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Supporting Online Material for

Lynx1, a Cholinergic Brake, Limits Plasticity in Adult Visual Cortex

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Supporting online material for "Lynx1, a cholinergic brake limits plasticity in adult visual cortex"

1. Materials and Methods

Animals Wild-type (C57 Bl/6; SLC Japan, and Charles River USA) and lynx1 knockout (KO) mice (*1*) were used. Light-reared (LR) animals were raised from birth on a 12-hour light/dark (L/D) cycle to various postnatal ages. N=3 to 7 mice were used per experimental condition for quantitative Western Blotting or immunohistochemistry.

Quantitative RT-PCR *Lynx1,2* mRNA and *β-actin* mRNA were quantified by real-time PCR using commercially available gene-specific primers and TaqMan gene expression assay (Applied Biosystems).

In situ hybridization Mouse cDNA fragments of *lynx1, lynx2, nAchR β2, PV, or GAD65* were amplified by PCR. Probes were synthesized using T3/T7 RNA polymerase (Roche) labeled with digoxigenin or fluorescein and hybridized to frozen sections. To amplify the signal, probes were detected using anti-digoxigenin or fluorescein antibody conjugated to Alkaline Phosphatase (Roche), or the TSA-Plus DNP System (PerkinElmer Life Sciences) in combination with fast red staining for double FISH.

Western Blotting Visual cortex from isoflurane-anesthetized mice was dissected and white matter stripped by aspiration before homogenizing by sonication in 50mM tris-HCl, pH7.4 containing 10mM EDTA and 1mM PMSF. Total protein concentration was determined by colorimetric (Bradford) assay system (BIO RAD) and used to normalize loading. Equal amounts of protein sample (20 or 40µg per lane) were separated by SDS/PAGE and transferred to PVDF membranes (ATTO Corporation) with semi-dry blotter. Membranes were blocked with 5% skim milk in TBS, pH7.6 containing 0.1% Tween20 for 2h, then treated with rabbit polyclonal anti-lynx1 (1:1000) (*2*) overnight at 4° C, washed and incubated with AP-conjugated secondary antibody. Blots were visualized by adding NBT/BCIP solution.

Immunohistochemistry. Mice were perfused transcardially with 0.9% saline then 4% para-formaldehyde, and brains removed into 30% sucrose/paraformaldehyde for cryoprotection. Brains were cut in coronal section (30µm) on a freezing microtome. Sections were rinsed in

PBS, then incubated overnight at 4°C in monoclonal antibody against Myelin Basic Protein (MBP: Chemicon, 1:200) or biotin-WFA (1:400), followed by secondary antibodies (anti-mouse IgG-Alexa-488, 594, streptavidin-488, 1:400).

Monocular deprivation (MD) procedure Eyelid margins were trimmed by iris scissor and eyes sutured shut under isoflurane anesthesia. Eyes were closed 4-5 days for short term MD (STMD), from P19 to >P60 for long term MD (CP-LTMD), and from P19 to P33 for recovery studies.

Extracellular recording in vivo Electrophysiological recording was performed under nembutal / chlorprothixene anesthesia using standard techniques for mice (*3, 4*). Ocular dominance in the binocular zone of each mouse was calculated as a contralateral bias index (CBI): [(n1-n7)+2/3(n2-n6)+1/3(n3-n5)+N]/2N, where N=total number of cells and nx=number of cells corresponding to ocular dominance score of x (*4*). For statistical comparison of OD distributions, normalized OD scores of single neurons were plotted as cumulative distribution for each experimental group. OD score was computed by PSTH analysis of peak to baseline spiking activity in response to each eye: {[Peak(ipsi)-baseline(ipsi)]–[Peak(contra)-baseline(contra)]} / {[Peak(ipsi)-baseline(ipsi)] +[Peak(contra)-baseline(contra)]} (*5*).

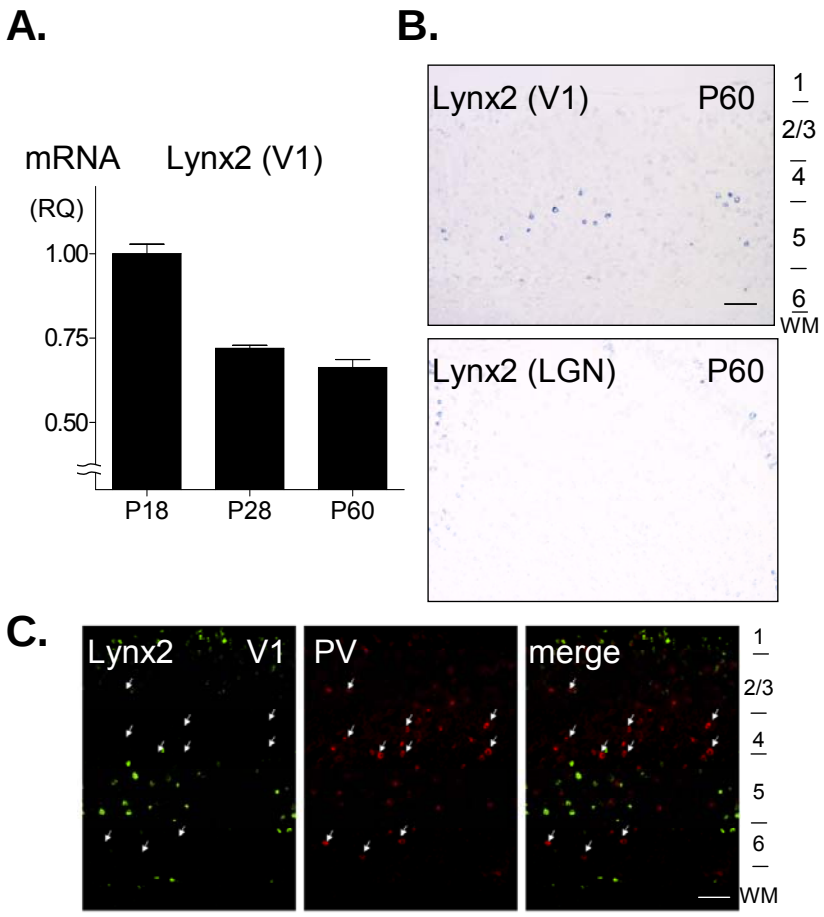
Visual Evoked Potentials VEPs were recorded under nembutal / chlorprothixene anesthesia using standard techniques in mice (*6*). A tungsten electrode was inserted into V1 where the maximal VEP response is located within the visual field 20° from the vertical meridian (usually 3mm from lambda). To record VEPs, the electrode was advanced to a depth of 100 - 400 μm within cortex where VEPs exhibit their maximal amplitude. Signals were band-pass-filtered (0.1–100 Hz), amplified, and fed to a computer for analysis. In brief, at least 20 events were averaged in synchrony with the stimulus contrast reversal. Transient VEPs in response to abrupt contrast reversal (1Hz) were evaluated in the time domain by measuring the peak-to-baseline amplitude of the major negative component. Visual stimuli were horizontal sinusoidal gratings of different spatial frequencies at 90% contrast. Visual acuity was obtained by extrapolation to zero amplitude of the linear regression through the last four to five data points along a curve of VEP amplitude plotted against log spatial frequency.

Drug administration. Nicotine (1mg/kg, s.c.) was injected during VEP recording from V1. Mecamylamine (2.5 mg/kg) or a mixture of α4- + α7-selective DHβE (2 mg/kg) + MLA (5 mg/kg)

were administered systemically (daily, i.p.) (*7*). Focal mecamylamine (50mM) or Diazepam (2 mg/ml in 50% propylene glycol) were administered via low-flow osmotic mini-pump infusion directly into V1 (1.0 µl/hr, 200 ml over 5-7 days; Alzet Model 2001, Alza) (*3*) before recording. Acetylcholinesterase Inhibitor (AchEI: physostigmine, 0.1mg/kg, i.p.) was injected daily from P45 until one day before recording.

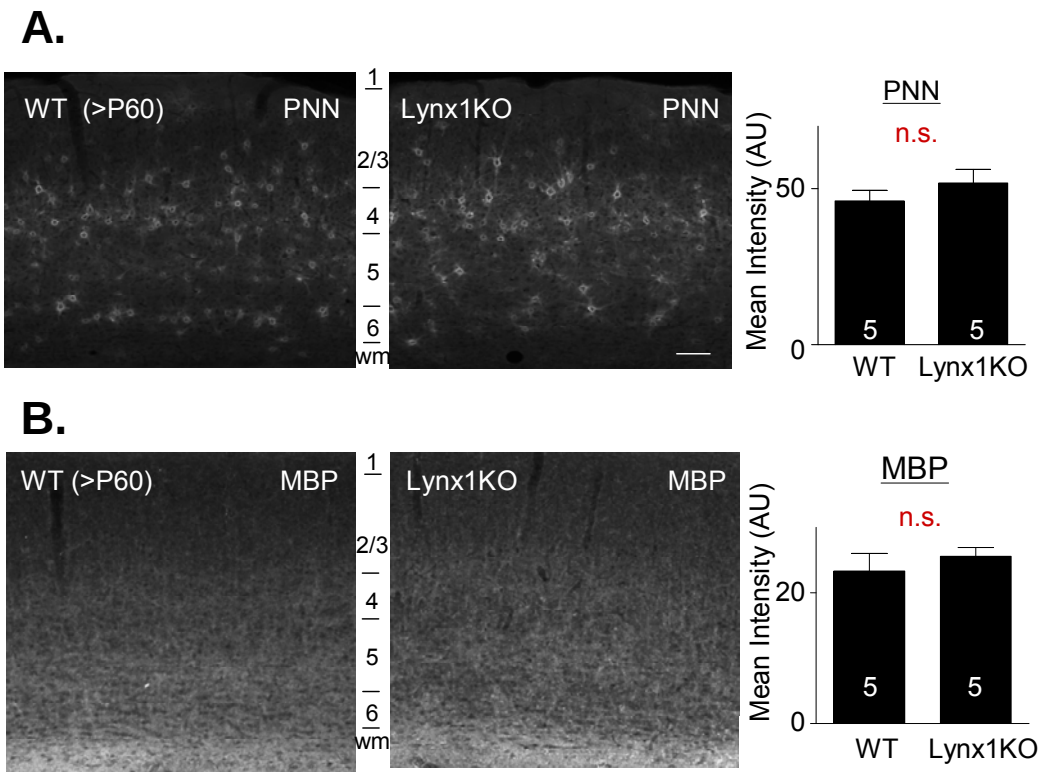
2. Supporting Figures

Supporting Figure 1. Lynx2 expression in visual system **(A)** Lynx2 mRNA expression decreases across CP. $P < 0.0001$, One-way ANOVA. Mean ± sem. **(B)** In situ hybridization of *lynx2* in adult V1 (upper panel) and LGN (lower panel). Scale, 100 μm **(C)** Double in situ hybridization of lynx2 (green) & PV (red) in adult V1. Note lynx2 does not co-localize with PV, while lynx1 does (Fig.4B).



Supp. Fig.1

Supporting Figure 2. Normal perineuronal nets and myelination in Lynx1 KO mice. (A) WFA staining of adult V1 in WT (left) and lynx1 KO (middle) mice. P > 0.3, t-test. (B) MBP staining of adult V1 in WT (left) and lynx1 KO (middle) mice. P > 0.4, t-test. Scale, 100 μm

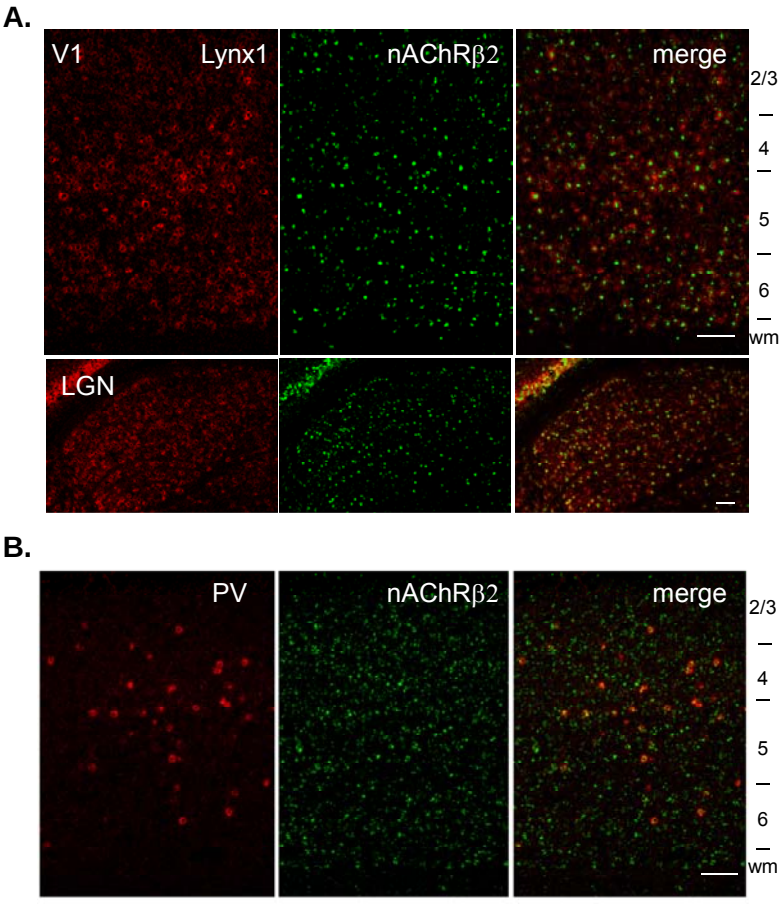


Supp. Fig.2

Supporting Figure 3. Co-localization of nAchR β2 subunit with lynx1 and PV in visual system.

(A) Double in situ hybridization of lynx1 (red) and nAChR β2 (green) in adult V1 and LGN. Scale, 100 μm

(B) Double in situ hybridization of PV (red) and nAChR β2 (green) in adult V1. Scale, 100 μm



Supp. Fig.3

3. Supporting References

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