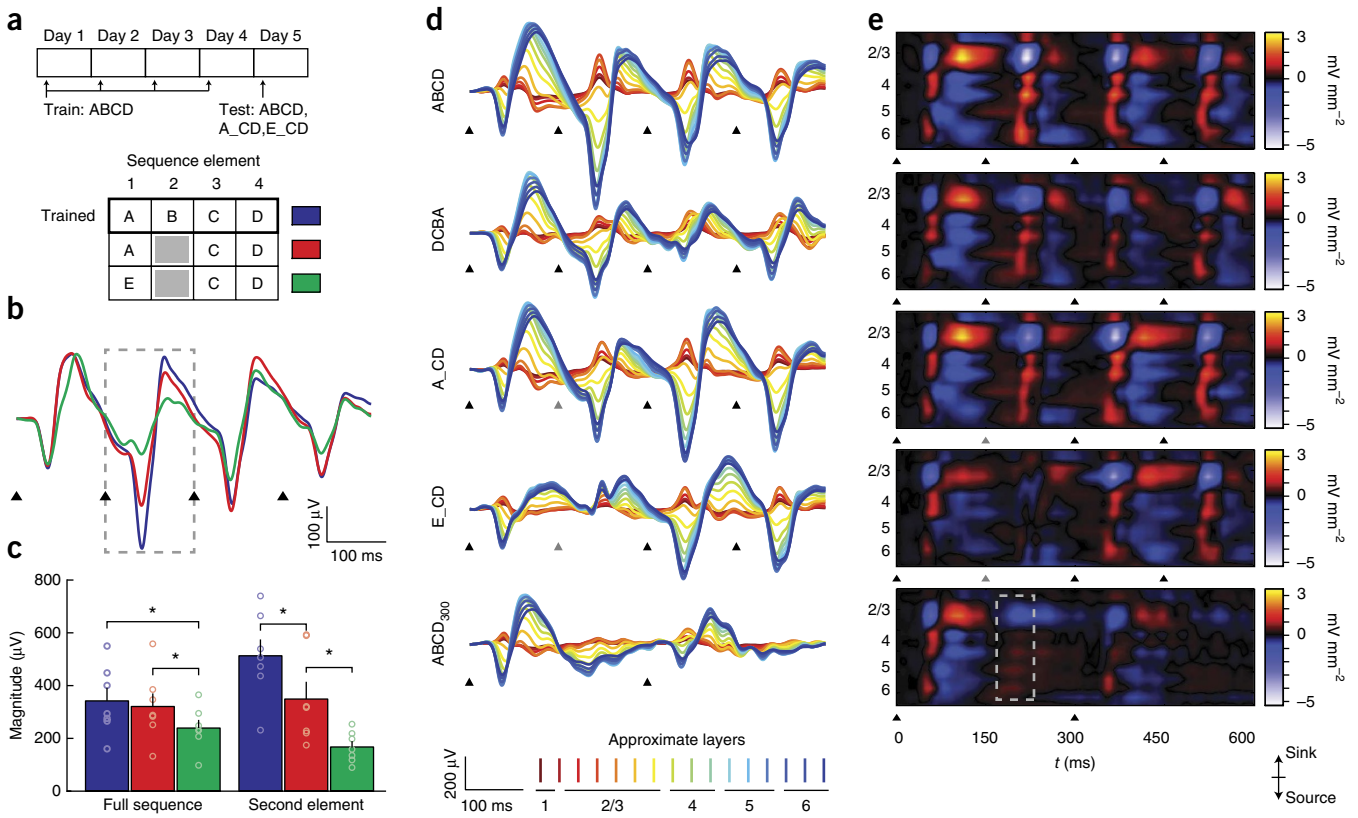

Figure 4 Learned sequence representations are predictive and involve multiple cortical layers. (**a**) Mice ($n = 7$) were trained with ABCD and tested with two sequences, A_CD and E_CD, where the second element was replaced with a gray screen. (**b**) When the omitted element was preceded by A (red), a response occurred in position 2 (marked by the dashed gray box) that was similar in form and latency to the response evoked when B was actually presented (blue). This predictive response was absent when the omitted element was preceded by the novel element E (green). (**c**) There was a significant effect of sequence on both the average magnitude across the four elements (left, one-way RM-ANOVA, $F_{2,6} = 12.186$, $P = 0.001$) and the response of the second element alone (right, one-way RM-ANOVA, $F_{2,6} = 31.597$, $P < 0.001$). Significant differences determined by *post hoc* analysis are indicated by brackets (full sequence: $t_6 = 4.675$, $P = 0.002$ and $t_6 = 3.711$, $P = 0.006$; Elmnt 2: $t_6 = 4.175$, $P = 0.003$ and $t_6 = 3.771$, $P = 0.003$). Error bars show s.e.m. $^*P < 0.05$. (**d**,**e**) Laminar field recordings (**d**) and CSD analysis (**e**) showed characteristic activation patterns evoked by trained and novel sequences. The DCBA sink-source pattern was similar to ABCD, but with smaller magnitudes. Activation patterns during omitted elements (marked gray triangles) closely matched those produced by real stimuli when the sequence was initiated with A, but not E. When each sequence element was held onscreen for twice the trained duration, activation patterns resembling those that would have occurred had element B been shown appeared at the expected time (highlighted with dashed gray box).

Restricting statistical analysis to the second element revealed that the anticipatory response following the predictive element A, while smaller than the response to the actual element B, was larger than the response following the nonpredictive element E. The data therefore suggest that a memory of stimulus element B is recalled in V1 when it is cued by stimulus element A.

To investigate how sequence evoked activity varies as a function of cortical depth, we implanted mice with linear arrays of 16 recording electrodes spanning the cortical layers from the surface to the white matter and trained them as before on the sequence ABCD (Online Methods). Sequence-driven VEPs spanned the cortical depth with positive-going responses in the superficial layers and relatively large negative-going responses in the middle and deeper layers. Both the familiar and novel sequences evoked clear responses, although those driven by the trained sequence were larger in all layers (**Fig. 4d**). Current source density (CSD) analysis, which estimates current source and sink locations and magnitudes by calculating the second spatial derivative of recorded voltages[13], was performed to determine the laminar distribution and temporal order of the transmembrane currents that produced the recorded field potentials. The earliest current sinks driven by the first sequence element occurred in thalamorecipient layers 4 and 6 approximately 50 ms after stimulus onset (**Fig. 4e**). These sinks then spread to layers 2/3 and were followed by deep layer sources. This characteristic activation pattern, with an additional initial superficial current sink, was repeated for subsequent sequence elements and was approximately the same, albeit with different magnitudes, for both ABCD and DCBA. CSD analysis also revealed the cued activation described above, with a clear differentiation between the omitted element response following the predictive element A and nonpredictive element E. Activation characteristic of the B response was also observed when A was held onscreen for twice the trained duration. The observation that anticipatory current sinks can be resolved at short latencies in the thalamorecipient layers suggests the possibility of anticipatory activation of thalamic relay neurons via corticothalamic feedback.

DISCUSSION

Spatiotemporal sequence learning has been reported in monkey area IT[14,15] and V4 (ref. 16), but never before in primary visual cortex. Several lines of evidence suggest that, in addition to being expressed in mouse V1, the underlying plasticity also occurs locally in V1. First, response potentiation driven by monocular experience did not transfer to the untrained eye (**Fig. 3**). This property requires that

plasticity occurs in a region where information from the two eyes is separable, consistent with a V1 locus. Second, spatiotemporal sequence potentiation (**Fig. 1**) and the cued anticipatory recall of an omitted stimulus (**Fig. 4**) were observed in short latency current sinks and spiking activity in thalamorecipient layer 4. The anticipatory activity is superficially similar to the 'omitted stimulus response' seen in the retina with periodic photic stimulation[17]; however, the orientation specificity suggests that the mechanism of sequence prediction requires participation of the central visual system and cannot be explained simply by entrainment of neural oscillators to the rhythm of preceding stimuli. Third, local microinfusion of the muscarinic acetylcholine receptor antagonist scopolamine directly into V1 blocked potentiation only in the infused hemisphere (**Fig. 3** and **Supplementary Fig. 6**). Although we cannot rule out the possibility that locally infused scopolamine spreads ipsilaterally outside of V1, we note that a previous study showed that infusion of an order of magnitude more scopolamine could be confined to the small volume of the rat amygdala[18]. A potential complication is that systemic and local scopolamine might reduce the amplitude of V1 VEPs. However, analysis revealed that small baseline VEPs did not preclude sequence potentiation in untreated mice (**Supplementary Fig. 6**). On the basis of these considerations, we propose that the mechanisms for both induction and expression of spatiotemporal sequence learning reside in V1.

Our findings are consistent with the hierarchical predictive coding hypothesis derived from studies in humans, which posits that the architecture of the cortex implements a prediction algorithm that anticipates incoming sensory stimuli[19–22], although they diverge from predictive coding models that assume NMDA receptor–mediated plasticity and predict that novel stimuli will drive larger responses than anticipated familiar stimuli[23]. Our discovery of a neurophysiological report of stimulus sequence prediction in an animal preparation that is amenable to invasive mechanistic studies makes possible the future refinement of such models to bring them into closer correspondence with the underlying biology.

Our data contribute to a growing body of knowledge that V1 is far more than a static feature detector. Previous work has shown that V1 responses in animals[8,12,24–26] and humans[10,27,28] can be rapidly, robustly and persistently modified by changes in the quality, trajectory and behavioral relevance of sensory stimulation. Collectively, these findings challenge the validity of visual processing models that assume V1 functions as a passive filter that conveys information to higher cortical areas where learning occurs[29]. The discovery of spatiotemporal sequence coding in V1 substantially expands the repertoire of plasticity expressed by primary sensory cortex and provides insight into how the brain learns to make intelligent guesses on the basis of past experience when confronted with limited sensory information. Hebbian plasticity in cortex, manifest as NMDA receptor–dependent long-term synaptic potentiation, readily accounts for SRP[9,30]. However, simple Hebbian principles do not predict the precise temporal dependence of the sequence representations that we observed[31], and the underlying mechanism is constrained by the observation that sequence learning occurs without a requirement for NMDA receptor activation. The cholinergic system's modulatory role in plasticity and attention is well documented[32], and the activity of cholinergic neurons in the basal forebrain projecting to V1 has been shown to enhance temporal[33] and spatial[34] discrimination in rodents, although it is surprising that it has such a specific role facilitating sequence response potentiation.

Cajal wrote in 1899 "that while there are very remarkable differences of organization of certain cortical areas, these points of difference do not go so far as to make impossible the reduction of the cortical structure to a general plan"[35]. Although the details of this structural plan continue to be debated[36], the idea that functionally disparate neocortical microcircuits use the same algorithmic 'primitives' remains attractive[20,37]. In this framework visual cortex is 'visual' not because it is specially suited to deconstruct the visual scene, but because it receives input from the eyes. It follows that many of the elementary operations underlying cognitive function in higher cortical regions may also exist in low-level sensory areas. Our results suggest that mouse V1 can be used to probe the mechanistic development of both learned sequence representations and temporal processing, which, we note, are impaired in several psychiatric and neurological disorders with genetic etiologies that can be modeled in mice[38–41].

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.P.G. and M.F.B contributed to study design and wrote the paper. J.P.G. performed all of the experiments.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Male C57BL/6 mice (Charles River Laboratories) were group housed with littermates (five mice per cage) on a 12-h light/dark cycle, and provided food and water *ad libitum*. Experimental and control groups were always selected randomly from littermates and yoked throughout the experiment. All experiments were performed during the light-cycle and animals were used for a single experiment only. All procedures were approved by the Institutional Animal Care and Use Committee of the Massachusetts Institute of Technology.

Electrode implantation. Mice were anesthetized with an intraperitoneal injection of 50 mg per kg ketamine and 10 mg per kg xylazine and prepared for chronic recording as described previously[8,9]. To facilitate head restraint, a steel headpost was affixed to the skull anterior to bregma using cyanoacrylate glue. Small (<0.5 mm) burr holes were drilled over binocular visual cortex (3 mm lateral from lambda) and either tungsten microelectrodes (for field recordings, FHC) or a custom-made recording bundle (for unit recordings, 20-µm outer diameter tungsten H-Formvar wire, California Fine Wire Company) was placed 450 µm below the cortical surface. For layer-specific analysis, a linear silicon probe (16 recording sites with 50-µm spacing, NeuroNexus) was placed with its shallowest electrode just below the cortical surface. In all cases, a reference electrode (silver wire, A-M systems) was placed below dura over prefrontal cortex. All electrodes were rigidly secured to the skull using cyanoacrylate glue. Dental cement was used to enclose exposed skull and electrodes in a protective head cap. Buprenex (0.1 mg per kg) was injected subcutaneously for postoperative pain amelioration. Except when otherwise noted (**Supplementary Fig. 7**), surgery was performed around postnatal day 30. Mice were monitored for signs of infection and allowed at least 24 h of recovery before habituation to the recording and restraint apparatus and were excluded from experiments before data collection only in the event of unsuccessful electrode implantation (that is, wrong location, bad grounding, etc.).

Stimulus presentation. Visual stimuli were generated using custom software written in Matlab (MathWorks) using the PsychToolbox extension (http://psychtoolbox.org/) to control stimulus drawing and timing. Sequences were constructed of four elements and an inter-sequence gray period. Each element consisted of a full-screen oriented high-contrast sinusoidal grating (0.5 cycles per deg). Sequence elements were separated by a minimum of 30 degrees and the order was restricted to prevent the appearance of rotation. For example, 30°-90°-60°-120° would be a valid sequence, but 30°-60°-90°-120° would not (note, this restriction did not apply to randomly generated sequences used when training the scrambled control group in **Fig. 1**). Different orientations were used with different experimental cohorts. Grating stimuli spanned the full range of monitor display values between black and white, with gamma correction to insure a linear gradient and constant total luminance in both gray-screen and patterned stimulus conditions. During experiments, animal handling consisted of placing each mouse (regardless of group membership, of which the investigator was aware) into the head-fixed presentation apparatus. Sequence presentation order was randomized using the Matlab pseudorandom number generator. Each sequence was presented 200 times per day in four groups of 50 presentations with each group separated by 30 s. There was also a 30-s interval between presentations of different sequences. In most experiments, all presentations of a single orientation were grouped together (for example, 200 presentations of the ABCD followed by 200 presentations of DCBA, etc.). In four mice in the variable timing experiment (**Fig. 2**), sequence presentation groupings were randomly interleaved (for example, 50 presentations of DCBA then 50 presentations of $ABCD_{300}$, etc.) during each of the four presentation cycles.

Data recording, analysis and presentation. All data was amplified and digitized using the Recorder-64 system (Plexon). Fields were recorded with 1-kHz sampling and a 200-Hz low-pass filter. Local field potential voltage traces in all figures show the average response of all mice in an experimental cohort. Spiking activity was digitized with 25-kHz sampling. Data was extracted from the binary storage files and analyzed using custom software written in C++ and Matlab. Sequence magnitude is defined as the average response magnitude, algorithmically scored peak-to-peak, for each of the four elements in a sequence (**Supplementary Fig. 1a**). VEPs associated with the SRP experiment were also scored peak to peak. Multi-unit spikes were isolated using Offline Sorter (Plexon).

Statistics. All statistics were performed using the SigmaPlot software package (Systat Software). Comparisons between groups were made using repeated-measures ANOVA (RM-ANOVA) with the *post hoc* Holm-Šidák test. Two-tailed, paired statistics were used for all in-group comparisons, sample sizes were based on effect size and confirmed via retrospective power calculations (minimizing the number of mice included in the experiments), normality and equal variance assumptions were confirmed before parametric analysis. In all figures, error bars indicate s.e.m. For field recordings, all mice were implanted with two recording electrodes in the left and right hemispheres. For the purpose of data presentation and statistics (except as noted for the monocular training experiment) the response of the two electrodes was averaged to produce a single response metric for each mouse and stats were always calculated using the number of mice as *n* rather than the number of recording electrodes.

CPP injections. Each training day, mice were treated with and intraperitoneal injection of either 10 mg per kg CPP (Sigma) or saline at least 30 min before sequence presentation. Recordings on the fifth day occurred at least 24 h after the last injection (on day 4). Mice injected with CPP were observed to be somewhat listless when placed in the recording apparatus, but no other obvious behavior changes were noted. It has previously been reported that CPP blocks SRP induction. As a positive control, after the fifth day of sequence presentations, all mice were allowed three full days of rest and recovery and were then regrouped into new CPP and control groups. The mice were then exposed to the SRP induction protocol described previously[8,9]. As during the sequence training, intraperitoneal injections were administered at least 30 min before stimulus presentation over the first 4 d of SRP induction. The same lots of CPP used during sequence training were also used for the positive control experiment.

Scopolamine injections. For the systemic scopolamine experiment, mice were treated with an intraperitoneal injection of either 3 mg per kg scopolamine hydrobromide (Tocris) or saline 25 min before sequence presentation on experimental days 1–4. To allow sufficient time for drug washout, recordings on the 5th day occurred at least 24 h after the last injection on day 4. For the local scopolamine experiment, 26 gauge bilateral guide cannulae (Plastics One) were implanted lateral to recording electrodes. The guide cannulae were angled at 45° relative to the recording electrode and positioned slightly below the cortical surface. Guides were affixed to the skull with cyanoacrylate and encased in the dental cement head cap and dummy cannulae were installed. After several days of recovery and habituation to the head-fixed restraints, the dummy cannulae were removed and infusion cannulae were lowered into the guides (the tip of the infusion cannulae were within 1 mm of the recording electrode tip; see schematic in **Supplementary Fig. 6a**). A Nanoject II (Warner Instruments), under the remote control of custom software, was used to infuse 1 µl of vehicle (NaCl) into the right hemisphere and 1 µl of scopolamine (0.6 mg ml^{-1}, 0.6 µg of the drug) in 2.3-nl pulses evenly spread over 10 min (6 µl h^{-1} infusion rate) immediately before stimulus presentation on experimental days 1 and 2. All mice received both drug and vehicle infusions, and all mice that evidenced clear visual responsiveness in both hemispheres (that is, no trauma caused by infusion) were included in the study. Local treatment was performed for only 2 d to minimize inherent cortical trauma associated with repeated insertions and removals of the infusion and dummy cannulae; training continued for 2 additional days after drug washout to verify that V1 was rendered aplastic as a result of drug treatment and not an unintentional cortical lesion.

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Erratum: Learned spatiotemporal sequence recognition and prediction in primary visual cortex

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In the version of this article initially published online, the asterisk was defined in the Figure 2 legend as $P > 0.05$ rather than $P < 0.05$, and the grant number in the Acknowledgments was given as NIMH:1K99MH099654-01 rather than K99MH099654. The errors have been corrected for the print, PDF and HTML versions of this article.