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A cortical disinhibitory circuit for enhancing adult plasticity

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The adult brain continues to learn and can recover from injury, but the elements and operation of the neural circuits responsible for this plasticity are not known. In previous work we have shown that locomotion dramatically enhances neural activity in the visual cortex (V1) of the mouse (Neill and Stryker, 2010); identified the cortical circuit responsible for this enhancement (Fu et al., 2014); and shown that locomotion also dramatically enhances adult plasticity (Kaneko & Stryker, 2014). The circuit responsible that is responsible for enhancing neural activity in the visual cortex contains both vasoactive intestinal peptide (VIP) and somatostatin (SST) neurons (Fu et al., 2014). Here we ask whether this VIP-SST circuit enhances plasticity directly, independent of locomotion and aerobic activity. Optogenetic activation or genetic blockade of this circuit reveal that it is both necessary and sufficient for rapidly increasing V1 cortical responses following manipulation of visual experience in adult mice. These findings reveal a disinhibitory circuit that regulates adult cortical plasticity.
Cortical plasticity declines with aging, accounting for decreased learning and memory, as well as reduced neural rehabilitation in aging brain (Park and Reuter-Lorenz, 2009; Singer, 1995). Running or other physical exercise has been suggested to improve many aspects of brain function in aging human beings, including brain plasticity (Voss et al., 2013). In aged animals, environmental enrichment has also been shown to improve learning and memory, as well as cortical plasticity; but the underlying circuit mechanisms are unknown (Greifzu et al., 2014; Vivar et al., 2013).

Our laboratory recently showed that running enhances both visual cortical responses and plasticity in adult mice (Kaneko and Stryker, 2014; Niell and Stryker, 2010). We also found that running potently activates VIP neurons in mouse primary visual cortex (V1), which in turn inhibit SST inhibitory neurons, thereby disinhibiting the excitatory pyramidal neurons and allowing them to respond more strongly to visual stimulus (Fu et al., 2014). We also showed that activating VIP neurons is both sufficient and necessary for enhancing visual response during running (Fu et al., 2014). Therefore, we set out to investigate whether the VIP-SST disinhibitory circuit, rather than general aerobic exercise, is responsible for enhanced cortical plasticity in adult mice.

To examine the function of VIP neurons in enhancing adult plasticity by running, we silenced their synaptic transmission in binocular zone of mouse V1 by injecting AAV-DIO-TetanusToxinLightChain-T2A-GFP (AAV-DIO-TcTx) into the VIP-Cre mice (Figure 1A) (Xu and Sudhof, 2013). We then compared the visual responses of stationary (running speed < 2 cm/s) and running (running speed > 5 cm/s) states, and found no significant difference (increase of peak response at preferred orientations: 4±33%, mean±SD, p=0.74) (Figure 1- figure supplement
indicating that silencing the transmission of VIP neurons by TeTx abolished the effect of running on visually responsive V1 neurons, consistent with our previous report (Fu et al., 2014).

We previously showed that running in conjunction with visual stimulation enhanced visual cortical plasticity in a mouse model of human amblyopia (Kaneko and Stryker, 2014). To test the requirement of VIP-cell synaptic transmission in this model, we first sutured closed one eyelid of VIP-Cre mice at the peak of critical period (~P25), and then after 4 months injected 1µl of AAV-DIO-TeTx or PBS (the vehicle solution for virus) into the binocular zone of V1. After an additional month, we re-opened the closed eye, and allowed the animals to run on Styrofoam balls floating on air while viewing a visual stimulus 4 hours per day for 21 days, as described previously (Kaneko and Stryker, 2014). Cortical responses through two eyes were measured by an experimenter blind to the treatment with intrinsic signal imaging immediately after eye opening and every 7 days during the recovery period (Figure 1B). Intrinsic signal imaging provides a reliable and non-invasive measurement of visual cortical responses to the two eyes, permitting long-term repeated measurement of the same animal to assess plasticity (Kaneko et al., 2008). Its results have been extensively validated by electrophysiological recordings in previous studies (Kaneko et al., 2008; Kaneko and Stryker, 2014). As expected, the deprived-eye visual responses recovered well in the control PBS-injected VIP-Cre mice, and the ocular dominance index (ODI) reached a level that is similar to non-deprived animals (Figure 1C-1E, black traces, compare to Figure 1 of Kaneko et al. (Kaneko and Stryker, 2014)). In contrast, V1 responses to the deprived eye in the AAV-DIO-TeTx injected animals recovered only modestly, even after 21 days (PBS: 1.82 ± 0.26, TeTx: 1.40 ± 0.20, p = 0.006), with an ODI significantly smaller than in the control animals (PBS: 0.249 ± 0.07, TeTx: 0.083 ± 0.02, p = 0.0004; Figure 1C-1E, green traces). Indeed, the poor recovery of AAV-DIO-TeTx injected animals was similar to that of...
animals that did not run in our previous report (Kaneko and Stryker, 2014), despite the fact that they ran as much as PBS-injected animals (% of running time: TeTx, 67.88 ± 23.6 vs. PBS, 68.14 ± 17.57; average running velocity in cm/s: TeTx, 10.78 ± 3.142 vs. PBS, 9.43 ± 5.29; Figure 1 – figure supplement 2). In these animals, as in all of the TeTx-treated animals described below, we noted no obvious changes in temperament or visual behavior; experimental and control animals appeared indistinguishable. Responses of the open-eye were not significantly changed in either group of animals (Figure 1D). Silencing the transmission of VIP neurons thus abolished the effect of running on adult plasticity in this mouse model of amblyopia by reducing the potentiation of deprived-eye responses during recovery.

During the critical period, 3 days of monocular deprivation (MD) is always sufficient to shift the ocular dominance by reducing the deprived-eye response. In adult mice (>3 months old), 7 days of MD is required for a reliable shift of the ocular dominance, and this shift is produced by increasing the open-eye responses (Sato and Stryker, 2008). Ocular dominance plasticity in adult mice is thus not only much slower but is also qualitatively different from that during the critical period (Sato and Stryker, 2008). To determine whether locomotion would enhance another measure of adult plasticity, we examined changes in visual responses after short-term MD in adult mice that had been reared normally. As expected, 4-day MD did not significantly change the ODI or the response of either eye in adult animals housed in standard conditions (Figure 2, ‘B6 Home cage’ group). However, 4-day MD combined with daily visual stimulation and running led to enhanced plasticity, including both increases in open-eye responses and decreases in deprived-eye responses (ODI before and after MD: 0.25 ± 0.03 vs. 0.09 ± 0.08, p < 0.001; closed-eye response before and after MD: 2.37 ± 0.28 vs. 1.95 ± 0.34, p < 0.01; open-eye response before and after MD: 1.41 ± 0.18 vs. 1.69 ± 0.25, p < 0.01; Figure 2, ‘B6 VS+run’
To examine the role of VIP-cell synaptic transmission in the effect of running on this measure of adult plasticity, we injected AAV-DIO-TeTx into the binocular V1 of adult VIP-Cre mice that had been reared normally, and measured the changes in visual responses after 4-day MD combined with daily visual stimulation and running. After silencing the synaptic transmission of VIP neurons, running in conjunction with visual stimulation still led to significant depression of the closed-eye response (2.51 ± 0.31 vs. 2.23 ± 0.29, p < 0.05; Figure 2A, ‘VIP-TeTx VS+run’ group) but failed to potentiate the open-eye response (1.51 ± 0.20 vs. 1.53 ± 0.18, p > 0.05; Figure 2B, ‘VIP-TeTx VS+run’ group), resulting in a much smaller change of ODI after 4D-MD (0.25 ± 0.03 vs. 0.18 ± 0.05, p < 0.05; Figure 2C, ‘VIP-TeTx VS+run’ group). These results indicate that the VIP neurons are specifically involved in potentiating the adult-form plasticity.

We next investigated whether activating VIP neurons would be sufficient to enhance adult plasticity even without the exercise of daily running. We expressed a variant of channelrhodopsin, ChETA, in VIP neurons of binocular V1 by infecting VIP-Cre mice with AAV-DIO-ChETA-YFP (Figure 3A); we had previously shown this treatment to enhance visual responses of excitatory cells in stationary mice upon stimulation with blue light (Fu et al., 2014). We imaged baseline visual responses ~2 weeks after viral injection. After implanting the fiber optic cannula in the binocular zone of V1, we sutured one eyelid and connected the cannula to a blue LED light source (20Hz pulses, 2s on, 1s off, 4h each day) while the mice were housed in their home cages. No behavioral response to the optogenetic stimulus was evident during delivery. After 5-day MD in conjunction with optogenetic stimulation, we found that the visual response of the open-eye was dramatically potentiated (1.39 ± 0.19 vs. 2.28 ± 0.81, p < 0.05; 64 ± 53% fold increase; Figure 3D, blue traces), significantly shifting ODI (0.22 ± 0.03 vs. 0.05 ±
0.08, p < 0.05; Figure 3E, blue traces), with no significant change in the visual response of the
closed eye (2.08 ± 0.12 vs. 2.10 ± 0.55, p > 0.05; Figure 3C, blue traces). To rule out the
possibility that the surgery procedure itself lead to enhanced plasticity, we injected VIP-Cre mice
with AAV-DIO-TdTomato and subjected these control animals to the same procedure of
optogenetic stimulation and 5-day MD (VIP-Tdtm group); no change was found in the open-eye
response (1.77 ± 0.37 vs. 1.62 ± 0.31, p > 0.05) and ODI (0.19 ± 0.06 vs. 0.21 ± 0.05, p > 0.05;
Figure 3C-3E, red traces). Because AAV-DIO-ChETA may have labeled a small number of non-
VIP neurons (Pi et al., 2013), we also examined whether stimulating non-specifically labeled,
mostly pyramidal neurons would enhance adult plasticity. We injected AAV-ChETA into VIP-
Cre mice and subjected the mice to the same procedure of optogenetic stimulation (pyr-ChETA
group). We found no change in the visual responses to the two eyes (open eye: 1.89 ± 0.37 vs.
1.78 ± 0.30, p > 0.05; closed eye: 2.64 ± 0.28 vs. 2.36 ± 0.18, p > 0.05) or ODI (0.20 ± 0.02 vs.
0.19 ± 0.02, p > 0.05) after 5-day MD (Figure 3C-3E, black traces). These results therefore
strongly support the hypothesis that activating VIP neurons is sufficient to enhance adult
plasticity in the home cage without exercise on the trackball.

Activating VIP neurons is thought to enhance visual responses by inhibiting SST neurons
and thereby disinhibiting pyramidal neurons (Fu et al., 2014; Pfeffer et al., 2013). We
hypothesized that silencing SST neurons directly would be as effective as activating VIP neurons
for enhancing adult plasticity. To test this hypothesis, we silenced SST neurons by injecting
AAV-DIO-TeTx into adult SST-Cre mice (Figure 4A), and examined visual responses and ODI
after 5-day MD (Figure 4B). We found that the open-eye response in SST-Cre mice injected with
AAV-DIO-TeTx (TeTx group) was significantly larger than that of either SST-Cre mice injected
with AAV-DIO-TdTomato (Tdtm group) or C57BL/6J mice (B6 group) (TeTx: 1.92 ± 0.35;
TdTomato: 1.42 ± 0.19; B6: 1.45 ± 0.27; Figure 4D), while the deprived-eye responses were indistinguishable between the three groups of mice (Figure 4C). Consequently, the ODI of TeTx group after 5-day MD was significantly different from those of the control Tdtm and B6 groups (TeTx: 0.06 ± 0.06, Tdtm: 0.22 ± 0.06, B6: 0.20 ± 0.07; Figure 4E). We also measured the changes in visual responses in a group of SST animals injected with AAV-DIO-TeTx before and after 5-day MD, yielding results that further confirmed that short-term silencing of SST neurons is sufficient to enhance adult plasticity by potentiating open-eye response (Figure 4 – figure supplement 1).

The VIP-SST disinhibitory circuit has recently been found to be a potent modulator of sensory responses and to regulate cortical states by integrating long-range inputs from other brain regions (Fu et al., 2014; Pi et al., 2013; Zhang et al., 2014). The present study reveals a new function of this disinhibitory circuit in regulating cortical plasticity.

Enhancing adult plasticity has long been a topic of great interest on account of its implications for human wellbeing. Inhibitory circuits have been implicated in regulating cortical plasticity. For example, both reducing GABA production and antagonizing GABA_A receptors have been found to enhance adult plasticity in rat visual cortex, possibly by facilitating the potentiation of the input from the open eye (Harauzov et al., 2010). Chronic treatment using the antidepressant drug fluoxetine enhances adult plasticity, accompanied by reduced GABA levels in visual cortex (Maya Vetencourt et al., 2008). Our results are consistent with the idea that reduced inhibition is permissive for enhancing adult plasticity.

Removing extracellular perineuronal nets is also reported to enhance adult plasticity in rats (Pizzorusso et al., 2002; but see Vorobyov et al., 2013). This and other findings suggest that simply destabilizing synaptic connections may enhance cortical plasticity, because reduced
GABA transmission de-stabilizes GABAergic synaptic terminals (Fu et al., 2012). Transplanted GABAergic progenitor cells may create another form of instability of inhibitory circuits by making widespread new connections with host neurons and causing a second critical period of plasticity in adult mice (Southwell et al., 2010).

Extensive evidence has accumulated indicating that parvalbumin (PV)-positive fast-spiking cells play an important role in cortical plasticity (Hensch, 2005; Kuhlman et al., 2013). Our study reveals that disinhibiting pyramidal neurons by either activating VIP neurons or silencing SST neurons also enhances adult plasticity and allows potentiation of open-eye responses in V1, revealing an important role for at least these other major types of inhibitory neurons in cortical plasticity. It should be noted that there are multiple classes of SST neurons (Hu et al., 2013; Markram et al., 2004), only some of which are suppressed during locomotion (Fu et al., 2014; Reimer et al., 2014). In the present experiments, TeTx was presumably not selective in silencing the different subgroups of SST neurons.

Enhancing serotonin signaling by chronic fluoxetine treatment has also been shown to enhance adult plasticity (Maya Vetencourt et al., 2008). Exposure to an enriched environment was reported to enhance serotonin levels in rat visual cortex as well as enhance adult plasticity (Baroncelli et al., 2010). Unlike PV and SST neurons, VIP neurons express the 5-HT$_3$ serotonin receptor, a channel that allows fast depolarization of neurons (Lee et al., 2010). VIP neurons also receive a direct nicotinic cholinergic input from the basal forebrain, which modulates cortical state and sensory responses (Fu et al., 2014; Lee and Dan, 2012). Therefore, the VIP-SST disinhibitory circuit is well poised as a target for manipulation of cortical plasticity by these two neuromodulators.
Adult ocular dominance plasticity in V1 consists of a potentiation of the open-eye response following MD; while critical period plasticity features an initial depression of the deprived-eye response followed by potentiation of the response to the open eye (Sato and Stryker, 2008). A number of manipulations in older animals mimic critical period plasticity in their effects on responses to the two eyes: transplantation of embryonic inhibitory neurons (Southwell et al., 2010) or acute suppression of the activity of PV neurons (Kuhlman et al., 2013) in mice, or chronic fluoxetine treatment in rats (Maya Vetencourt et al., 2008). Other manipulations, such as brief dark exposure (He et al., 2006; Stodieck et al., 2014), repeated MD (Hofer et al., 2006), visuomotor experience (Tschetter et al., 2013), or activation of Rho GTPases (Cerri et al., 2011), lead to a potentiation of open-eye responses that resembles normal adult plasticity following more prolonged MD. The present findings reveal that activation of the VIP-SST disinhibitory pathway is both necessary (Figure 1 and Figure 2) and sufficient (Figures 3 and 4) for cortical plasticity that potentiates visual responses, and that aerobic exercise is neither necessary nor sufficient. Running, on the other hand, produces both potentiation of open eye responses and depression of responses to the closed eye. Running is associated not only with activation of cortical VIP neurons, but also with increases in multiple neuromodulators including serotonin and noradrenalin (Meeusen and De Meirleir, 1995), both of which have been implicated in modulating adult plasticity (Gu, 2002). It is therefore not surprising that enhancement of adult plasticity by locomotion is more complex than simply activating VIP-SST disinhibitory circuit, even though silencing VIP transmission abolished the effect of running on enhancing visual response (Figure 1 – figure supplement 1) (Fu et al., 2014). More importantly, the present results also suggest that the potentiation of open-eye response and the depression of closed-eye response are separable from each other and are mediated by distinct mechanisms.
The difference between the plasticity produced by reducing the activity of PV- (Kuhlman et al., 2013) or SST- (Figure 4) neurons during MD suggests that different inhibitory circuits are engaged in gating distinct aspects of cortical plasticity. PV- and SST-cells innervate different compartments of pyramidal neurons and exert distinct physiological effects (Hu et al., 2014; Markram et al., 2004). For example, inhibitory synapses on dendritic spines (van Versendaal et al., 2012), which are preferentially innervated by SST neurons, are specifically lost during potentiation of the open-eye response in adult plasticity (Chiu et al., 2013). The different responses to reduced inhibition of distinct elements of the cortical circuit may be fundamental to understanding the differences between critical period and adult plasticity.

**Materials and Methods**

**Animals and monocular deprivation**

VIP-Cre (stock No. 010908), SST-Cre (stock No. 013044), and C57B/L6 mice were from Jackson Lab. Experiments were performed on adult (age 3-6 months) mice of both sexes. The animals were maintained in the animal facility at University of California, San Francisco (UCSF) and used in accordance with protocol AN098080-02A-G approved by the UCSF Institutional Animal Care and Use Committee. Animals were maintained on a 14hr light / 10hr dark cycle. Experiments were performed during the light phase of the cycle.

Monocular deprivation (MD) was performed as described previously (Gordon et al., 1996) except that 2–3% isoflurane in oxygen was used for anesthesia. For long-term monocular deprivation, the lid of the right eye was sutured shut at P22-24. Mice were housed in the standard condition until P135~150, at which time a custom stainless steel plate for head fixation was attached to the skull with dental acrylic under isoflurane anesthesia. The exposed surface of
the skull was covered with a thin coat of nitrocellulose (New-Skin, Medtech Products Inc., NY) to prevent desiccation, reactive cell growth, and destruction of the bone structure. Animals were given a subcutaneous injection of carprofen (5 mg/kg) as a post-operative analgesic. 5–7 days after head-plate implantation, the closed eyelid was re-opened and groups of mice for the intrinsic signal imaging study underwent the imaging session. The re-opened eyelid was left open afterward to allow binocular vision while animals were subjected to the visual stimulation and locomotion as described previously (Kaneko and Stryker, 2014).

Virus and viral injection

AAV-DIO-TeTx (AAV_{DJ}-DIO-TeTxlC-T2A-GFP) was a gift from Dr. Wei Xu and Dr. Thomas Sudhof at Stanford University (Xu and Sudhof, 2013). AAV-DIO-TdTomato (AAV_{2.9}-CAG-LSL-TdTomato) (Cat. No. AV-9-ALL856), AAV-DIO-ChETA (AAV_{2.9}-EF1a-DIO-ChETA-YFP) (Cat. No. AV-9-26968P) and AAV-ChETA (AAV_{2.2}-hSyn.ChETA) (Cat. No. AV-9-2-26967M) were purchased from University of Pennsylvania Vector Core, and injected as previously described (Fu et al., 2014). For viral injection, a small bur hole was drilled into the skull using a dental drill over the binocular zone of primary visual cortex in anesthetized mice. A glass micropipette (tip size ~10-30um) attached to a Picospritzer was lowered below the pia surface to the specified coordinates. 0.5-1ul of the virus was injected with short pulses (50ms) over 5min. The glass pipette was left in place for an additional 3 min to allow viral diffusion. After removal of the injection pipette, the scalp was closed with vetbond (3M) and the animals were allowed to recover.

Tetrode recording in awake mice

Our spherical treadmill was modified from the design described in (Niell and Stryker, 2010). Briefly, a closed-cell foam ball (Plasteel Corporation) was placed on a base foam bowl
with a single air inlet at the bottom. The base foam bowl was trimmed to allow the close
placement of 2 USB-optical mice mouse for sensing the rotation of the floating styrofoam ball,
which transmitted the USB signals to our data analysis system. The animal’s head was fixed via
a surgically attached steel headplate screwed into a rigid crossbar above the floating ball. The
recording was performed as described previously (Niell and Stryker, 2010). On the day of
recording, the animal was anesthetized with isoflurane in oxygen (3% induction, 1.5-2%
maintenance). The skull was thinned above the area of viral infection so that it was nearly
transparent. A small opening in the skull was made with a 27 gauge needle to allow insertion of a
16 channel probe (Neuronexus model a2X2-tet-2mm-150-121). The electrode was placed at an
angle of ~45 deg relative to the cortical surface, to increase the distance between the insertion
and recording sites. The electrodes were inserted to a depth of < 400um below the cortical
surface to record cells in layer 2/3. For each animal, the electrode was inserted only once. The
animals recovered for > 3 hours after craniotomy before recording data was collected.

Visual stimulation, data acquisition, and analysis

Visual stimuli were presented as described previously (Niell and Stryker, 2008). Briefly,
stimuli were generated in MatLab using the Psychophysics Toolbox extensions (Brainard, 1997;
Pelli, 1997) and displayed with gamma correction on a monitor (Dell, 30X40cm, 60Hz refresh
rate, 32cd/m² mean luminance) placed 25cm from the mouse, subtending ~60-75° of visual space.
For drifting sinusoidal gratings, the spatial frequency was 0.05 cpd and the temporal frequency
was 1Hz. Stimulation was presented at nominal 100% contrast for 3sec with 0.5sec gray interval.
For recovery from long-term MD, the visual stimulus was contrast modulated Gaussian noise,
with a randomly generated spatiotemporal spectrum having low-pass spatial and temporal cutoffs
applied at 0.05 cpd and 4 Hz, respectively, as previously described (Kaneko and Stryker, 2014).
To provide contrast modulation, the noise pattern was multiplied by a sinusoid with a 10-s period. Movies were generated at 60 × 60 pixels and then smoothly interpolated by the video card to 480 × 480 to appear 30 × 30 cm on the monitor and played at 30 frames per second. Each movie was 5 min long and repeated for 4 hr total presentation.

Data acquisition was performed as described by Niell and Stryker (Niell and Stryker, 2008). Signals were acquired using a System 3 workstation (Tucker-Davis Technologies). For single-unit activity, the extracellular signal was filtered from 0.7 to 7 kHz and sampled at 25 kHz. Spiking events were detected on-time by voltage threshold crossing, and a 1 ms waveform sample on all 4 recording surfaces of the tetrode was acquired around the time of threshold crossing. Single-unit clustering and spike waveform analysis was performed as described previously (Niell and Stryker, 2008), using Klusta-Kwik (Harris et al., 2000, available at http://git.debian.org). Quality of separation was determined based on the Mahalanobis distance and L-ratio (Schmitzer-Torbert et al., 2005) and evidence of clear refractory period. Units were also checked to assure that they responded similarly at the beginning and end of recording to ensure that they had not drifted or suffered mechanical damage. As previously described (Niell and Stryker, 2008), units were classified as narrow or broad spiking based on properties of their average waveforms, at the electrode site with largest amplitude.

For drifting gratings, responses at each orientation were calculated by averaging the spike rate during the 3s presentation and subtracting the spontaneous rate. The preferred orientation was determined by averaging the response across all spatial frequencies, and calculating half the complex phase of the value

\[ \frac{\sum F(\theta) e^{2i\theta}}{\sum F(\theta)} \]

The orientation tuning curve was fitted as the sum of two Gaussians centered on \( \theta_{\text{pref}} \) and \( \theta_{\text{pref}} + \pi \), of different amplitudes A1 and A2 but equal width \( \sigma \), with a constant baseline.
Optical imaging of intrinsic signals

After the headplate implantation, the first imaging of intrinsic signals was performed to measure baseline responses through each eye. The mouse was anesthetized with isoflurane (3% for induction and 0.7% during recording) supplemented with intramuscular injection of chlorprothixene chloride (2 µg/g body weight), and the closed eyelid was carefully opened by slitting horizontally at the center of the fused lid just before the imaging session. Repeated optical imaging of intrinsic signals was performed as described (Kaneko et al., 2008). We monitored the concentration of isoflurane using an Ohmeda 5250 RGM (Datex-Ohmeda, Madison, WI) throughout each imaging session. Images were recorded transcranially through the window of the implanted headplate. Intrinsic signal images were obtained with a Dalsa 1M30 CCD camera (Dalsa, Waterloo, Canada) with a 135 × 50 mm tandem lens (Nikon Inc., Melville, NY) and red interference filter (610 ± 10 nm). Frames were acquired at a rate of 30 fps, temporally binned by four frames, and stored as 512 × 512 pixel images after binning the 1024 × 1024 camera pixels by 2 × 2 pixels spatially. We used two kinds of visual stimuli presented between −5° and 15° (azimuth) on the stimulus monitor (0° = center of the monitor aligned to center of the mouse) to record intrinsic signals in the binocular visual cortex: (1) 2°-wide bars, moving continuously and periodically upward or downward at a speed of 10°/sec; (2) the contrast-modulated noise movie, as described above. To record in the monocular visual area, the contrast-modulated noise was presented between 50° and 70° (azimuth) of the visual field. Visual stimuli were presented on a 40 × 30 cm monitor placed 25 cm in front of the mouse. The phase and amplitude of cortical responses at the stimulus frequency were extracted by Fourier analysis as described (Kalatsky and Stryker, 2003). Response amplitude was an average of at least four measurements. Ocular dominance index was computed as \( \frac{R - L}{R + L} \), where R
and L are the peak response amplitudes through the right eye and the left eye, respectively, as described (Kaneko et al., 2008). All mice were kept under standard housing conditions with free access to food and water between recordings, and daily running on the treadmill in indicated experiments.

**Fiber optic cannula implantation and optogenetic stimulation**

Three weeks after viral injection, a cannula with optic fiber in the center (0.2 mm in diameter for the optical fiber) (Thorlabs, CFMC12U-20; the optic fiber was cut to be 1mm in length) was implanted on top of the center of the binocular zone and secured to the skull with dental cement. A 470nm LED (Thorlabs, M470F1) with 400um diameter core fiber output was used to generate blue light. The fiber optic inserted to the cannula was illuminated by the LED through a flexible optic fiber with rotatory joint that allowed the animal to move freely in its home-cage, at a final intensity emitted from the fiber of 15 mW/mm². Light pulse stimuli (2 seconds of 20Hz (25ms on, 25ms off) and 1 second off) were generated by connecting the LED controller with a Master-8 pulse stimulator. The mice were given daily optogenetic stimulation for 4 hours per day for 5 consecutive days.

**Immunohistochemistry**

Animals were transcardially perfused with saline and 4% formaldehyde. The brains were removed, cryoprotected in 30% sucrose, and cut into 30μm coronal sections on a frozen sliding microtome (Physitemp Instruments). Floating sections were blocked for 1 hour at room temperature in Tris buffered saline (TBS) containing 10% normal goat serum and 1% Tween-100, then incubated overnight at 4 °C using in the same solution with the following antibodies: chicken anti-GFP, 1:1000 (Aves); rabbit anti-SST, 1:300 (Swant); and rabbit anti-VIP, 1:1000 (Immunostar). The sections were then washed in TBS with 1% Tween-100 3 times for 15
minutes, incubated for 1 hour at room temperature in blocking solution with 1:1000 each of Alexa Fluor 488 goat anti-chicken and Alexa Fluor 568 goat anti-rabbit (Life Technologies). The sections were washed in phosphate buffered saline 3 times for 10 minutes, mounted on glass slides, dried, and covered with coverslips. Images of the visual cortex were captured using a Zeiss Axiovert-200 microscope and AxioCam Mrm (Zeiss). Contrast was adjusted in ImageJ.

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Figure 1. Synaptic transmission from VIP neurons is necessary for the enhancement of recovery of the amblyopic eye by running. (A) Representative fluorescent images of binocular V1 area from a VIP-Cre mouse injected with AAV-DIO-TeTx-GFP. Slices were immunostained for GFP to indicate viral infected neurons and VIP for VIP-positive neurons. White arrows indicate the cells positive for both GFP and VIP staining. Red arrows indicate the cells stained positive for VIP only; green arrow indicates a cell positive only for GFP. (B) Experimental schedule. (C, D) Changes in intrinsic signal responses evoked by the visual stimulation through the closed eye (C) and the open eye (D) in AAV-DIO-TeTx-injected (VIP-TeTx, n = 5) experimental and PBS-injected control mice (PBS, n = 6). (E) Ocular dominance index (ODI) computed from responses to contralateral (closed) and ipsilateral (open) eyes shown in (C) and (D). ODI represents normalized difference in response magnitude between the two eyes; higher ODI indicates more domination of the contralateral eye. Open circles represent measurements in individual animals, solid circles indicate mean of the open circles. (Data are plotted as mean ± S.D., *** P < 0.001, ** P < 0.01 between groups at given time point; Two-way ANOVA followed by multiple comparisons with Bonferroni correction)
Figure 2. Synaptic transmission from VIP neurons facilitates the enhancement of ocular dominance plasticity in adult mice by running. (A) Experimental schedule. (B, C) Amplitudes of intrinsic signal responses evoked by the visual stimulation through the closed eye (B) and the open eye (C) before (baseline) and after 4-day monocular deprivation of the contralateral eye (MD 4d). (D) Ocular dominance index (ODI) computed from response amplitude to contralateral (closed) and ipsilateral (open) eyes shown in (B) and (C). Open circles and triangles represent measurements in individual animals. B6: C56BL6 wild type mice (VS+run: n=7; home-cage: n=5); VIP-TeTx: VIP-Cre mice that received a cortical injection of AAV-DIO-TeTx and treated with VS+run during MD (n=5). Solid circles or triangles represent the average of the corresponding open circles or open triangles (± S.D.). * P < 0.05, ** P < 0.01 between baseline and after MD-4d; two-way ANOVA followed by multiple comparisons with Bonferroni correction.
Figure 3. Activation of VIP neurons is sufficient to enhance visual cortical plasticity in adult mice. (A) Representative fluorescent images of binocular V1 area from a VIP-Cre mouse injected with AAV-DIO-ChETA-YFP. The slices were immunostained for YFP to indicate viral infected neurons and VIP for VIP-positive neurons. White arrows indicate the cells positive for both YFP and VIP staining. (B) Experimental schedule. (C, D) Changes in intrinsic signal responses evoked by the visual stimulation through the closed eye (C) and the open eye (D). VIP-Cre mice were injected with AAV-DIO-ChETA (VIP-ChETA, n=4), AAV-DIO-TdTomato (VIP-Tdtm, n=4), or AAV-ChETA (pry-ChETA, n=3). (E) Ocular dominance index (ODI) computed from response amplitude to contralateral (closed) and ipsilateral (open) eyes shown in (C) and (D). Open circles represent measurements in individual animals, and solid circles indicate mean of the open circles. (Data are plotted as mean ± S.D., * P < 0.05, ** P < 0.01; paired t-test for comparing baseline and MD 5d of the VIP-ChETA group; others comparisons were analyzed with the two-way ANOVA followed by multiple comparisons with Bonferroni correction)
Figure 4. Short-term silencing of SST neurons is sufficient to enhance visual cortical plasticity in adult mice. (A) Representative fluorescent images of area in binocular V1 from SST-Cre mouse injected with AAV-DIO-TeTx-GFP. The slices were immunostained for GFP to indicate viral infected neurons and SST for SST-positive neurons. White arrows indicate the cells positive for both GFP and SST staining, and green arrow indicates a cell positive only for GFP. (B) Experimental schedule. (C, D) Amplitudes of intrinsic signal responses evoked by visual stimulation through the closed eye (C) and the open eye (D), in SST-Cre mice treated with AAV-DIO-TeTx-GFP (TeTx, n=5) or with AAV-DIO-TdTomato (Tdtm, n=7) and in C57B/L6 mice (B6, n=5). (E) Ocular dominance index (ODI) computed from response amplitude to contralateral (closed) and ipsilateral (open) eyes shown in (C) and (D). Open circles represent measurements in individual animals. (Data are plotted as mean ± S.D., * P < 0.05, ** P < 0.01; One-way ANOVA followed by multiple comparisons with Bonferroni correction)
Figure 1 – figure supplement 1. Silencing the synaptic transmission of VIP neurons abolished the effect of running on visually responsive neurons. (A) AAV-DIO-TeTx-GFP was injected into the V1 of VIP-Cre mice. The visual response to the drifting gratings was examined by silicon tetrode recording 4 weeks after viral injection. Peak responses of the preferred orientation were compared between running and stationary states. Each dot represents visual responses on one cell (n=13 from 2 animals). (B) Data showing representative visual responses of 6 cells. Orientation tuning curves (blue traces) were plotted using the average visual responses during stationary state (blue open circles, each circle represent one repetition of visual stimulation at indicated orientation). Open red circles represent visual responses during running state, and the solid red circles represent the average of open red circles at indicated orientation.
Figure 1 – figure supplement 2. VIP-Cre mice injected with AAV-DIO-TeTx-GFP (TeTx) ran similarly to PBS-injected control mice (PBS). (A) The percentage of running time during the 4-hour visual stimulation on day 14 of recovery from amblyopia. (B) The average running velocity during the running state. Each circle represents one mouse. (Data are plotted as mean ± S.D., P>0.05 between TeTx and PBS groups)
Figure 4 -- figure supplement 1. Chronic intrinsic signal imaging confirms that the short-term silencing of SST neurons is sufficient to enhance visual cortical plasticity in adult mice by specifically potentiating open-eye response. (A) Experimental schedule. (B, C) Amplitudes of intrinsic signal responses evoked by visual stimulation through the closed eye (B) and the open eye (C) in SST-Cre mice treated with AAV-DIO-TeTx-GFP (n=5) before (baseline) and after 5 days of monocular deprivation of the contralateral eye (MD5d). (D) Ocular dominance index (ODI) computed from response amplitude to contralateral (closed) and ipsilateral (open) eyes shown in (B) and (C). Open circles represent measurements in individual animals. Error bars represent mean ± S.D., * P < 0.05, ** P < 0.01; paired t-test.
Figure 1

A

TeTx-GFP

VIP

Merge

100 μm

B

right eyelid suture

P22~24

virus or PBS injection

~P120

headplate implantation

~P150

Monocular Deprivation (MD)

Binocular Vision (BV)

and trackball running

C

D

E

close-eye response (ΔF/F x 10^-4)

open-eye response (ΔF/F x 10^-4)

ODI

recovery days

recovery days

recovery days

TeTx

PBS

*** *** ***
Figure 2

A

-21 -10 -5 0 5 (d)

-21 -10 -5 0 5 (d)

Monocular Deprivation (MD)
VS+run or home-cage

B

C

D

closed-eye response (ΔF/Fx10^-4)
open-eye response (ΔF/Fx10^-4)
ODI

B6: VS+run
B6: home-cage
VIP-TeTx: VS+run

baseline
MD 4d
baseline
MD 4d
baseline
MD 4d
baseline
MD 4d
baseline
MD 4d
baseline
MD 4d
baseline
MD 4d

virus
implantation
intrinsic signal imaging

injection
implantation
imaging

~P120

headplate
implantation
intrinsic signal imaging

-21 -10 -5 0 5 (d)

Monocular Deprivation (MD)
VS+run or home-cage

**
*
Figure 3

A

Monocular Deprivation (MD) and optogenetic stimulation

B

-21 -10 -5 -2 0 5 (d)

virus injection
headplate implantation
intrinsic signal imaging
removal of cannula implantation
make craniotomy
intrinsic signal imaging

C

D

E

VIP-ChETA
VIP-Tdtm
pyr-ChETA

VIP-ChETA-YFP
VIP
Merge

ChETA-YFP
VIP
Merge

ChETA-YFP
VIP
Merge

ChETA-YFP
VIP
Merge

ChETA-YFP
VIP
Merge

ChETA-YFP
VIP
Merge

Monocular Deprivation (MD) and optogenetic stimulation
Figure 4

A. Monocular Deprivation (MD)

-5                                   -2                    0                                                     5 (d)

headplate implantation

virus injection

~ P100

TeTx-GFP

B. Intrinsic signal imaging

Merge

100 µm

C. 

Closed-eye response (ΔF/F x 10^-4)

TeTx  Tdtm  B6

1.0  1.5  2.0  2.5  3.0

D. 

Open-eye response (ΔF/F x 10^-4)

TeTx  Tdtm  B6

1.0  1.5  2.0  2.5  3.0

E. 

ODI

TeTx  Tdtm  B6

0.0  0.1  0.2  0.3  0.4

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