Dissociating instructive from permissive roles of brain circuits with reversible neural activity manipulations
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ABSTRACT
Neuroscientists rely on targeted perturbations and lesions to causally map functions in the brain1. Yet, since the brain is highly interconnected, manipulation of one area can impact behavior through indirect effects on many other brain regions, complicating the interpretation of such results2,3. On the other hand, the often-observed recovery of behavior performance after lesion can cast doubt on whether the lesioned area was ever directly involved4,5. Recent studies have highlighted how the results of acute and irreversible inactivation can directly conflict4-6, making it unclear whether a brain area is instructive or merely permissive in a specific brain function. To overcome this challenge, we developed a three-stage optogenetic approach which leverages the ability to precisely control the temporal period of regional inactivation with either brief or sustained illumination. Using a visual detection task, we found that acute optogenetic inactivation of the primary visual cortex (V1) suppressed task performance if cortical inactivation was intermittent across trials within each behavioral session. However, when we inactivated V1 for entire behavioral sessions, animals quickly recovered performance in just one to two days. Most importantly, after returning these recovered animals to intermittent cortical inactivation, they quickly reverted to failing on optogenetic inactivation trials. These data support a revised model where the cortex is the default circuit that instructs perceptual performance in basic sensory tasks. More generally, this novel, temporally controllable optogenetic perturbation paradigm can be broadly applied to brain circuits and specific cell types to assess whether they are instructive or merely permissive in a brain function or behavior.

Main
Recent work has demonstrated that acute inactivation experiments can lead to potentially erroneous conclusions about the causal role of brain areas, casting doubt on a major avenue by which neuroscientists study the brain. For example, acute inactivation of forebrain motor or sensory areas in animals can lead to profound behavioral deficits, but animals with permanent lesions are either unaffected or can recover performance within just a few days4-6. In one striking example, the behavioral deficit during acute inactivation of one forebrain region of the songbird was instead due to an indirect effect on activity in a downstream area4. Once the downstream area adjusted its activity, presumably homeostatically, the animal’s behavior recovered, demonstrating that the lesioned area was merely permissive in the behavior rather than instructive (Fig. 1a). In a second study, acutely optogenetically silencing the barrel cortex
impaired performance on a whisker-dependent task, but an irreversible lesion led to rapid (~2 day) recovery that was experience dependent\textsuperscript{5}. This latter study proposed two explanations for their results: either barrel cortex was redundant with another area for the task\textsuperscript{7} (Fig. 1b), or its inactivation transiently disrupted the instructive area (as in the case of Otchy et. al. 2015). Both studies demonstrate that acute manipulations alone may lead to erroneous or ambiguous conclusions about the instructive role of a brain area, such as the cortex, in a specific behavior. Yet acute, rapid, and reversible manipulations, such as with optogenetics\textsuperscript{8-10}, have become central to the investigation of the brain and behavior. Compared to irreversible lesions, acute manipulations avoid potential indirect neurodegenerative effects\textsuperscript{2,11} (diaschisis), permit rigorously interleaved control trials, and can address the precise timescales for when neural activity in specific regions is necessary for behavioral output\textsuperscript{12,13}. However, the current inability to properly interpret the results of such acute manipulations is a major roadblock in neuroscience. Thus, we sought to develop a new paradigm that can resolve the critical ambiguities associated with acute perturbations.

**A flexible optogenetic paradigm for probing instructive roles of brain areas**

We reasoned that we could overcome the ambiguities of existing perturbation approaches by employing a flexible optogenetic inactivation strategy in which one could acutely or chronically silence a brain area. With such an approach, one could first measure the acute effects of inactivation of a brain area on a behavior, then chronically silence it to mimic an irreversible lesion, and finally return the brain area to acute inactivation (Fig. 1c). In cases where acute inactivation impairs behavior, animals might recover behavioral function during chronic optogenetic silencing, as they often do after irreversible lesions. In such cases, one can resolve the apparent conflict by finally returning to acute, intermittent silencing in the recovered animals. This last test is critically informative: if recovered animals no longer showed any effect of acute silencing, one could conclude that the brain region only played a permissive role in the initial condition, such as by providing a tonic level of drive that supports the computation but does not instruct it (Fig. 1c). The supporting drive would no longer be necessary because during the chronic silencing phase the brain homeostatically compensated for its loss. Conversely, if in recovered animals a return to acute silencing re-instated the behavioral deficit, one may conclude that the brain region instructs the behavior, directly contributing to the computational process in a critical manner.

To develop and leverage the proposed optogenetic strategy, we focused on the primary visual cortex (V1), which is among the best studied areas in the mammalian brain, but whose role in different aspects of visual perception remains in doubt. Prior work has shown that inactivating or lesioning V1 results in severely compromised visual function\textsuperscript{3,12,14-16}, including in contrast sensitivity\textsuperscript{17-19}, but that visual performance can recover spontaneously, with further training, or sometimes even with lesions to other brain areas\textsuperscript{20-22}. We therefore sought to clarify the role of V1 in basic visual functions in mice, using a combination of acute and sustained inactivation protocols.

To reversibly silence neural activity in V1, we virally expressed the potent optogenetic silencer, GtACR2\textsuperscript{23}, through intracortical injection (via the viral vector AAV-CamKII-somBiPOLES\textsuperscript{24}, see Methods). To test the efficacy of our silencing under both intermittent and sustained illumination conditions, we recorded neural activity with multielectrode arrays across the layers
of V1 while illuminating the cortex briefly on every third trial, and then persistently for 40 continuous minutes (2mW/mm², 455 nm) (Fig. 2a-c). In GtACR2-expressing mice, brief illumination resulted in immediate and strong suppression of both spontaneous and visually evoked neural activity (Fig. 2c-e; 83±4% reduction at 100% contrast, p<0.05, Wilcoxon signed rank test). Next, we asked if uninterrupted sustained illumination could effectively silence cortical activity for the entire duration of the illumination, and whether neural activity would rapidly recover after cessation of illumination to rule out potential photo-toxic effects. Indeed, during persistent illumination, the mean firing rate averaged over all recorded units was suppressed by 76±4% (at 100% contrast, p<0.05, Wilcoxon sign-rank test, Fig. 2d,e). After the cessation of sustained illumination, the mean activity was indistinguishable from baseline levels (p=0.53, Wilcoxon signed rank test, Fig. 2e), arguing against potential issues with prolonged illumination at this light level, 2mW/mm², or that prolonged flux of ionic currents might lead to cellular alterations. These experiments confirm the efficacy, stability, and reversibility of these optogenetic manipulations.

Equipped with this optogenetic approach, we trained GtACR2-expressing mice on a visual contrast detection task (Fig. 3a). Animals learned to detect a small drifting grating of varying contrasts and respond to visual stimuli by licking for reward. After mice achieved stable performance (see Methods), we illuminated V1 on 33% of trials and found that this severely impaired performance (Fig. 3c-e3c-f, Fig. S1; intermittent silencing day 1 (I1), I1_off: 62±3% hit, I1_on 41±3% hit; p=0.00011; paired t-test, n=9 mice), consistent with prior results in related visual tasks. Similar illumination in mice not expressing GtACR2, even at double the light power, did not affect performance (Fig. 3c3d, no light: 65±4% hit, light: 65±3% hit; p = 1.00, Paired t-test, n=2 mice), controlling for any direct effects of the illumination on brain activity or visual perception. This is consistent with our previous findings that illumination alone does not impact performance in this task.

On their own, these results demonstrate that activity in V1 is acutely required for normal performance on the contrast detection task. However, it does not dissociate whether this activity is instructive in the task or simply permissive, as it may cause indirect effects on downstream areas that may instead be instructive (e.g., superior colliculus, dorsal striatum, motor cortex, etc.). Thus, we next asked whether animals might spontaneously recover performance if we repeated this intermittent silencing for several days. We observed continued impairment and no recovery, even over six consecutive days of intermittent silencing (Fig. 3d; intermittent silencing days, light on, effect of day: p=0.45, rmANOVA). This demonstrates that if V1 is only intermittently silenced, even over many days, animals will not recover performance on the contrast detection task during V1 silencing. Although the lack of recovery supports the notion that V1 is required for this task, it still does not dissociate an instructive from a permissive role.

Sustained optogenetic inactivation reveals rapid behavioral recovery
To better mimic the permanent lesion that other studies have used, we then illuminated V1 for the entire duration of each behavioral session. Notably, this avoids irreversibly lesioning the tissue so that silencing can be switched off in future sessions. Moreover, it avoids silencing V1 outside the task, such as in the home cage, which might on its own cause local or downstream circuit reorganizations.
We applied this persistent illumination scheme to the same mice as above to test if mice might recover performance over time despite continued suppression of V1. Between the last day of intermittent silencing and the first day of persistent silencing, we included a ‘transition day’ in which the first half of the session employed intermittent silencing and the second half employed persistent silencing to look for any potential fast changes in the role of V1 in behavior. After this day, the optogenetic light was on for the entirety of each session for six consecutive days session (of persistent silencing, 57±13 minutes; mean ± SD). During the transition day, we observed no significant recovery (Fig. S2a; Transition Day 1; Intermittent light on (I_on): 48±4%, Intermittent light off (I_off): 67±3%, Persistent Light (P): 47±4%; p<0.0001 rmANOVA, with post-hoc Tukey HSD; Pairwise comparisons, T1: I_on vs PP: p=0.9, I_on vs I_off: p=0.0025, I_off vs P: p=0.0019; n=9 mice). However, on the first full day of persistent silencing there was substantial recovery of performance (Fig. 3f-h; I6_on: 38±4% P1: 52±4%, p= 0.0012, paired t-test, n=9 mice). This demonstrates that mice can recover much of their ability to solve the task even in the continued absence of V1 activity. These data further show that whatever plasticity is required for the performance improvement must develop over more than one behavioral session. Furthermore, we did not see improvement within a session; performance at the start of a new session was not worse than the performance of the end of the prior day’s session (Fig. S2c, first fifth of session: 52±3%, last fifth of prior session: 50±2%; p=0.311, paired t-test; n=9 mice), suggesting that whatever process occurs does not evolve within a session, but over one to two days.

To better characterize the dynamics of the changes in contrast sensitivity between intermittent and persistent silencing, we fit psychometric functions to the behavioral data and calculated the percent contrast for half maximal performance (C50). During intermittent silencing, C50 increased nearly three-fold, changing from 6±1%, to 17±3%. However, during persistent inactivation, C50 recovered to the original control levels 7±1% (Fig. 3f,h, S1, p<0.00001, 1-way rmANCOVA, with post-hoc Tukey HSD; Pairwise comparisons: I_on vs I_off: p=0.0002, I_on vs P: p=0.0006, I_off vs P: p=0.9231, n=9 mice).

These results imply that V1 is not absolutely required for performance on the task, and that following sustained inactivation, other brain circuits can instruct appropriate behavior independently of V1 with a short timescale of recovery. Alternatively, it remains possible that mice can adapt to using the small remaining amount of activity in V1 to solve the task. If the latter were true, then animals should show no recovery of behavioral performance following a mechanical lesion. To distinguish between these possibilities, we mechanically removed V1 in a different set of trained mice and compared their performance before and after lesion (Fig. S3a-d). Consistent with the results of the chronic optogenetic suppression experiments, mechanically lesioned mice exhibited substantial recovery in performance, starting within two days of surgery (Fig. S3d, post-lesion days 0-10: p=0.0016, rmANOVA, n=7 mice; Fig S3b-c; Day 0 Post-Lesion: 22±4%, Day 2 Post Lesion: 42±4%; p=0.0018, Paired t-test; n=8 mice). As a control, in a separate group of mice we lesioned the somatosensory cortex (S1) and tracked performance on the visual task. S1 lesion caused only a much smaller drop in performance, even on the day of surgery, and performance quickly recovered to pre-lesion levels (V1, Day 0: 22±4%; S1, Day 0: 50±5%; p=0.0042; t-test; n=11 mice, 8 V1 lesion and 3 S1 lesion). These results demonstrate that other brain regions can compensate for the absence of V1 in the visual detection task16.

Return to intermittent suppression identifies an instructive role of V1 in visual perception
The data above show that the mice can adapt to solve the task when V1 is very strongly suppressed or even entirely ablated by exploiting alternative brain circuits. The next key question is whether V1 or an alternative area is the default circuit with which the animals normally solve the task. We reasoned that we could address this by reverting the persistently silenced and behaviorally recovered mice to intermittent silencing. Since mice performed well on the task during persistent silencing, we expected that reverting these animals to intermittent silencing would no longer reveal any effects of acutely inactivating V1. Indeed, it would be quite astonishing if on one day V1 activity was largely unnecessary, but on a subsequent day, V1 activity would again become critical. If intermittent silencing of V1 no longer had a behavioral effect, this would imply that the cortex is not the default circuit instructing performance on this visual task and may be merely permissive. Conversely, if mice instead return to failing on intermittent V1 silencing trials, this would imply that V1 is not merely permissive but is the default circuit that actively instructs perceptual behavior.

To discriminate between these possibilities, we reverted the persistently silenced animals to intermittent silencing the cortex only on 303% of trials as before (intermittent repeat, IR). To resolve any fast changes in behavioral performance, we started with a second transition day composed of persistent silencing in the first half and intermittent silencing in the second half. Then we began the intermittent repeat block of six sessions of intermittent silencing alone. Surprisingly, we found that during the first day of intermittent repeat (IR1) block, all mice returned to showing a dramatic reduction in performance during intermittent silencing trials (Fig. 3i-k, P6: 58±4%, IR1on: 45±4%, p=0.0039, paired t-test, n=7 mice). The performance in intermittent silencing trials was the same before and after the behavioral recovery during persistent illumination (Fig 3i; Ion: 40±3%, IRon: intermittent silencing block 42±3%, intermittent repeat silencing block p=0.50, paired t-test, n=9 mice). This reversion to requiring V1 activity data is consistent with V1 playing an instructive rather than a merely permissive role in the visual task.

Again, to address how fast this reversion to relying on V1 activity might occur, we examined the transition session between the P and IR blocks. During the intermittent half of the session, there was already a significant difference between light off and light on (Fig. S2b), and there was a trend for lower performance during intermittent silencing trials compared to persistent trials in this same session (Fig S2b; Transition Day 2; P: 57±4, Ion 52±3, Ioff 66±3; p=0.0007 rmANOVA, with post-hoc Tukey HSD; Pairwise comparisons: Ion vs P: p=0.5, IRon vs Ioff: p=0.01, Ioff vs P: p=0.13; n=9 mice). These findings emphasize how quickly mice go back to relying on V1 activity. More generally, these results with a return to intermittent silencing demonstrate that the behavioral recovery observed during persistent inactivation of V1 is itself reversible: once the animal regains access to normal V1 activity it goes back to relying on it.

**Discussion**
Here, we developed a simple and potentially broadly applicable three stage paradigm for using optogenetics to determine when a given brain circuit is instructive or permissive in a behavior. In stage one, acute inactivation assesses whether the target circuit is involved at all but does not discriminate between an instructive or permissive role. In stage two, sustained inactivation assesses whether the behavior can recover, either due to relearning with alternative circuits or due to homeostasis. Finally, when there is behavioral recovery, stage three returns to acute inactivation to determine whether the targeted circuit is instructive or permissive.
Our data on mouse V1 supports a model in which V1 is not merely permissive but instead instructive in perceptual behavior. It is the result from the return to intermittent inactivation that distinguishes between the multiple competing explanations for why acute cortical suppression impairs behavior, but persistent silencing yields recovery. First, the performance recovery could have been explained by homeostatic recovery of a downstream area whose normal activity level depends on sensory cortical input. If this were true, then when the mice were reverted to intermittent silencing, one would have expected the mice to be resistant to cortical inactivation since the downstream area had homeostatically become independent of cortical drive. However, this was not the case as mice quickly reverted to depending on V1 activity. It is possible that the homeostatic recovery could itself have disappeared in the intervening time between the last persistent inactivation session and the first intermittent inactivation session of the subsequent block. However, if this were true it should have happened between each of the persistent inactivation sessions, and then mice should have shown transient poor performance at the beginning of each persistent inactivation session. However, this was not the case (Fig. S2c, first fifth of session: 52±3%, last fifth of prior session: 50±2%; p=0.311, paired t-test; n=9 mice), arguing against any spontaneous recovery between days of persistent illumination. This implies that whatever circuit changes mediate the behavioral recovery during chronic silencing did not spontaneously reverse between days. Thus, we must consider alternate explanations.

In a second scenario, the recovery of performance during persistent silencing could have been explained by the animals permanently relearning the task using alternative circuits that do not depend on primary sensory cortical activity. Visual information is routed to other subcortical structures that can mediate perceptual behaviors, such as the superior colliculus or secondary sensory cortical areas that can receive input that bypasses V1. In this scenario, task relevant information is available to the animal in both the primary sensory cortex and alternative structures independently, and though originally the animal depended on V1 activity to solve the task, over several days of training in its absence, the animal would have relearned the task via these alternate circuits. In this scheme, again one would have expected that the animals would be resistant to reversion to intermittent inactivation of V1 since they had learned to solve the task independent of the primary sensory cortex. However, this was not the case, as again, animals very quickly returned to showing poor performance upon reversion to intermittent silencing (Fig. 3j-m). This negates the second explanation.

In a third scenario V1 is the default brain area that instructs the animal’s downstream decision circuits to solve the perceptual task. When V1 is inactivated intermittently, the animal fails on the task since it is relying on primary sensory cortical output for instructing performance. When V1 activity is unavailable for long enough, the downstream decision circuits eventually adapt to employing an alternate source of information to solve the task that may always have been present but was originally ineffective at driving these circuits. This learned reliance on alternate circuits is, unlike in scenario two, impermanent: when primary sensory cortical activity again becomes available, the downstream circuits revert to ignoring this alternative source of information even on trials in which the primary sensory cortex is acutely inactivated. Our results support this third scenario; they argue that V1 is the dominant and default source of instructive information for solving a simple contrast detection task.

We note that the persistent optogenetic silencing with GtACR2 did not completely suppress activity in V1. It is possible that the mice recovered performance during this phase of
the task not by relying on an alternative circuit (e.g., the superior colliculus), but by better exploiting the small, residual activity in V1. However, removal of V1 also led to recovery, arguing that alternate circuits, at least in principle, are sufficient to support task performance. Furthermore, if mice could exploit the small, residual activity, it is surprising that upon return to intermittent silencing they quickly (within a single session) returned to failing on silencing trials. It is possible that whatever plasticity occurred in the downstream readout circuit to compensate for the strong loss of V1 activity also rapidly reversed. However, regardless of this outstanding possibility, such a scenario also supports that V1 is instructive and not permissive in the task. In the future, exploiting even more potent optogenetic silencers that have been recently discovered\(^{33,34,35}\) could entirely remove activity in expressing neurons if the opsins can be expressed transgenically to overcome issues with incomplete viral infection.

The new optogenetic methodology we introduce here will enable investigators to resolve conflicting results between acute and irreversible manipulations of brain circuits on behavior. In this study, we showed how this paradigm can resolve an apparent conflict on the role of V1 in visual contrast detection. But this paradigm should be broadly useful throughout the nervous system and across species for dissociating instructive versus permissive roles of brain circuits in specific behaviors. It might even be applied in any experimental system in which sustained optogenetic inactivation is effective, whether illuminating cell bodies or axonal fibers. The only important technical requirement is that the optogenetic silencing is just as effective during sustained as during intermittent illumination (something we found to be true for GtACR2 but may not be true for other inactivation schemes) and causes no toxicity at the required illumination levels. These are necessary controls that can be done with standard physiological approaches such as electrophysiology or functional imaging. It might be possible to execute the three-stage paradigm described here with alternative inactivation methods, including thermal\(^{36}\), ultrasonic\(^{37}\), magnetic\(^{38}\) or radiofrequency modulation\(^{39}\), provided the approach has sufficient temporal precision (seconds to minutes) to rigorously compare behavioral effects between acute and sustained inactivation.

Taken together, this new multi-stage optogenetic approach can generally help address the challenges posed by potential off-target effects of acute circuit perturbations. In principle, any study already using optogenetic inactivation can readily execute this paradigm with minimal additional technical complexity. The main advantage is that using this paradigm should lead to much more interpretable results. This should obviate the need to exhaustively search for potential alternative neural pathways with which an animal can solve a task (although such a search would still be valuable). Moreover, it may aid in understanding the mechanisms of behavioral recovery after brain lesions\(^{40}\), helping to delineate the default versus accessory circuits that can contribute to specific behaviors.

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Author contributions
D.Q., J.B., H.A.B. and H.A. conceived the experiments. D.Q. performed all behavioral experiments with assistance from M.W. H.A. performed all electrophysiological recordings. H.A.B. developed the task and performed quantitative analysis. J.B. performed an initial series of behavioral and optogenetics experiments to define illumination parameters. N.B. performed cortical lesions. J.S.W. developed and contributed the AAV-somBiPOLES vector. H.A. wrote the manuscript.
Fig. 1 | A three-stage optogenetic inactivation paradigm for dissociating instructive from permissive roles of a targeted brain circuit. a) A permissive model for the circuit. In this model, Area A is only needed to keep Area B in a functional state so that Area B instructs correct performance. When Area A is removed or permanently silenced (bottom), Area B can homeostatically recover (curved green arrow) from its loss of drive from Area A and restore correct behavior. b) An instructive model for the circuit. In this model, Area A directly instructs correct performance, but when it is removed or permanently silenced, a redundant accessory circuit through Area B restores performance (bottom). c) An approach to distinguish among these models with chronic, but reversible inactivation: three successive stages of intermittent, persistent, and repeat intermittent silencing of the targeted circuit. Bottom: schematic plots of behavioral performance on a relevant task showing performance in each stage for light off (gray) and light on (blue) trials. For stage 2, the dotted lines are the schematized performance data from stage one replotted for comparison. In this example set of results, in Stage 1 inactivation reduces performance, but in stage 2 performance recovers. In stage 3, intermittent inactivation either returns to reducing performance (top) or no longer has any effect (bottom).
Figure 2. Acute and persistent optogenetic suppression of cortical activity. a) Left: schematic of the recording configuration. Right: Example image of a post-mortem slice of visual cortex expressing the inhibitory opsin throughout the cortical layers. Scale bar: 100 μm. Bottom: schematic of the illumination procedure with an intermittent illumination block, followed by 40 minutes of continuous persistent illumination, followed by a return to intermittent illumination. b) Example raster plot for all spikes on an example electrode during the acute/persistent optogenetic suppression protocol. Left: Trials with no illumination during the intermittent phases. Right: trials during the illumination phases (blue rectangle); intermittent (pre), persistent, and intermittent (post), as indicated by the blue shaded regions. c) Averaged normalized spiking activity across all recorded tetrodes across all experiments (3 mice, 6 sessions, 25 tetrodes) during each phase of the optogenetic protocol for the 100% contrast stimulus. Intermittent illumination for ~40 trials, followed by continuous illumination for ~130 trials, followed by a return to intermittent illumination. Black dots correspond to no light trials, blue dots indicate illumination trials. Each phase of illumination is schematized by the blue bars above the plot; broken lines: intermittent; solid bar: persistent light. d) Average contrast response plots across all recorded units in each phase of the silencing experiment. Left: control (black) and intermittent illumination trials (blue). Middle: persistent illumination trials. Right: return to the intermittent illumination.
illumination. e) Scatter plots of recording unit firing rates between the indicated conditions; 3 mice, 6 sessions, 25 tetrodes; Wilcoxon sign rank test. All error bars are s.e.m.
Fig. 3 | Reversible recovery from persistent inactivation of visual cortex. a) Left: Schematic of visual detection task. Right: Optogenetic trial structure: Light is on transiently (~2 seconds) for 30% of trials (intermittent and intermittent repeat, left) or chronically (persistent, right) for the
duration of the session. Gray bar represents stimulus presentation time, black line represents response window, time referenced to the start of the stimulus window. **b)** Schematic of progression through stages of silencing, not including transition days. For intermittent block (sessions I1-I6), silencing occurs on only a subset of trials shortly prior to stimulus presentation through response window. For the persistent block (sessions P1-P6), silencing occurs for the entire duration of the session. For the intermittent repeat block (sessions IR1-IR6), sessions are structured exactly like the intermittent block. **c)** Left, Performance in light off (black) vs light on (blue) trials for intermittent day 1, ‘I1’ (p=0.00011; paired t-test, n=9 mice). Right, Performance of non-opsin expressing mice during intermittent illumination (p=.99; paired t-test; n=2 mice) **d)** Performance over each day of intermittent silencing. **e)** Psychometric curves of performance during light on and light off trials across all intermittent days (n=6 mice). **f)** Left, performance during light off vs light on trials for intermittent day 6 vs persistent day 1 (p= 0.0012, paired t-test, n=9 mice). Right, c50 of light on and light off intermittent sessions vs persistent sessions (p<0.00001, 1-way rmANOVA, with post-hoc Tukey HSD; Pairwise comparisons: Intermittent (I) l_{on} vs l_{off}: p=0.0002, l_{on} vs Persistent (P): p=0.0006, l_{off} vs P: p=0.9231). **g)** As in D). but during each day of persistent silencing. **h)** As in E), but for persistent silencing. Overlayed dashed curves are performance for l_{on} and l_{off} performance from panel e). **i)** Left, Light on performance for intermittent vs intermittent repeat blocks (p=0.50, paired t-test, n=9 mice). Right, Performance during day 6 of persistent vs day 1 of intermittent (p=0.0039, paired t-test, n=7 mice). **j)** As in D) but after returning to intermittent silencing. **k)** As in E), I but for intermittent repeat block. Data are group mean ± s.e.m.
Fig. S1 | Performance curves for each mouse. a-i) Psychometric curves of performance for each mouse during each stage of the task, fit with a Weibull function. Data are group mean ± s.e.m.
Fig. S2 Performance in transition days and within sessions. a) Performance during the first transition day between stage one (intermittent) and stage two (persistent). p<0.0001 rmANOVA,
with post-hoc Tukey HSD; Pairwise comparisons: I\textsubscript{on} vs P: p=0.9, I\textsubscript{on} vs I\textsubscript{off}: p=0.0025, I\textsubscript{off} vs P: p=0.0019, n=9 mice. b) As in A) but for the second transition day, between stage two (persistent) and stage three (intermittent repeat). p=0.0007 rmANOVA, with post-hoc Tukey HSD; Pairwise comparisons: I\textsubscript{on} vs P: p=0.5, I\textsubscript{on} vs I\textsubscript{off}: p=0.01, I\textsubscript{off} vs P: p=0.13, n=9 mice. c) Comparison of, for persistent silencing days, last bin of performance on prior days vs bin on next day (first fifth of session: 52±3%, last fifth of prior session: 50±2%; p=0.311, paired t-test; n=9 mice). d) Binned performance over each block (top: intermittent, middle: persistent, bottom: intermittent repeat) and for each session (1 to 6; left to right) for no light (black) and light (color) trials. Group mean ± s.e.m.
Fig. S3 Lesion of visual cortex causes acute impairment followed by incomplete recovery. a) coronal section stained with anti-NeuN showing lesion of V1 from a representative example mouse. b) Psychometric curves of performance of mice with V1 lesions on the day immediately prior to lesion (Day -1, black), day of lesion (Day 0, yellow), and Day 10 (orange) after lesion, n=8 mice for Day -1&0 and 7 for Day 10. Lines, Weibull function fits of the psychometric curve. c) Performance of mice for six days before lesion (circlescircles), tested hours after lesion (Day 0), and then tested for ten days after lesion (squaresquares). Orange, V1 lesion, n=8 mice, gray, S1 lesion, n=3 mice.

Materials and methods

Animals
Mice used for experiments in this study were C57/BL6. Mice were housed in cohorts of five or fewer in a reverse light:dark cycle of 12:12 hours, with experiments occurring during the dark phase. Both Female and male mice were used. For optogenetic experiments, a total of 9 mice were used (6 males).

Viral injection
Neonatal mice (P3-P5) were cryoanesthetized and their head was placed in a ceramic mold and secured with non-abrasive tape. Viral solutions (AAV9-CaMKII-somBiPOLES-mCerulean, Addgene) were loaded into beveled glass injection needles (WPI) and mounted in a NanoJect II
(Drummond) and the needle was inserted through the skin and scalp using a micromanipulator (Sutter, MP-285). We injected 3 locations at 3 depths with 27 nl viral solution per location.

**Headpost surgery**

All experiments were performed in accordance with the guidelines and regulations of the Animal Care and Use Committee of the University of California, Berkeley. For head fixation during behavioral and physiological experiments, a small custom stainless-steel headplate was surgically implanted. Briefly, adult mice (P35-P60) were anesthetized with 2-3% isoflurane and mounted in a stereotaxic apparatus. Body temperature was monitored and maintained at 37°C. The scalp was removed, the fascia retracted, and the skull lightly scored with a drill bit. Vetbond was applied to the skull surface, and the headplate was fixed to the skull with dental cement (Metabond). A fine-point marker was used to note the approximate location of the left primary visual cortex (V1; 2.7mm lateral, 0mm posterior to lambda). For all behavioral experiments we implanted a glass cranial window centered on the marked site for optical access. For electrophysiological experiments, the skull was thinned to transparency on the day of recording. Mice received buprenorphine and meloxicam for pain management and could recover for at least three days before being placed on water restriction.

**Lesion surgery**

Water restricted adult mice (P75-P180) previously headplated (see headpost surgery for procedure) and trained to perform the behavioral paradigm were anesthetized using isoflurane 2.5% and mounted in a stereotaxic apparatus. Buprenorphine (0.05-0.1 mg/kg SC) was administered at anesthetic induction. The eyes of the animal are covered with ocular lubricant to keep eyes moist. A dental burr was used to perform a 3 mm × 4 mm rectangular craniotomy over the left visual cortex (V1; 2.7mm lateral, 0mm posterior to lambda). Exposed cortical tissue was aspirated using a vacuum-attached blunt needle (16-30 awg). The lesion cavity is extended ventrally until the alveus surface of the hippocampus is exposed. The cavity was covered and sealed using a glass coverslip. Animal received meloxicam and buprenorphine post-surgery for analgesia. Mice performed on the behavioral paradigm 2-4 hours post-surgery and performance is reported as lesion day.

**In vivo electrophysiology recordings**

Mice were anesthetized with isoflurane (2.5%), and a small craniotomy (<250 microns) was opened over V1 with a fine needle. Mice were transferred to the electrophysiology rig where they were head fixed and allowed to wake and run freely on a circular treadmill. A 32-channel multielectrode array (NeuroNexus, A1x32-5mm-25-177-a32) was guided into the brain using a micromanipulator (Sutter Instruments) and a stereo microscope (Leica). Electrical activity was amplified and digitized at 30kHz (spikeGadgets Inc.) and stored on a computer hard drive. The bottom of the electrode was zeroed with the surface of the brain to determine z depth. The electrode array was lowered ~650-750 microns into V1. A multimode fiber (400 μm diameter) was positioned ~2-3mm from brain next to the recording electrode to deliver 455 nm LED light during optogenetic illumination at a total of 2mW/mm² measured at the tip of the fiber.
Spike detection was performed across assigned tetrodes (four adjacent electrode contacts) using SpikeGadgets threshold detection algorithm. Analysis was performed on all detected spikes on each tetrode. Not all assigned tetrodes were used because the top tetrodes were often located in L1 or outside of the brain. Statistically significant differences between conditions were determined using the Wilcoxon sign rank test across all tetrodes. Since mCerulean was not readily visible during adult surgeries, all experiments were initially performed blind to successful opsin expression, and animals with no obvious physiological optogenetic effects (strong activation by red light via the Chrimson in somBiPOLES), were excluded, which correlated with weak or no expression observed post-mortem.

**Water restriction**

Initial animal weight was recorded for 3 days before water restriction to establish baseline weight. Mice were then placed water restriction. On training days, mice received most water during the task. Mice were weighed after training and given additional water if their weight dropped below 70% initial weight. Food was available *ad libitum*. The weight and health (quality of the fur and nails, gait, posture) of the mice were monitored daily.

**Behavioral training on the contrast detection task:**

**Apparatus**

For the contrast detection task, the visual behavioral apparatus was controlled in real time using an Arduino DUE synchronized with Raspberry Pi 3+/4+ and a custom software in Python, Arduino and Java (Bounds et al., 2021). The mouse was head fixed bilaterally and placed on a 3D-printed holder tube. Mouse licking was measured through the detection of changes in the capacitive load in a touch sensor (Adafruit, AT42QT1070) caused by the contact of the mouse’s tongue and a 0.05-inch diameter steel lickport. Water was delivered through the lickport using a 2-way normally closed isolation valve control (Neptune Research Inc.). Visual stimuli were displayed in a gamma corrected LCD monitor (Podofo, 15.7 x 8.9 cm, 1024x600 pixels, 60 Hz refresh rate) positioned 12 cm from the right eye with a 30 degree angle from the longitudinal axis.

**Behavioral training and task design**

Head fixed mice were trained to lick when they detected a visual stimulus of variable contrasts displayed on the monitor. The training started after weight dropped below 80% of the initial weight from water restriction. Prior to training, mice were habituated to head-fixation for two daily sessions lasting 30 minutes each. The behavioral training protocol consisted of four stages.

In stage 1, mice were classically conditioned to lick in response to a visual stimulus that consisted in a square patch with drifting sinusoidal grating (2Hz, 0.08 cycles/degree, 600 ms, 100% contrast, 34 visual degrees, black background luminescence). Each visual stimulus was paired with a water reward delivered at the beginning of a 1000 ms response window. Mice who achieved a performance of more than 90% of correct trials were transitioned to the next phase.
In stage 2, mice were operantly conditioned to respond every time the same stimulus from stage 1 was displayed (2Hz, 0.08 cycles/degree, 600 ms, 100% contrast, 34 visual degrees, black background luminescence). Mice received a water reward when they licked within a 1000 ms response window after the visual stimulus presentation. A no-lick period was enforced after two seconds of the trial. Catch trials were introduced and consisted of 25% total trials. Mice licking in a no-lick period or a catch trial received a 5–9 seconds time out punishment. Mice achieving performance with a hit rate >90% and false alarm rate <30% for two consecutive sessions were switched to the next phase.

In stage 3, mice continued to train in the same behavioral paradigm with increased background luminescence at different sessions until it reached mean luminescence. Then, the visual stimulus size was decreased in different sessions until it reached 18 VD. The same performance criteria as in stage 2 was used in every session before transitioning to any change.

In stage 4, mice started to detect stimuli of six different contrasts, pseudo-randomly distributed. The intensities were customized for each mouse to provide a reliable detection threshold. Mice were introduced to the optogenetic protocol once performance was stable for more than 3 sessions. During photostimulation experiments, catch trials were 25% of all trials and response window was reduced to 500 ms.

**Optogenetic stimulation during behavior:**

For V1 one-photon optogenetic experiments, an optical fiber (400μm diameter, 0.39 nA; Thorlabs Inc.) coupled to a 455 nm LED (M4553, Thorlabs) and attached to a LED driver (LEDD1B, Thorlabs), was placed above the mouse’s window using a micromanipulator. Light power was calibrated daily and measured from the tip end of the fiber using a power meter (PM160T, Thorlabs). The light power used during photostimulation was 2.0 mW for all mice. Optogenetic stimulation trials were 33% of all trials in intermittent blocks, pseudo-randomly distributed in blocks. Light was delivered at a random time prior to the stimulus onset, between 300 - 3000 ms, to avoid cueing the mouse to the trial start. Light was sustained until the end of the response window. A custom designed removable light blocking system was attached to the head of the mouse to prevent the mice from seeing the optogenetic stimulation light. A masking light and a plastic eye patch over the left eye were also placed to prevent additional extraneous cues.

**Analysis of behavioral data:**

% Hit was defined as the number of trials go trials (Contrast > 0) where the mouse correctly licked within the response window divided by the total number of go trials. Days where mice were run on the incorrect contrast levels due to experimenter error were excluded. For detection threshold analysis, data for individual sessions were fit using a Weibull cumulative density function that fit the false alarm (lower asymptote), lapse rate (upper asymptote), slope and C63. Threshold was defined as the contrast at which the mouse was performing with performance halfway between the false alarm (lower asymptote) and lapse rate (upper asymptote).
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed using MATLAB or python. The analyses performed were repeated measures analysis of variance (rmANOVAs), with Tukey post-hoc tests as well as paired t-tests and Wilcoxon rank sign tests. Unless otherwise noted, all tests were two-tailed and all plots with error bars were reported as mean ± SEM. Sample size was not predetermined using power analysis.

Data availability

All behavioral and electrophysiological data will be made available upon publication.


