Alpha-1 Adrenergic Receptors Gate Rapid Orientation-Specific Reduction in Visual Discrimination

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Prolonged imbalance in sensory experience leads to dramatic readjustments in cortical representation. Neuromodulatory systems play a critical role in habituating experience-induced plasticity and regulate memory processes in vivo. Here, we show that a brief period of intense patterned visual stimulation combined with systemic activation of alpha-1 adrenergic neuromodulator receptors (α1-ARs) leads to a rapid, reversible, and NMDAR-dependent depression of AMPAR-mediated transmission from ascending inputs to layer II/III pyramidal cells in the visual cortex of young and adult mice. The magnitude of this form of α1-AR long-term depression (LTD), measured ex vivo with miniature EPSC recordings, is graded by the number of orientations used during visual experience. Moreover, behavioral tests of visual function following the induction of α1-AR LTD reveal that discrimination accuracy of sinusoidal drifting gratings is selectively reduced at high spatial frequencies in a reversible, orientation-specific, and NMDAR-dependent manner. Thus, α1-ARs enable rapid cortical synaptic depression which correlates with an orientation-specific decrease in visual discrimination. These findings contribute to our understanding of how adrenergic receptors interact with neuronal networks in response to changes in active sensory experience to produce adaptive behavior.

Keywords: experience-induced plasticity, mEPSC, mouse, NMDA receptors, visual cortex

Introduction

Experience-induced plasticity modifies sensory representations and leaves a lasting trace in the brain. It shapes the developmental history of the organism and adjusts neuronal circuits to the nature of inputs to optimize information processing and adapt to the environment. The visual cortex has long been used for the study of experience-dependent plasticity because visual experience can be easily controlled and the consequences of these manipulations can be readily measured at the anatomical, physiological, and molecular levels (Tropea et al. 2009). For example, prolonged absence of visual experience leads to a deprivation-induced weakening of cortical connections through plastic processes like homosynaptic long-term depression (LTD; Smith et al. 2009). Neuromodulatory systems regulating attention and emotion participate in habituating this and other forms of plasticity and memory processes in vivo (Bear and Singer 1986; Manunta and Edeline 2004; Froemke et al. 2007; Hu et al. 2007; Maya Vetencourt et al. 2008; Sara 2009). Neuromodulator receptors are G protein-coupled receptors (GPCRs) linked to different Ca2+ -dependent kinases and phosphatases that gate and control the expression of long-term potentiation (LTP) and LTD. Accordingly, the magnitude and polarity of the synaptic change depend on the specific balance between these GPCR-mediated cascades (Scheiderer et al. 2004; Choi et al. 2005; Wang et al. 2006; Seol et al. 2007; Abraham 2008; Shen et al. 2008). For example, in hippocampal and cortical slices, Gs-linked GPCRs gate LTP, while Gq-linked GPCRs gate LTD (Choi et al. 2005; Zhang et al. 2009; McElligott et al. 2010).

Behavioral studies designed to measure attention propose that the noradrenergic system, mainly through activation of Gs-linked beta adrenergic receptors, gates and enhances perceptual acuity, a phenomenon that has been referred to as "sensory gating" (Sara 2009). Yet, the exact "gating" properties of Gq-linked alpha-1 adrenergic neuromodulator receptors (α1-ARs) remain unknown. Here, we show that systemic activation of α1-ARs primes visual cortical neurons of young and adult mice. This priming leads to a postsynaptic form of LTD (α1-AR LTD) only when it is combined with brief and intense visual experience in vivo. α1-AR LTD is expressed at ascending inputs carrying visual information to layer II/III of the mouse visual cortex and correlates with a selective and orientation-specific decrease in visual discrimination performance of sinusoidal drifting gratings at high spatial frequencies. Thus, α1-ARs allow visual information to produce cortical LTD and decrease visual acuity in a stimulus-specific manner. These results indicate that a fraction of noradrenergic receptors plays an active role in regulating LTD, thereby serving adaptive behavioral change.

Materials and Methods

Animals

We used male C57BL/6 mice for our experiments (Charles River, Sulzfeld, Germany). All experimental procedures in this study were performed according to the animal welfare guidelines of the Max Planck Society.

Controlled Visual Experience

Visual stimulation to drive spikes in the visual cortex (Niell and Stryker 2008) consisted of computer-generated square-wave gratings (black and white bars: width, 3.72°; length, 71°; contrast, 100%) drifting at 1 cycle per second and back-projected from 2 flat screens 20 cm in front of the mice at ±60° with respect to the animals’ midline (effective spatial frequency of 0.13 cycles per degree [c/deg]; frame rate, 60 Hz; mean illuminance, 90 ± 2 lux; background illuminance, 5 ± 2 lux; Voltcraft MS-1500, Hirschau, Germany; frame size, 71° by 71°). Gratings were displayed with 1 (restricted to 0 or 90°), 8, or 16 different directions (random multiples of 22.5°, always including 0 and 90°; the 8 directions being a subset of the 16 directions), switched every minute with total duration of stimulation as indicated in the results. Angles were measured counterclockwise with respect to the ground. The animals were lightly anesthetized (pentobarbital, 30-35 mg/kg intraperitoneally [i.p.]; Merial GmbH), injected with the α1-AR agonist methoxamine (MTX, 5 mg/kg, i.p.; Sigma, 0.37K) or vehicle (9% NaCl),...
10 min before subjected to patterned visual experience, and kept in the dark afterward. Eye drops were administered to maintain a good level of eye moisture. Ex vivo electrophysiological recordings (2-7 h) or behavioral measurements (7-9 h) were performed following visual stimulation of the animals. Some mice were i.p. injected with the competitive NMDAR antagonist carboxyphenylbenzyl-4-methylpyridine-1-phenolic acid (CPP, 10 mg/kg i.p.; Sigma) or with vehicle (9% NaCl) 30 min before visual stimulation. No change in visual discrimination or motor behavior was detected 7 h after pentobarbital injection alone (data not shown) or after pentobarbital + CPP (Fig. 7C) (Frenkel et al. 2006).

**Electrophysiological Experiments**

Coronal visual cortical slices (350 μm) were prepared from postnatal day (P) 22 ± 1 (10 ± 1 g) and P40 ± 2 (± 2 ± 2 g) male C57BL/6 mice (Charles River; Sulzfeld, Germany) in dissection buffer (4°C) containing (in mM): 212.7 sucrose, 5 KCl, 1.25 NaH2PO4, 10 MgCl2, 0.5 CaCl2, 26 NaHCO3, 10 dextrose. Individual slices were gently stored in normal artificial cerebrospinal fluid (ACSF) for at least 1.5 h before recording. Normal ACSF was similar to the dissection buffer except that sucrose was replaced by 119 mM NaCl, MgCl2 lowered to 1 mM, and CaCl2 raised to 2 mM. The slices were transferred to a submerged recording chamber and perfused with ACSF (2 mL/min at 30°C). Both cutting and normal ACSF solutions were saturated with 95% O2/5% CO2 (pH 7.4). Visualized whole-cell recordings (IR-DIC, Axioskop 2 FS; Carl Zeiss AG, Göttingen, Germany) were made from layer II/III pyramidal cells (LII/III-PyrCs) located in the monocular visual cortex (V1M, ~350 μm lateral of lambda, ~35° depth from the pia). Pyramidal cells were identified by their pyramidal shaped somata, prominent apical dendrites, and regular-spiking pattern in response to depolarizing current (0.05-0.5 nA, 1 s). A subset of 20 pyramidal-like cells was filled with 0.1% Lucifer yellow, and we confirmed that some were spine-containing pyramidal cells, validating the selection criteria. Labeled cells were not used for electrophysiology. Borosilicate recording pipettes (4-6 MΩ) were filled with intracellular solution containing (in mM): 130 (Cs)Gluconate, 5.5 KCl, 1 ethylene glycol-bis(2-aminoethyl ether)-N,N,N’,N’-tetra acetic acid (pH = 8), 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 4 (Mg)ATP, 0.5 (Na4)GTP, 10 (Na2)phosphocreatine, and 5 QX-314 (Charles River; Sulzfeld, Germany) in dissection buffer (4°C; 290-290 mOsm) and a liquid junction potential of 16.3 mV.

Electrophysiological Experiments: Table 1

<table>
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<th>Age (d)</th>
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<th>Cells</th>
<th>Mice</th>
<th>Amplitude (μA)</th>
<th>Frequency (Hz)</th>
<th>Rise time 10-90% (ms)</th>
<th>Decay time (ms)</th>
<th>Rm (MΩ)</th>
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<td>2.6 ± 0.5</td>
<td>2.1 ± 0.2</td>
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<td>30</td>
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<td>470 ± 33</td>
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<td>4</td>
<td>10.7 ± 0.5</td>
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<td>2.2 ± 0.1</td>
<td>6.3 ± 0.5</td>
<td>517 ± 43</td>
<td>7.8 ± 1.2</td>
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<td>10.7 ± 0.7</td>
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<td>595 ± 74</td>
<td>7.8 ± 0.3</td>
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</table>

Statistically significant difference from NR condition at P < 0.05 with one-way ANOVA.

Behavioral Experiments

Adult male mice (P40-P50) were caged under a 12:12 h light/dark cycle with 35–40% relative humidity and food and water ad libitum. Mice were handled in a single session per day consisting of 3 blocks of 10 trials with 10 min breaks. Visual discrimination performance was quantified using a well-established two-alternative, forced choice, water discrimination task developed by Glen T. Prusky and collaborators (Prusky and Douglas 2004). In this task, the grating-versus-gray visual acuity threshold is determined by measuring the animal’s ability to discriminate 100% contrast, full cycle, sine-wave gratings of different spatial frequencies from isolated 50% gray (85 ± 5 lux, 46 cm from the monitor at water level). Gratings in the water task drifted at 1 Hz. With exception of experiments shown in Figure 8, visual stimulation always included the grating direction that was used during visual discrimination training. Mice were trained through 5 phases (Prusky and Douglas 2001; Iny et al. 2006) and learned to associate that swimming toward the gratings (i.e., the reinforced stimulus, CS+) at 0.148 c/d and reaching the platform was rewarded with escape from water, whereas swimming to the gray screen (i.e., the nonreinforced stimulus, CS−) was not. Swimming side-biases during training were corrected by early shaping (Prusky and Douglas 2004; Iny et al. 2006), and training was finalized when the animals reached a stable discrimination accuracy of ≥90% over 2 consecutive days (0.148 c/d; pre-1, 92.3 ± 1.1%, pre-2, 92.7 ± 1.0%, n = 59, paired t-test P = 0.626; 0.444 c/d; pre-1, 72.2 ± 1.2%, pre-2, 73.1 ± 1.0%, n = 59, paired t-test P = 0.894). On average, all experimental groups were visually tested after the same number of training days ([MTX+V16], 5.4 ± 0.6, n = 10; [MTX+V1], 4.9 ± 0.4, n = 49; [V16], 5.3 ± 0.3, n = 10; [V1], 5.1 ± 0.2, n = 24; [CPP+MTX+V16], 5.0 ± 0.3, n = 13; [MTX], 5.3 ± 0.5, n = 12; [CPP], 5.5 ± 0.4, n = 11; one-way ANOVA, F1,61 = 0.136, P = 0.762). “Warm-ups” of 5 training trials
Results

α_1-ARs Depress AMPAR-Mediated mEPSCs in Visually Stimulated Cortex

We first explored whether systemic injection of methoxamine, an α_1-adrenergic receptor (α_1-AR) agonist which crosses the blood-brain barrier, enables an operative form of experience-induced plasticity in the visual cortex of young mice. We injected young mice (P22 ± 1) with methoxamine (MTX, 5 mg/kg i.p.) or vehicle (9% NaCl) 10 min before visually stimulating with binocular drifting gratings for 1 h with 16 random directions (V_16, see Materials and Methods) (Fig. 1A). Changes in synaptic strength were monitored ex vivo by recording synaptic activity in the monocular portion of the primary visual cortex (V1M) (Fig. 1B). After reaching stable recording conditions, the first 300 spontaneous mEPSCs were aligned with respect to their rise time, and an averaged mEPSC per cell was used as readout for in vivo-induced cortical plasticity. The average mEPSC amplitude from all visually stimulated animals preinjected with methoxamine [MTX+V_16] was reduced by ~17% compared with control, normal reared mice injected with vehicle [NR] (P < 0.001; KS test) (Fig. 1C). This result indicates that systemic pharmacological activation of α_1-ARs in young mice enables LTD of cortical synapses after visual stimulation in vivo, hereafter referred to as α_1-AR LTD. The induction of α_1-AR LTD required the combination of active visual experience with α_1-AR activation because visual stimulation alone [V_16] or methoxamine alone [MTX] did not depress cortical synapses ([NR], 11.1 ± 0.4 pA, n = 54; [MTX+V_16], 9.2 ± 0.2 pA, n = 49; [V_16], 11.3 ± 0.3 pA, n = 40; [MTX], 10.8 ± 0.3 pA, n = 30; F_3,169 = 16.326, P < 0.001) (Fig. 1C and Table 1).

Many forms of synaptic plasticity are operative only during “critical periods” in the young postnatal brain (Iny et al. 2006; Goel and Lee 2007; Jiang et al. 2007; Mrsic-Flogel et al. 2007; Turrigiano 2008; Smith et al. 2009). We explored whether α_1-AR LTD persists into the adult visual cortex. In adult mice (P40 ± 1), the average mEPSC amplitude from LII/III-PyrCs in [MTX+V_16]-treated animals was reduced by ~21% as compared with [NR] (KS test, P < 0.001) (Fig. 1D). Also, consistent with the data from young mice, no changes in [V_16]- or [MTX]-treated groups were detected ([NR], 11.1 ± 0.6 pA, n = 27; [MTX+V_16], 8.7 ± 0.2 pA, n = 22; [V_16], 10.7 ± 0.5 pA, n = 38; [MTX], 10.7 ± 0.7 pA, n = 26; F_3,108 = 20.462, P < 0.001) (Fig. 1D and Table 1). Generally, a decrease in amplitude requires a decrease in postsynaptic sensitivity, whereas a change in mEPSC frequency is indicative of a modification in the...
probability of glutamate release. The mEPSC frequency at both age-groups was not affected by any of these manipulations (P22: F3,108 = 1.480, P = 0.242; P40: F3,108 = 2.132, P = 0.543) (Fig. 1C.D). In slices, neither brief bath application of MTX in vitro (5 µM, 10 min) nor its combination with a protocol that induces LTD affect paired-pulse depression at LII/III-PyrCs synapses (Trevino and Kirkwood 2008). All these observations speak in favor of a postsynaptic locus for the effect. Hence, z1-AR LTD is operative in LII/III beyond puberty, it is postsynaptically expressed and does not involve changes in mEPSC kinetics (decay time constant against that of [NR], P > 0.100 for all cases) (Table 1).

This form of experience-induced plasticity may follow the rules described for homeostatic synaptic scaling, a non-Hebbian form of plasticity that regulates neuronal excitability and synaptic strength under different regimes of network activity (Goel and Lee 2007; Turrigiano 2008). Homeostatic plasticity is considered to be global and multiplicative, such that all synapses in one cell are either increased or decreased in the same proportion, preserving the relative weights between synapses and optimizing the neuron’s “dynamic range” (Turrigiano 2008). To test for this possibility, we: 1) multiplied the mEPSC amplitude distributions from untreated [NR] animals by the appropriate scaling factor (P22: 83%; P40: 79%) and 2) removed scaled events that fell below the smallest mEPSC for the treated group at each age but still found significant differences against the distributions from aged-matched [MTX+V16]-treated mice (P < 0.001 both age groups) (Fig. 1E.F). These differences became evident at a wide range of amplitudes when subtracting either the [NR] or the [NR-scaled] distributions from the [MTX+V16] distributions (Fig. 1E,F), indicating that z1-AR LTD does not completely follow the rules of multiplicative scaling. Yet, this does not preclude the possibility of a homeostatic component. In sum, z1-AR agonists combined with grating stimulation produce a form of LTD, which is not fully homeostatic and is postsynaptically expressed in LII/III-PyrCs from juvenile and adult mice.

### z1-AR LTD is Blocked by an z1-AR Antagonist

If systemic methoxamine gates plasticity exclusively through z1-AR, then an z1-AR antagonist should block z1-AR LTD. To test for this, we injected adult mice (P40 ± 1) with either vehicle or prazosin (1 mg/kg i.p.), an z1-AR antagonist which crosses the blood-brain barrier, 10 min before applying the standard protocol for induction of z1-AR LTD (see above). As opposed to vehicle-, prazosin-treated mice did not present any change in average mEPSC amplitude ([NR], 10.2 ± 0.1 pA, n = 14; [vehicle+MTX+V16], 9.0 ± 0.1 pA, n = 21; [prazosin+MTX+V16], 10.7 ± 0.1 pA, n = 22; F2,53 = 13.318, P < 0.001) (Fig. 2). This result shows that z1-AR LTD is reproducible across visually stimulated mice, and it can be blocked by an z1-AR antagonist.

### Behavioral Assessment Of Mouse Visual Discrimination

The function of the mammalian visual cortex is plastic and shaped by visual experience throughout life (Prusky and Douglas 2004; Iny et al. 2006; Li et al. 2008). Thus, visual cortical plasticity should reconfigure sensory detection and perception. We investigated whether z1-AR LTD leads to changes in visual discrimination in adult mice. To quantify visual discrimination, we used a well-established water task designed to measure the visual acuity in rodents (Prusky and Douglas 2004). Visual discrimination performance depends on the retina but is also profoundly sensitive to pharmacological inactivation and ablation of the primary monocular and binocular visual cortex (Paxinos and Franklin 2001; Prusky et al. 2008). This justifies the usage of such a visual discrimination task to test for relevant modifications in the function of the visual cortex. Figure 3A shows a scheme of the visual task. Two monitors face into the arms of the Y-maze and simultaneously display a positive (CS⁺, conditioned or reinforced, i.e., the sinusoidal grating) and a negative (CS⁻, nonreinforced, i.e., 50% gray) stimulus (with 100% contrast). A submerged transparent platform below the CS⁺ serves as the corresponding unconditioned stimulus (US), whose position (together with CS⁻) in either arm of the maze varies pseudorandomly over trials. In the task, mice were released into the pool from a release chute and learned to swim toward the CS⁺ (correct choice) and to reach the platform because they were rewarded with escape from water. Otherwise, by choosing CS⁻, the mice had to continue swimming to reach the platform in the opposite arm (incorrect choice). By using this task, we trained the animals during 5 to 6 days until they reached a stable baseline level of visual discrimination performance ≥90% for 2 consecutive training sessions at low spatial frequency (0.148 c/d; correct, 95 ± 1%; n = 138, P = 0.620) (Fig. 3B). A “probe test” revealed that task performance was 96 ± 2% correct when the platform was hidden below CS⁺, but it fell to 6 ± 2% when the platform was placed below CS⁻ (n = 13 mice, t-test, P < 0.001) (Fig. 3C). The task performance was at chance levels of 48 ± 3% and 49 ± 2% when both monitors displayed either the CS⁺ or CS⁻ stimulus, respectively (n = 7 mice each) (Fig. 3C). These results demonstrate that visual discrimination with our experimental conditions is invariant to visual spatial cues other than those displayed by the monitors, excluding the possibility that the mice saw the hidden transparent platform. Given that the mice lacked sensory access to the US, the training trials can be regarded as retention trials.

We next assayed grating-versus-gray visual acuity using either vertically or horizontally oriented drifting gratings with 7 different frequencies versus gray. Figure 3D shows a sample frequency-of-seeing curve for a mouse trained with vertical gratings. The point at which the curve intersects 75% accuracy (0.49 c/d for this case) was adopted as the acuity threshold for each mouse (Prusky and Douglas 2004; Iny et al. 2006). On average, the binocular acuity threshold was 0.45 ± 0.01 c/d (n = 9) and 0.45 ± 0.02 c/d (n = 6) for mice trained with...
vertical and horizontal gratings, respectively (Fig. 3E). These estimations did not differ from each other and were pooled together ($F_{1,12} = 0.685$, $P = 0.403$) (Fig. 3E), indicating that the mice discriminate vertically and horizontally oriented sine-wave drifting gratings against 50% gray equally well.

**$z_1$-ARs Decrease Visual Discrimination Accuracy in Visually Stimulated Adult Mice**

Maximum contrast sensitivity occurs at low spatial frequencies (Niel and Stryker 2008), but visual acuity is only sensitive at higher spatial frequencies (Prusky and Douglas 2004; Iny et al. 2006). This means that plastic changes of cortical function should be robust at 0.45 c/d, where mice discriminate at ~75%, allowing bidirectional modification (Fig. 3E). We therefore directed our measurements to monitor putative changes in visual discrimination accuracy at high (0.444 c/d) and, for reference, at low (0.148 c/d) spatial frequencies. This approach was advantageous as it permitted us to quantify visual discrimination within a single training session. Before visually stimulating, we allowed the mice to achieve stable visual performance at both frequencies. Discrimination behavior reveals no apparent changes by any treatment at a low spatial frequency of 0.148 c/d (Fig. 4). At a high spatial frequency of 0.444 c/d, however, we found that the [MTX+V16] treatment reduced visual discrimination accuracy by ~18% (0.444 c/d: pre, 75.09 ± 2.72%, post, 63.00 ± 3.35%), but no changes were detected in [V16]- or [MTX]-treated mice (pre vs. post; [MTX+V16], 0.148 c/d, $n = 10$, $P = 0.07$; 0.444 c/d, $P < 0.01$; [V16], 0.148 c/d, $n = 10$, $P = 0.514$; 0.444 c/d, $P = 0.913$; [MTX], 0.148 c/d, $n = 12$, $P = 0.728$; 0.444 c/d, $P = 0.674$) (Fig. 4). A one-way ANOVA test confirmed the significance of this difference ($F_{2,28} = 7.423$, $P = 0.021$). Notably, this form of visual discrimination plasticity was not accompanied by changes in other behavioral indicators that we quantified for all groups. These included path length (pre vs. post; correct choices, 0.148 c/d, $P > 0.1$; 0.444 c/d, $P > 0.18$; incorrect choices, 0.444 c/d, $P > 0.09$) and latency that is, the time interval required to reach the platform from the release chute (pre vs. post; correct choices, 0.148 c/d, $P > 0.1$; 0.444 c/d, $P > 0.13$; incorrect choices, 0.444 c/d, $P > 0.1$) (Table 2). Taken together, the reduction in visual discrimination accuracy is a behavioral correlate of $z_1$-AR LTD and may reflect its strong dependency on local V1 microcircuit processing.

**$z_1$-AR LTD Reduces the Quantal Amplitude of Ascending Inputs to Layer II/III**

Visual stimulation activates only a subset of inputs in LII/III (Chung and Ferster 1998; Fiser et al. 2004), and visual experience should act at these synapses. Average mEPSCs are derived from a mixed population of synapses contacting the cell, but it is not clear whether $z_1$-AR LTD is expressed only at specific synaptic inputs. From the work of R. Miledi (Miledi and Slater 1966), we know that when Ca$^{2+}$ is replaced by strontium (Sr$^{2+}$) in normal ACSF, electrical stimulation of presynaptic fibers leads to a combination of synchronous and a barrage of asynchronous EPSCs (SrEPSCs, see Materials and Methods), which occur for up to 1 s after stimulation. In this way, it is assumed that the majority of the evoked asynchronous SrEPSCs originate from the stimulated axons, allowing an estimation of quantal properties of transmission at different inputs (Oliet et al. 1996; Bender et al. 2006). We used this approach by using monopolar electrical stimulation of LIV and LII/III to determine the number of quantal events responsible for the asynchronous EPSCs (SrEPSCs, see Materials and Methods).
characterize the SrEPSC quantal amplitude biased toward 1) ascending (IV → II/III) and 2) intralaminar (II/III → II/III) synapses to LII/III-PyrCs, respectively (Fig. 5A,B). For each "pathway,"

![Figure 4.](attachment:image)

**Figure 4.** β1-AR agonists decrease visual discrimination accuracy in visually stimulated mice. (A–C) Visual discrimination performance for individual mice at 0.148 c/d (left) and 0.444 c/d (middle) for 2 consecutive days before (pre-1, white dots; pre, gray dots) and after visual manipulations (post, black dots; see Materials and Methods). (A) Combining methoxamine with visual stimulation [MTX+V16] reduced visual discrimination accuracy at high spatial frequencies, but no reduction was found with [V16] alone (C). Plots on the right depict the normalized cumulative probability distributions for discrimination at 0.444 c/d 1 d before (pre, gray) and 7–9 h after (post, black) visual experience. Stable performance before plastic manipulations (pre-1 vs. pre; [MTX+V16], 0.148 c/d, n = 10, P = 0.779; 0.444 c/d, P = 0.724; [V16], 0.148 c/d, n = 10, P = 0.586; 0.444 c/d, P = 0.929; [MTX], 0.148 c/d, n = 12, P = 0.864, 0.444 c/d, P = 0.823). The number of animals is indicated in parenthesis. Asterisks depict statistical significance.

![Table 2](attachment:table)

**Table 2.**

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asynchronous SrEPSCs were plotted for each cell (Fig. 5C) and averaged for each group (Fig. 5D). In normal reared mice (NR), no significant difference between the asynchronous SrEPSC amplitudes was detected between pathways (ascending: 11.93 ± 0.92 pA; intralaminar: 12.65 ± 0.87 pA, n = 12) (Fig. 5E). These average amplitudes were similar to the average amplitude of spontaneous SrEPSCs, recorded before the evoked ones (spontaneous: 12.04 ± 1.10 pA, n = 12; [MTX+V16], 8.46 ± 0.51 pA, n = 13; F1,22 = 7.690, P = 0.005) (Fig. 5E,F). This difference was also detected for asynchronous SrEPSCs from ascending but not from intralaminar inputs (ascending: [NR], 11.93 ± 0.91 pA, n = 12; [MTX+V16], 9.29 ± 0.76 pA, n = 13; F1,22 = 4.058, P = 0.044; intralaminar: [NR], 12.65 ± 0.87 pA, n = 12; [MTX+V16], 11.51 ± 0.73 pA, n = 13; F1,22 = 0.950, P = 0.324) (Fig. 5E,F). Accordingly, in [MTX+V16]-treated mice, asynchronous SrEPSCs from intralaminar inputs were bigger than those evoked from ascending inputs or from spontaneous SrEPSCs (F2,35 = 9.129, P = 0.011) (Fig. 5E,F). Notably, the amplitudes for spontaneous and asynchronous SrEPSCs evoked by layer IV stimulation were not statistically different (P = 0.357) possibly because in our conditions asynchronous SrEPSCs are evoked in a background of spontaneous SrEPSCs. These results suggest that β1-AR LTD in vivo can be associated with a specific decrease in quantal size of ascending inputs to LII/III.

### β1-AR LTD is Graded by the Number of Orientations Used During Visual Stimulation and is NMDAR-Dependent

β1-AR LTD may act homosynaptically over a subset of synapses that are orientation specific (Kirkwood and Bear 1994; Jia et al. 2010). If so, the cumulative amount of depression, reflected here as a change in average mEPSC amplitude, should be graded by the number of directions used during visual stimulation. We tested this idea by visually stimulating MTX-injected mice with 1, 8, or 16 random directions (see Materials and Methods) at a fixed duration of 3.75 min/direction, as given by our previous stimulation paradigm with 16 directions for 1 h. We found that mEPSCs were depressed by ~0.5%, ~12.9%, and ~21.8% when stimulating with 1, 8, and 16 directions, respectively (P40: [NR], ~21.8%).
11.1 ± 0.6 pA, n = 27; [MTX+V1], 11.0 ± 0.2 pA, n = 28; [MTX+V8], 9.6 ± 0.1 pA, n = 29; [MTX+V16], 8.7 ± 0.2 pA, n = 22; F9,288 = 53.992, P < 0.001 (Fig. 6A–D and Table 1). Plasticity induced within 30 or 60 min by visual stimulation with 8 or 16 directions was not fully homeostatic (KS test against scaled [NR], P < 0.001 in both cases) (Fig. 6B). There was no change in mEPSC frequency (Fig. 6E and Table 1) or kinetics (against decay time constant of [NR], P > 0.1 for all cases) (Table 1). These results confirm that α1-AR LTD in vivo is not fully homeostatic and is reflected as a graded reduction in mEPSC amplitude proportional to the number of nonoverlapping grating directions used during visual experience.

Changes at the mEPSC level may require robust plasticity occurring at multiple synapses, and the amount and specificity of synaptic plasticity could be affected by the duration of visual experience. We asked whether the graded changes in mEPSC amplitude by α1-AR LTD could be explained rather by the duration but not by the number of orientations used during visual stimulation. For this, we compared 2 stimulation paradigms that maximized the difference between the number of orientations, namely V1 and V16, since the closer these numbers are, the more difficult it would be to detect a putative dependency on duration. We found that visual stimulation of [MTX]-injected mice for 1 h with 1 direction [MTX+V1 × 1 h] rendered an average mEPSC that was similar to [MTX+V13.75 min]- but was different to [MTX+V16 × 1 h]-treated mice. Also, doubling the time of stimulation with 16 directions [MTX+V16 × 2 h] did not produce additional effects compared with [MTX+V16 × 1 h]-treated mice ([NR], 11.1 ± 0.6 pA, n = 27; [MTX+V1 × 3.75 min], 11.0 ± 0.2 pA, n = 28; [MTX+V1 × 1 h],...
Visual Discrimination Plasticity with Single-Oriented Gratings by α1-ARs Requires NMDAR Activation

It is possible that α1-AR LTD produced with single-oriented gratings at specific inputs (V1, Fig. 6) may have remained undetected at the mEPSC recording level, simply because the contributions of affected and unaffected synapses were averaged. Yet, we hypothesized that α1-AR LTD induced with single-oriented gratings in vivo could be detected at the behavioral level. We combined systemic methoxamine with 1 h of vertically (or horizontally) oriented grating stimulation (i.e., a single direction) and tested for visual discrimination changes using gratings with the same direction. We first trained a naïve set of mice until their visual performance was stable at both spatial frequencies for 2 days before visual manipulations (pre-1 vs. pre) (Fig. 7). Similar to Figure 4, single-oriented stimulation in the presence of MTX produced no discrimination changes at 0.148 c/d ([MTX+V1], n = 21, P = 0.322; [V1], n = 24, P = 0.287; [CPP], n = 11, P = 0.170; [CPP+MTX+V1], n = 13, P = 0.633) (Fig. 7). However, at 0.444 c/d discrimination was reduced by ~18%, as observed before (pre, 73.09 ± 2.20%; post, 60.00 ± 2.13%, n = 21, P < 0.001; KS test, P = 0.004) (Fig. 7A). Accordingly, this discrimination plasticity required NMDARs: systemic CPP (or vehicle alone) did not change the discrimination behavior of trained mice when tested 7 h after injection (pre vs. post; [V1], n = 24, P = 0.925; [CPP], n = 11, P = 0.603) (Fig. 7B,C) (Frenkel et al. 2006) but prevented discrimination changes produced by [MTX+V1] (n = 13, P = 0.697; KS test, P = 0.974) (Fig. 7D). A one-way ANOVA test confirmed the significant difference at 0.444 c/d between experimental groups (F_{6,64} = 17.033, P < 0.001). We conclude that in MTX-injected animals, stimulation with single-oriented gratings leads to a reduction in visual discrimination of gratings with the same orientation, and this form of plasticity is blocked by an NMDAR antagonist.

Visual Discrimination Plasticity by α1-ARs is Orientation Specific

If the plastic change in visual discrimination induced with single-oriented gratings relies exclusively on activated synapses, and if these changes do not transfer to nonactivated synapses during sensory experience, then discrimination should remain intact when animals are stimulated and tested with orthogonal gratings. To test for this, we trained mice to discriminate either vertical (V) or horizontal (H) gratings and stimulated them with 1 h of V- or H-gratings in the presence of MTX. Animals discriminated stably before visual manipulations (pre-1 vs. pre; [VH], 0.148 c/d, n = 12, P = 0.258; 0.444 c/d, P = 0.743; [VH], 0.148 c/d, n = 12, P = 0.542; 0.444 c/d, P = 0.854; [HH], 0.148 c/d, n = 13, P = 0.205; 0.444 c/d, P = 0.648; [HV], 0.148 c/d, n = 13, P = 0.205; 0.444 c/d, P = 0.648) that were preinjected with CPP (P40: [NR], 11.1 ± 0.6 pA, n = 27; [MTX+V16], 8.7 ± 0.2 pA, n = 22; [CPP+MTX+V16], 10.7 ± 0.2 pA, n = 35; F_{2,88} = 53.992, P < 0.001) (Fig. 6D). However, CPP could interfere with the induction of experience-induced plasticity indirectly, by decreasing the level of network activity. We doubled the duration of visual stimulation in the presence of MTX and found that CPP still blocked the induction of α1-AR LTD in vivo ([CPP+MTX+V16, × 2 h], 10.9 ± 0.4 pA, n = 20; paired t-test against [NR], P = 0.379) (Fig. 6D). Altogether, these results indicate that α1-AR LTD in vivo is orientation dependent and blocked by NMDAR antagonists.

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0.148 c/d, n = 12, P = 0.993; 0.444 c/d, P = 0.439) (Fig. 8). Visual discrimination accuracy was reduced when the mice were stimulated and tested with equally oriented gratings (Fig. 8A,C; see also Fig. 7A). However, no change in discrimination was detected when the animals were stimulated and tested with orthogonal gratings (Fig. 8B,D) (pre vs. post, [V], 0.148 c/d, P = 0.573; 0.444 c/d, n = 12, P = 0.002; [VII], 0.148 c/d, n = 12, P = 0.264; 0.444 c/d, P = 0.927; [VIII], 0.148 c/d, n = 13, P = 0.019; 0.444 c/d, P = 0.002; [HV], 0.148 c/d, n = 12, P = 0.243; 0.444 c/d, P = 0.728). We conclude that visual discrimination plasticity by z₁-Rs is reproducible and orientation specific because it is not transferred across orthogonal orientations.

z₁-AR LTD in Visually Stimulated Cortex is Reversible at Synaptic and Behavioral Level

Electrophysiological and behavioral correlates of visual deprivation can be reversed by reexposure to light (Iny et al. 2006; He et al. 2007; Turrigiano 2008). For instance, the plastic changes in mEPSC amplitude by 2 days of dark rearing (DR) in aged mice are rapidly reversed by reexposing the animals to light for 1 day (Goel and Lee 2007). With this in mind, we measured the changes in mEPSCs (Fig. 9A) and tested visual discrimination (Fig. 9B,C) 1 day after inducing z₁-AR LTD. During that day, animals were reared under normal (12/12 h) lighting conditions (+1d NR). Indeed, as it occurs with deprivation-induced plasticity, we found that z₁-AR LTD was synthetically and behaviorally reversed after 1 day of normal rearing conditions (mEPSCs: [NR], 11.1 ± 0.6 pA, n = 27; [MTX+V16], 8.7 ± 0.2 pA, n = 22; [MTX+V16+1d NR], 11.1 ± 0.4 pA, n = 31; F3,28 = 53.399, P < 0.001; 0.444 c/d, [MTX+V16], pre, 75.55 ± 0.03%; post, 50.00 ± 4.14%; post + 1d NR, 73.88 ± 3.72%; n = 9, P = 0.741; [MTX+V16], pre, 75.00 ± 0.03%; post, 63.00 ± 3.35%; post + 1d NR, 73.00 ± 3.06%; n = 10, P = 0.373). We confirmed this conclusion by using KS tests on the appropriate electrophysiological and behavioral distributions (mEPSCs: P = 0.870; 0.444 c/d, [MTX+V16], P = 0.957; [MTX+V16], P = 0.974) (Fig. 9A–C). Reversal of z₁-AR LTD depression did not occur when mice were kept in complete darkness for 1 day after induction (+1d DR) but reexposure to light (+1d NR) fully reversed z₁-AR LTD (mEPSCs: [NR], 11.0 ± 0.2 pA, n = 12; [MTX+V16 + 1d DR], 8.6 ± 0.1 pA, n = 13; [MTX+V16+1d DR + 1d NR], 12.8 ± 0.2 pA, n = 13; F3,28 = 19.394, P < 0.001; 0.444 c/d, [MTX+V16], pre, 78.12 ± 2.24%; post + 1d DR, 62.50 ± 3.10%; post + 1d DR + 1d NR, 75.62 ± 4.45%; n = 9, P = 0.606) (Fig. 9D,E). Note that dark rearing for 1 day does not produce homeostatic changes at the mEPSC-recording level (Goel and Lee 2007). Therefore, our results indicate that z₁-AR LTD is rapidly reversed by light at synaptic and behavioral levels.

Discussion

Previous studies have shown that experience-induced plasticity requires the obligatory participation of neuromodulatory systems regulating attention and emotion (Bear and Singer 1986; Manunta and Edeline 2004; Froemke et al. 2007; Hu et al. 2007; Maya Vetencourt et al. 2008; Sara 2009). Here, we explored how z₁-ARs interact with visual experience to gate and produce an operative form of synaptic plasticity in the visual cortex in vivo. Our approach combined ex vivo electrophysiological recordings with behavioral tests, allowing us to characterize the synaptic properties and behavioral correlates of z₁-AR LTD.

Binocular deprivation reduces network activity and leads to slow forms of homeostatic plasticity of cellular intrinsic and synaptic (i.e., mEPSC) properties (Goel and Lee 2007; Turrigiano 2008). In contrast, z₁-AR LTD was rapidly expressed at specific synapses from LII/III-PyrfCs of juvenile and adult mice (see also Frenkel et al. 2006). z₁-AR LTD operated in a feedforward and synapse-specific manner. It was induced after a very short period of intense visual grating stimulation (as brief as 30 min) and did not fully satisfy the scaling property.
described for “global” homeostatic plasticity. Alternatively, α1-AR LTD could be understood as a form of non-multiplicative scaling (see Goel and Lee 2007), although an interaction with a slower homeostatic component cannot be discarded. The apparent lack of global changes in synaptic strength following visual manipulations can be easily explained by the limited synaptic coverage of visual inputs into the cortex (Chung and Ferster 1998; Fiser et al. 2004). In agreement, α1-AR LTD was expressed at ascending inputs carrying visual information to LII/III. We focused on AMPAR-mediated transmission into LII/III-PyrCs in the visual cortex. Concomitant changes in and into local inhibition (Maffei et al. 2006; Morishita et al. 2010; Trevino et al. 2011; but see Jiang et al. 2007), during induction and/or expression, or in the peripheral visual system, cannot be discarded. Future studies will be necessary to elucidate how, where, and over which time scales these mechanisms interact to modify and stabilize cortical circuitry.

As opposed to other mammals, orientation selective cells are dispersed in the mouse visual cortex, forming intermingled ensembles of cells that are tuned to different orientations. Cellular orientation selectivity in the cortex may arise from complex interactions between geniculate projections and intracortical axons, but at least a portion of the excitatory inputs reaching LII/III is also “tuned” and codes for different grating orientations (Nelson et al. 1994; Jia et al. 2010). Our results evidenced that α1-AR LTD was orientation-dependent at LII/III synapses, where neurons have small receptive fields and sharp orientation tuning. As expected for plasticity occurring at different sets of orientation-tuned synapses (Jia et al. 2010), plastic changes detected at the mEPSC recording level were graded by the amount of orientations used during visual stimulation. Favoring the notion of Hebbian plasticity (Kirkwood and Bear 1994; Smith et al. 2009), α1-AR LTD was electrophysiologically and behaviorally blocked by systemic CPP, a selective NMDAR-antagonist (Clem et al. 2008; Sato and Stryker 2008). It could be argued that CPP “blocked” the experience-induced plasticity by decreasing network activity. We doubled the duration of visual stimulation, but CPP still blocked α1-AR LTD.

Rodents possess a sophisticated visual system and constitute an excellent model organism to study the cellular and molecular mechanisms of experience-dependent plasticity (Smith et al. 2009). In rodents, as in other mammals, deprivation from visual experience affects synaptic strength at the cellular (Goel and Lee 2007) and network (He et al. 2007) levels and reduces the

Figure 8. Visual discrimination plasticity is orientation specific. Mice trained with vertical (A,B) and horizontal (C,D) gratings were injected with methoxamine (5 mg/kg, i.p.) and subjected to 1 h of stimulation with either vertical (V) or horizontal (H) gratings. (A–D) Visual discrimination performance for individual mice at 0.148 c/d (center-left panel) and 0.444 c/d (center-right panel) for 2 consecutive days before (pre-1, white dots; pre, gray dots) and after visual manipulations (post, black dots; see Materials and Methods). Plasticity in visual discrimination accuracy was detected in those animals that were stimulated and tested with equally oriented gratings (A,C) but not with orthogonal-oriented gratings (B,D). The number of animals is indicated in parenthesis. Asterisks depict statistical significance.
capacity to make visual discriminations (Prusky et al. 2000). Our results indicate that $\alpha_1$-AR LTD in the primary visual cortex correlates with a rapid drop in discrimination accuracy at high spatial frequencies in adult mice. Most likely, $\alpha_1$-AR LTD entails multiple changes at the synaptic, network, and perceptual level. One could argue that stress associated with our experimental protocols could modulate attention and affect the measurement of visual discrimination responses. This scenario would imply that changes in visual discrimination should be observed only in those mice that were treated with agonists and that paid less attention to testing stimuli. However, this is unlikely as the motor performance before and after visual manipulations did not differ between sessions or conditions. Since visual discrimination at high spatial frequencies was neither at ceiling nor at chance level, differences in stress level or attention would have been expected to produce differences in behavior and choice, but this was not the case for mice treated with the agonist only (Table 2). A plausible explanation is that the depression at cortical excitatory synapses by $\alpha_1$-AR LTD is responsible for the rapid detriment in visuomotor performance. Supporting this view, visual discrimination plasticity of high-frequency patterns was orientation specific, that is, it was not transferred between orthogonal gratings, thereby reflecting a direct contribution of the visual cortical circuit (Gilbert et al. 2001). Indeed, the changes in the visual discrimination performance correlate with stimulus orientation during visual experience, suggesting that feature-specific changes were initiated in early visual cortex. Despite the fact that visual discrimination per se was independent of NMDARs, discrimination plasticity was blocked by CPP reflecting similarities with the canonical homosynaptic LTD (Kirkwood and Bear 1994; Smith et al. 2009).

Susceptibility to reversal by spontaneous activity is a common feature of cortical plasticity in vivo (Xu et al. 1998; Yao and Dan 2001). As it occurs for homeostatic scaling up of synapses by visual deprivation (Goel and Lee 2007; Turrigiano 2008; Smith et al. 2009), the synaptic and behavioral manifestations of $\alpha_1$-AR LTD were both reversed by light after 1 day of normal rearing. Thus, homeostatic plasticity and $\alpha_1$-AR LTD may share some of the molecular mechanisms that underlie structural and functional plasticity in the upper visual cortex: they both depend on visual experience and do not involve changes in mEPSC frequency or kinetics (Goel and Lee 2007). Notably, the rapid reversal in visual discrimination performance after induction of $\alpha_1$-AR LTD is in contrast with the delayed acuity recovery after longer periods of deprivation (Iny et al. 2006), which may reflect slower anatomical compensations.

Plasticity in discrimination of grating-orientation and complex contours alters V1 tuning for the trained feature (Gilbert et al. 2001; Furmanski et al. 2004; Meliza and Dan 2006). Yet, the exact neural mechanisms of visual learning still remain controversial. At the level of input detection, training can increase neural responses to reinforced stimuli, shift tuning curves toward (or away from) trained stimuli, or sharpen these curves (and their slopes) to improve discrimination. Some studies favor the notion that changes in V1 orientation-tuning and in discrimination of orientation result from an increase in the number of neurons responding to the trained stimulus.

Figure 9. $\alpha_1$-AR LTD and decrease in visual discrimination are reversible. (A) $\alpha_1$-AR LTD (black) is reversed after 1 day of rearing the animals in normal lighting conditions (+1d NR; dotted). [NR]-trace from vehicle-injected animals (gray) as reference. (B, C) Course for visual discrimination accuracy at low (0.148 c/d; thin line) and high (0.444 c/d; thick line) spatial frequencies, measured before (pre-1, pre) and after (post, post+1) visual manipulations. In methoxamine-injected mice, the plasticity in visual discrimination produced by stimulation with $V_{16}$-gratings (B) or $V_{1}$-gratings (C) is fully reversed after 1d NR. Dark rearing the animals for 1 day after induction (+1d DR) delays the reversal produced by 1d NR at the electrophysiological (D) and behavioral (E) level. Line plots on the right depict the normalized cumulative probability distributions for discrimination accuracy at 0.444 c/d: 1 d before (pre, gray), 7–9 h after (post, black), 1 day after (post+1, black discontinuous), and 2 day after (post+2, gray discontinuous) controlled visual experience. The number of animals is indicated in parenthesis. Asterisks depict statistical significance.
(Furmaniski et al. 2004), while others suggest that perceptual improvement can be explained by changes in synaptic strength (Yao and Dan 2001; Meliza and Dan 2006). Frenkel et al. (2006) show that repeated exposure of awake, restrained, alert mice to grating stimuli of certain orientation induces LTP of visually evoked potentials (VEPs) to the trained orientation. The potentiation occurs in juveniles and adults, is specific to the trained eye (i.e., does not transfer to the untrained eye), and develops over multiple days of training (Frenkel et al. 2006). The fact that VEP plasticity occurs only for previously presented but not for novel stimuli indicates that grating-induced plasticity does not generalize in the mouse visual cortex. This lack of transfer in learning from one stimulus to another strongly suggests that the underlying plasticity occurs in primary sensory cortex (Gilbert et al. 2001) and thus meets the criterion of input specificity. Our results are compatible with these observations but highlight an important additional feature: grating stimulation can also lead to a rapid orientation-specific depression provided that synapses are primed by Gq-linked 1-ARs. Thus, the difference in the polarity of plasticity may rely in the neuromodulator levels associated with alert mice (Jones 2005; Ramos and Arnsten 2007; Sara 2009). A tempting prediction would be that bidirectional VEP plasticity turns into VEP LTD when 1-ARs override the action Gs-linked GPCRs.

Mood, attention, and motivation covary with the activity of neuromodulatory systems in the brain to influence behavior. The "tones" of arousal-promoting neuromodulators are high during wakefulness and low during sleep (Hill and Tononi 2005; Jones 2005), gating, and regulating plasticity (Vyazovskiy et al. 2008). Thus, volume transmission by release of neuromodulators in response to environmental imperatives serves to modify the activity of neuronal networks and facilitate their reorganization. This work illustrates how systemic activation of 1-ARs combined with patterned visual stimulation leads to a form of stimulus-specific LTD which rapidly reconfigures the transfer of sensory information into layer II/III of the visual cortex, thus affecting visual discrimination of similar stimuli. Plasticity mechanisms translate information from the external world into networks that are adaptively shaped to process information (Tropea et al. 2009). Elucidating how these mechanisms operate and interact is fundamental to understand how the brain allocates attention and apprehends the environment to select, store, and retrieve information for generating adaptive behavior.

**Funding**

M.T. was supported by a Max Planck Fellowship.

**Notes**

We thank Dr R. J. De Marco (R.M.) for helpful discussions; R. M., J. Ledderose, and H. Salgado for useful comments on the manuscript; K. Schmidt for constructing components of the water maze; R. Rödel, S.-B. Li, C. Roome, and S. Hundemer for excellent help during the development of this project. We thank Prof. P.H. Seeburg for constant support. **Conflict of Interest:** None declared.

**References**


