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Learned spatiotemporal sequence recognition and prediction in primary visual cortex

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Abstract

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 causes evoked response potentiation in mouse V1 that is highly specific for stimulus order and timing. Remarkably, after V1 is trained to recognize a sequence, cortical activity regenerates the full sequence even when individual stimulus elements are omitted. This novel neurophysiological report of sequence learning advances the understanding of how the brain makes “intelligent guesses” based on limited information to form visual percepts and suggests that it is possible to study the mechanistic basis of this high–level cognitive ability by studying low–level sensory systems.

Introduction

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 implicate 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. In this work we show that repeated exposure to sequential visual stimuli over multiple days is sufficient to encode

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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 experiments.

Author Information

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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, mice were assigned 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). Visual evoked potentials (VEPs) recorded in binocular layer 4 (see methods) reveal that ABCD elicits a dramatically larger response after training than DCBA in the experimental group (Fig. 1c), but not in the control animals which, due to the randomized nature of their training, had no reason to expect the sequence elements to appear in any particular order (see also Supplementary Fig. 1). Thus, repeated exposure to a visual sequence is sufficient to encode a neural representation of that sequence.

The same animals 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 is very similar to that seen with the trained timing but responses to subsequent sequence elements are clearly smaller (Fig. 1d). Comparing the average sequence evoked response magnitudes (Fig. 1e) confirms what is qualitatively obvious from the VEP waveforms; the training regime has a highly significant effect on sequence–specific response potentiation. Within the experimental group, serial order and timing both significantly influence evoked response magnitudes. The effects of reordering are not specific to sequence reversal; other tested sequence permutations also cause decreased response magnitudes similar to that shown in Fig. 1 for DCBA (see, e.g., Supplementary Fig. 4). The data suggest that any manipulation of sequence content after training disrupts the response magnitude. In contrast, there is no significant effect of sequence order or timing within the control group, although there is a magnitude increase relative to day 1 (Fig. 1f). Sequence–specific effects are also visible in cortical spiking activity, as demonstrated in Fig. 1g where the trained sequence drives higher multi–unit spike rates than does a novel sequence (see also 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, a cohort of animals was trained using a protocol where the four sequence elements were held on–screen with alternating short and long durations (Fig. 2a). After training, the animals 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 is subtle, the cortical response to the trained sequence presented with familiar timing is significantly larger than the response to either a reordered or re–timed sequence (Fig. 2b,c). That this specificity is a consequence of training

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

One striking aspect of this plasticity is the small amount of sensory experience necessary to markedly potentiate the cortical response. The largest increase in sequence magnitude occurs after the first training day (Fig. 1f), at which point each mouse has seen the sequence only 200 times (corresponding to 2 minutes of active visual stimulation). This rapid change is similar to a form of cortical plasticity called SRP (stimulus selective response potentiation) that is characterized by a daily increase in VEP magnitude following repeated exposure to a sinusoidal grating[8]. This increase is stimulus specific and involves local plasticity within V1[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 if sequence learning shares this property, mice were trained with sequence presentation restricted to one eye and tested with monocular presentation to both eyes (Fig. 3a). While there was a clear and statistically significant 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 (LTP), 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, mice were systemically treated with either the NMDA receptor antagonist CPP (3–(2–carboxypiperazin–4yl)propyl–1–phosphonic acid, 10mg/kg i.p.) or saline prior to sequence presentation on each training day. Surprisingly, there was no significant effect of CPP treatment relative to vehicle control in the expression of sequence learning (Fig. 3d). To confirm the effectiveness of the CPP in blocking NMDA receptors under our experimental conditions, the same animals were subsequently reassigned after a 3–day washout period into new CPP and vehicle control groups and exposed to the SRP induction protocol. We found that the same CPP prevented induction of SRP (Fig. 3d, 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, mice were treated systemically 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, 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, Supplementary Fig. 6). These results demonstrate involvement of the cortical cholinergic system in the mechanisms underlying sequence learning.

It is clear from the data above that the mice learn neural representations of the familiar visual sequence, but it is not clear whether this representation is sufficient to reproduce the

sequence absent external stimulation. To test this possibility, a cohort of animals was trained 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, while 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 is small and consists solely of a late positive–going bump (Fig. 4b). By contrast, the response following A shares a similar morphology and timing with the response actually evoked by the element B: the average latency to peak negativity during the second element is almost identical when the response is driven by element B (60.8 ±2.6 ms) or anticipatory based on the presence of element A (60.3 ±3.5 ms). There is no statistical difference in the average sequence magnitude between ABCD and A_CD, but both are significantly larger than E_CD (Fig. 4c). Restricting statistical analysis to the second element reveals that the anticipatory response following the predictive element A, while smaller than the response to the actual element B, is larger than the response following the non–predictive 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, mice were implanted with linear arrays of 16 recording electrodes spanning the cortical layers from surface to the white matter and trained as before on the sequence ABCD (see methods). Sequence driven VEPs span 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 evoke clear responses although those driven by the trained sequence are larger in all layers (Fig. 4d). Current source density (CSD) analysis, which estimates current source and sink locations and magnitudes by calculating the 2nd 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 occur in thalamorecipient layers 4 and 6 approximately 50 ms after stimulus onset (Fig. 4e). These sinks then spread to layers 2/3 and are followed by deep layer sources. This characteristic activation pattern, with an additional initial superficial current sink, is repeated for subsequent sequence elements and is approximately the same, albeit with different magnitudes, for both ABCD and DCBA. CSD analysis also demonstrates the cued activation described above, with a clear differentiation between the omitted element response following the predictive element A and non–predictive element E. Activation characteristic of the B response is also observed when A is 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 [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 within V1. First,

response potentiation driven by monocular experience does not transfer to the untrained eye (see 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 (see Fig. 1) and the cued anticipatory recall of an omitted stimulus (see Fig. 4) are 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 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 blocks potentiation only in the infused hemisphere (see 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 reduce the amplitude of V1 VEPs. However, analysis shows that small baseline VEPs do not preclude sequence potentiation in untreated mice (Supplementary Fig. 6). Based on these considerations, we propose that the mechanisms for both induction and expression of spatiotemporal sequence learning reside within 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 which 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 based on 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 observed in our study[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 plays 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 the exciting possibility 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

Animals

Male C57BL/6 mice (Charles River Laboratories) were group housed with littermates (5 mice per cage) on a 12–hour 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/kg ketamine and 10 mg/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 (< 0.5mm) 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) were 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 within a protective head cap. Buprenex (0.1 mg/kg) was injected subcutaneously for postoperative pain amelioration. Except when otherwise noted (Supplementary Fig. 7), surgery was performed around post–natal day 30. Animals were monitored for signs of infection and allowed at least 24 hours 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 (i.e. wrong location, bad grounding, etc.).

Stimulus Presentation

Visual stimuli were generated using custom software written in Matlab (MathWorks) utilizing the PsychToolbox extension (http://psychtoolbox.org) to control stimulus drawing and timing. Sequences were constructed of four elements and a inter–sequence gray period. Each element consisted of a full–screen oriented high–contrast sinusoidal grating (0.5 cy/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 sec. There was also a 30 second interval between presentations of different sequences. In most experiments, all presentations of a single orientation were grouped together (e.g. 200 presentations of the ABCD followed by 200 presentations of DCBA, etc.). In four animals in the variable timing experiment (Fig. 2) sequence presentation groupings were randomly interleaved (e.g. 50 presentations of DCBA then 50 presentations of $ABCD_{300}$ etc.) during each of the 4 presentation cycles.

Data Recording, Analysis and Presentation

All data was amplified and digitized using the Recorder–64 system (Plexon Inc.). 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 animals 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 (see Supplementary Fig. 1a). VEPs associated with the SRP experiment were also scored peak to peak. Multi–unit spikes were isolated using Offline Sorter (Plexon Inc.).

Statistics

All statistics were performed using the SigmaPlot software package (Systat Software Inc). 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 prior to 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/kg CPP (Sigma) or saline at least 30 minutes before sequence presentation. Recordings on the 5th day occurred at least 24 hours 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 5th 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, i.p. injections were administered at least 30 minutes before stimulus presentation over the first 4 days or 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/kg scopolamine hydrobromide (Tocris) or saline 25 minutes before sequence presentation on experimental days 1 through 4. To allow sufficient time for drug washout, recordings on the 5th day occurred at least 24 hours 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, 0.6 μg of the drug) in 2.3 nL pulses evenly spread over 10 minutes (6 μL per hour infusion rate) immediately prior to 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 (i.e. no trauma caused by infusion) were included in the study. Local treatment was performed for only 2 days 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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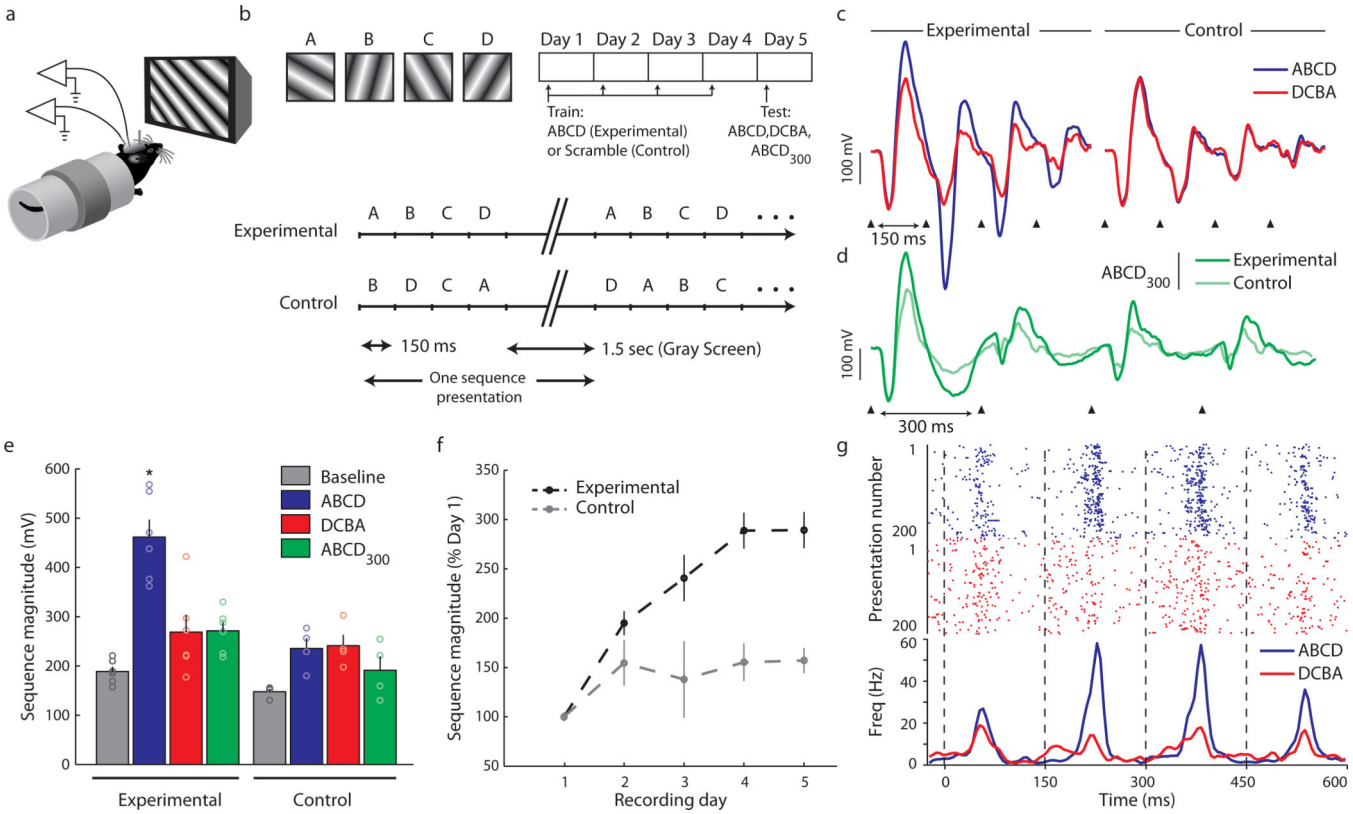

Fig. 1.

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) was shown 200 presentations of the sequence ABCD (where each letter indicates a uniquely oriented sinusoidal grating) and the control group (n=4) 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 animals were tested on the 5th 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 5th day show that ABCD drives larger responses (blue) than DCBA (red) in the experimental animals, while there is no differential responses in control animals. Voltage traces represent the average response of all animals in each group and triangles mark the onset of each sequence element. (**d**) $ABCD_{300}$ drives relatively small responses in both groups. (**e**) Training regime has a significant effect on sequence response magnitude (quantified as the average peak–to–peak response to each of the four elements, see Supplementary Fig. 1) potentiation (2–way RM ANOVA, $F_{(1,8)}$=22.560, P=0.001). There is a significant interaction (2–way RM–ANOVA, $F_{(1,8)}$=6.638, P=0.008) between sequence and experimental group on day 5 and post–hoc analysis shows that the response to ABCD is significantly larger than either DCBA ($t_{(5)}$=5.738, P=0.002) or $ABCD_{300}$ ($t_{(5)}$=4.923, P=0.005). Sequence effects are not significant within the control group. Error bars show s.e.m. (**f**) Potentiation time course. (**g**) Sequence effects are evident in spiking neural activity. In this representative example, ABCD drives higher peak firing rates than DCBA (multi–unit spike rasters above peristimulus time histograms, dashed lines indicate element onset times).

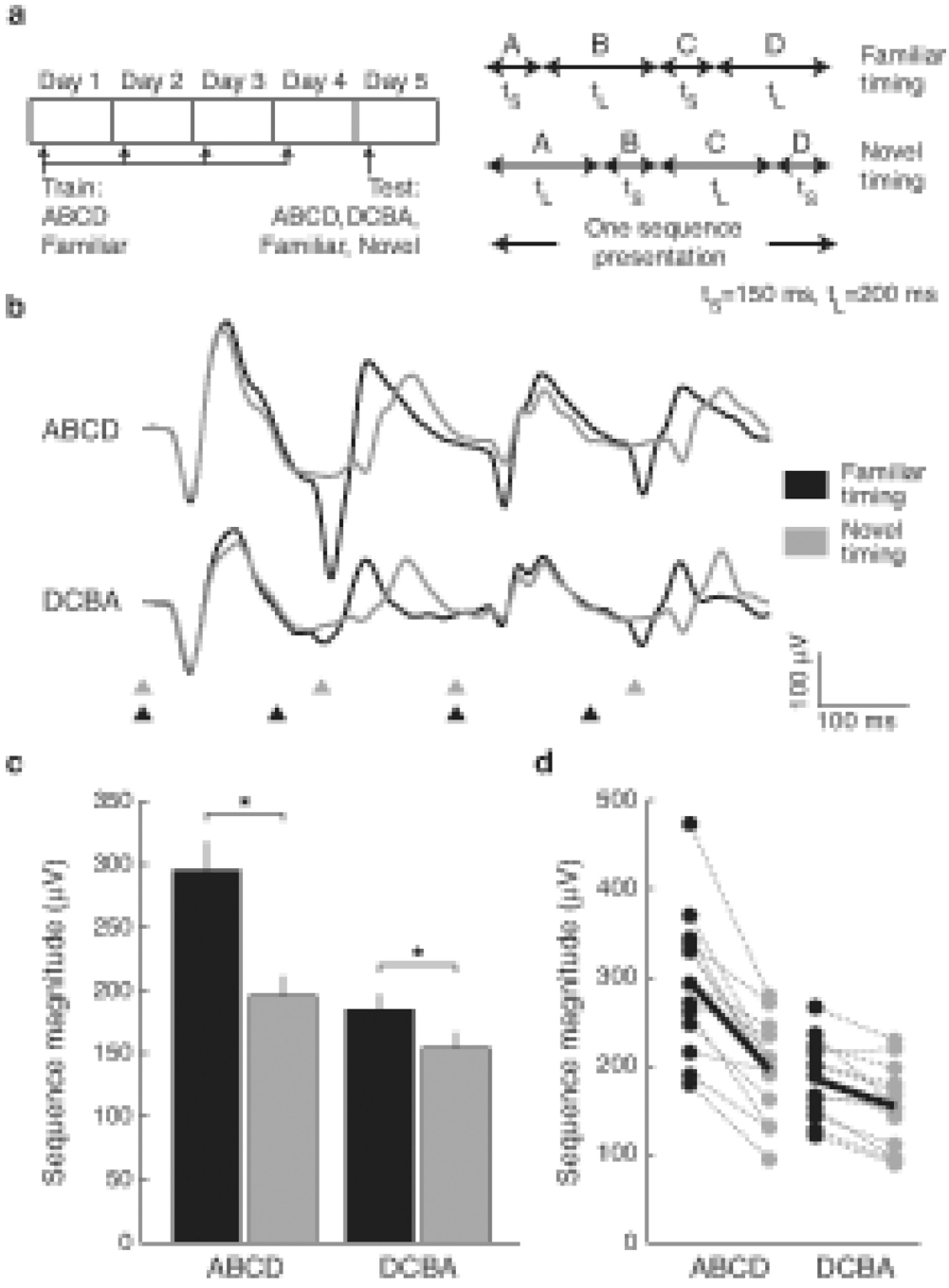

Fig. 2.

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 occur when the trained sequence is presented with the trained timing (top). Timing makes little apparent difference when a novel sequence is shown (bottom). (**c**) There is a significant interaction between sequence order and timing (2–way RM ANOVA, $F_{(1,12)}$=22.925, P<0.001). Post–hoc analysis shows

the response to ABCD with trained timing is significantly larger than ABCD with novel timing ($t_{(12)}$=8.760, P<0.001). There is also a small effect of timing within DCBA ($t_{(12)}$=2.722, P=0.012). Error bars show s.e.m. (**d**) The relative effect of timing as a function of sequence is demonstrated by paired–response plots (dashed lines connect responses for single animals, black indicates the mean).

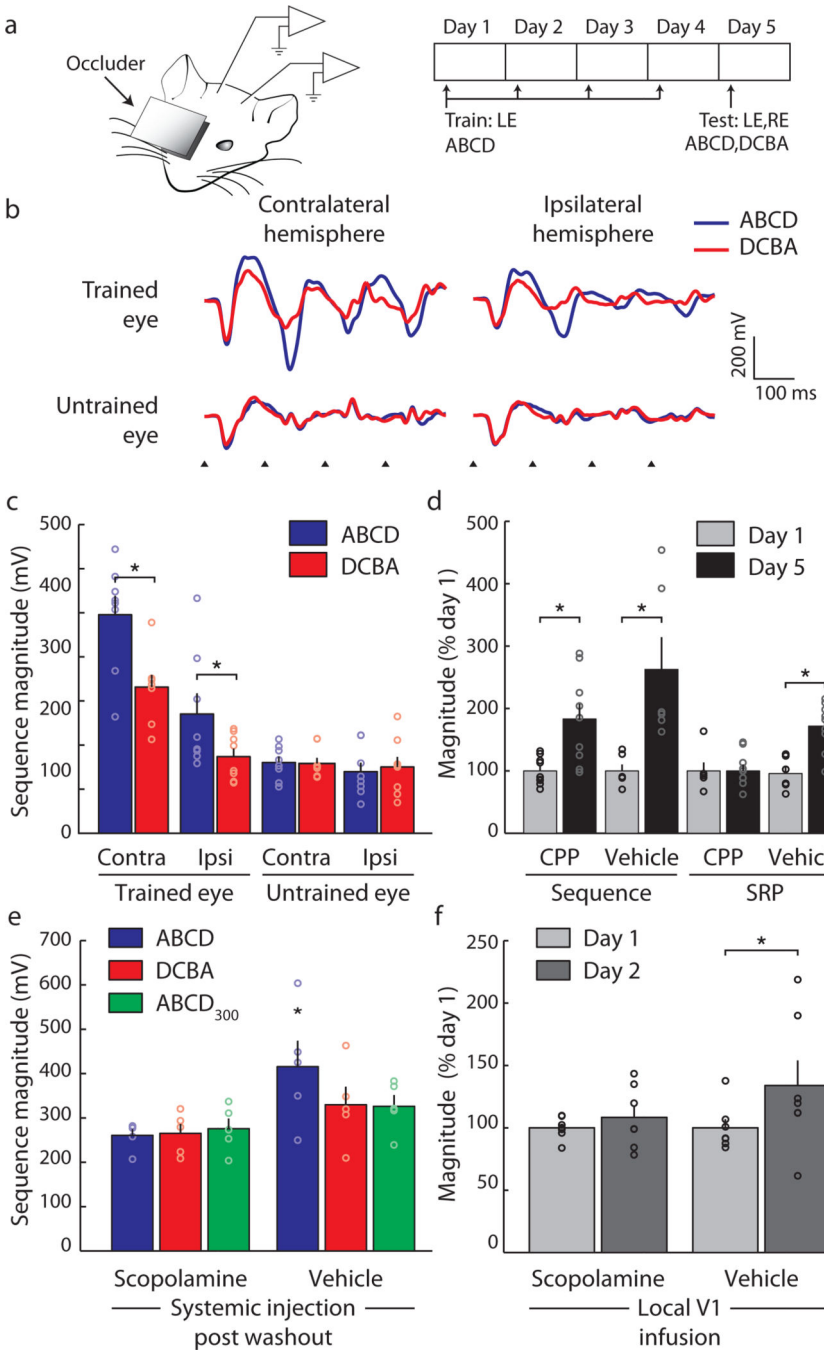

Fig. 3.

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 5th 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 is a significant interaction between viewing eye and sequence in both hemispheres (2–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 is 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 prior to stimulus presentation during training) has no significant effect on sequence potentiation compared to vehicle (n=6) and response potentiation is 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 is a significant interaction between treatment and SRP recording session (2–way RM–ANOVA, $F_{(1,13)}$=42.210, P<0.001) and potentiation is significant only in the vehicle control group ($t_{(8)}$=9.692, P<0.001). (**e)** Muscarinic receptor antagonism during training blocks sequence potentiation. There is a significant day 5 interaction between treatment and sequence in scopolamine (n=5) and vehicle (n=5) treated mice (2–way RM ANOVA, $F_{(1,8)}$=5.827, P=0.013) and ABCD is 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) blocks potentiation relative to the opposite vehicle treated hemisphere (2–way RM ANOVA, $F_{(1,6)}$=30.189, P=0.002). Error bars show s.e.m.

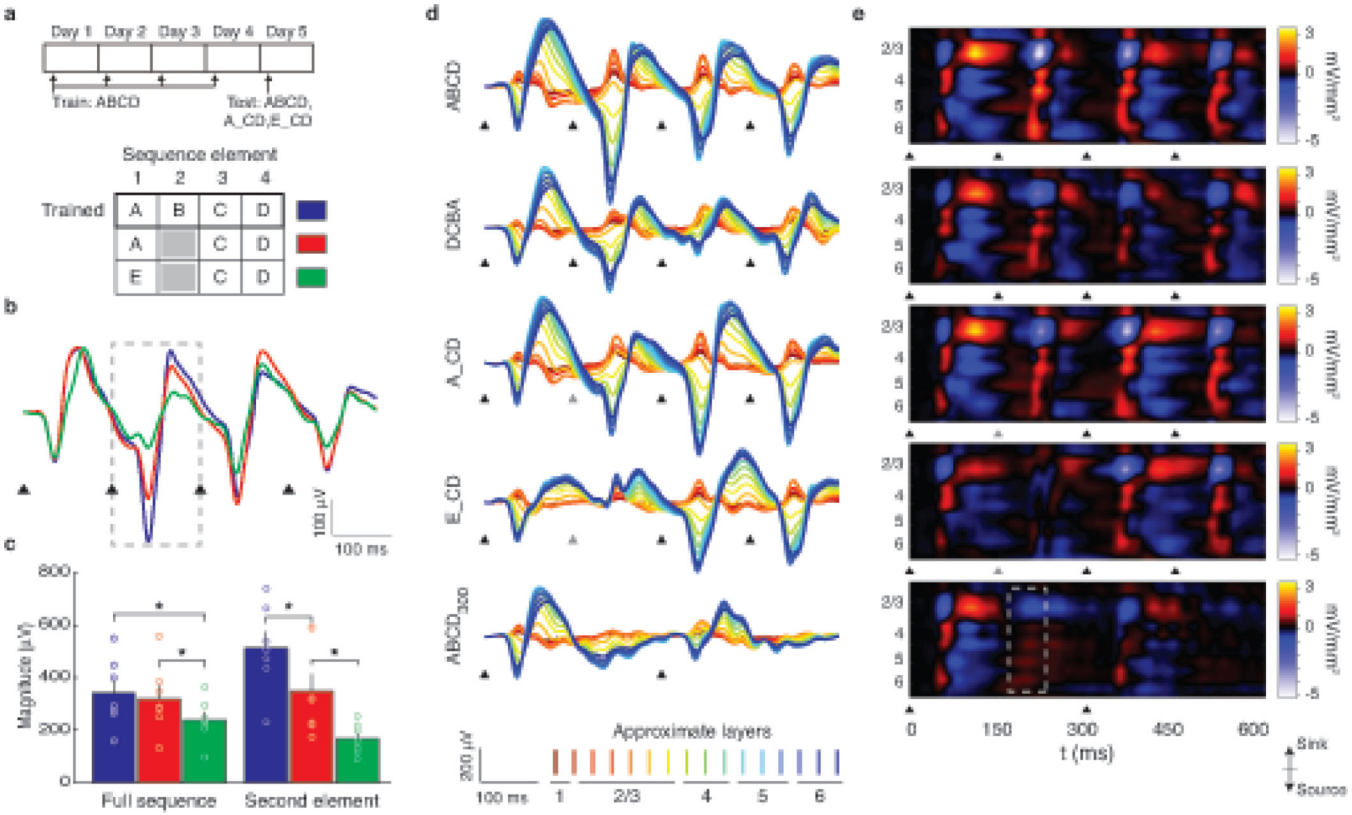

Fig. 4.

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 is preceded by A (red), a response occurs in position 2 (marked by the dashed gray box) that is similar in form and latency to the response evoked when B is actually presented (blue). This predictive response is absent when the omitted element is preceded by the novel element E (green). (**c**) There is a significant effect of sequence on both the average magnitude across the four elements (left, 1–way RM ANOVA, $F_{(2,6)}$=12.186, P=0.001) and the response of the second element alone (right, 1–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. Laminar field recordings (**d**) and CSD analysis (**e**) show characteristic activation patterns evoked by trained and novel sequences. The DCBA sink–source pattern is similar to ABCD but with smaller magnitudes. Activation patterns during omitted elements (marked gray triangles) closely match those produced by real stimuli when the sequence is initiated with A but not E. When each sequence element is held onscreen for twice the trained duration, activation patterns resembling those that would have occurred had element B been shown appear at the expected time (highlighted with dashed gray box).