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

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

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Experience-dependent brain plasticity typically declines after an early critical period during which circuits are established. Loss of plasticity with closure of the critical period limits improvement of function in adulthood, but the mechanisms that change the brain's plasticity remain poorly understood. Here, we identified an increase in expression of Lynx1 protein in mice that prevented plasticity in the primary visual cortex late in life. Removal of this molecular brake enhanced nicotinic acetylcholine receptor signaling. Lynx1 expression thus maintains stability of mature cortical networks in the presence of cholinergic innervation. The results suggest that modulating the balance between excitatory and inhibitory circuits reactivates visual plasticity and may present a therapeutic target.

The waxing and waning of cortical plasticity during a postnatal critical period serves to consolidate neural circuits and behavior (*1*), but in turn limits recovery of function in the adult brain (*2*). For example, discordant vision through the two eyes during an early critical period results in the enduring loss of visual acuity (amblyopia) that reflects aberrant circuit remodeling within primary visual cortex (V1). Amblyopia, which affects 2 to 4% of the human population, exhibits little recovery in adulthood (*3*). Identifying specific biological mechanisms that restrict adult plasticity would inspire potentially novel strategies for therapy.

We hypothesized that the gradual emergence of molecular "brakes" might actively prevent plasticity in the adult brain. The only molecules previously reported to play a role in closing the critical period are related to axonal growth inhibition, such as chondroitin sulfate proteoglycans and the myelin-signaling proteins NgR and PirB (*4*–*6*). To identify further targets, we analyzed the transcriptome of the binocular zone in mouse V1 for molecules that are expressed more in adulthood than during the critical period (*7*). Here, we characterize one of these, Lynx1, which is an endogenous prototoxin similar to α-bungarotoxin in snake venom and binds to the nicotinic acetylcholine receptor (nAChR) (*8*).

Lynx1 expression increases only after the critical period for amblyopia in adult V1 both at the protein and mRNA level (Fig. 1A). Along the visual pathway, *Lynx1* transcripts were expressed both in V1 and the lateral geniculate nucleus (LGN) (Fig. 1B). In contrast, expression of another member of the lynx family, *Lynx2*, declined over the critical period and was hardly found in the visual pathway (fig. S1). We therefore directly assessed Lynx1 function in the binocular region by electrophysiological recordings from knockout mice.

In mice lacking the *Lynx1* gene, the eye preference of single neurons (ocular dominance) was no different from that of wild-type mice (Fig. 1E). Upon short-term (4 day) monocular deprivation (MD) in mature wild-type animals (>postnatal day 60, P60), there was little change in the visual spiking response (*3*). Instead, adult Lynx1 knockout mice exhibited a robust shift in responsiveness away from the deprived eye (Fig. 1, C to E). This heightened plasticity was specific to older ages, because short-term MD was equally effective in both wild-type and Lynx1 knockout mice during the critical period (Fig. 2A).

Lynx1 protein directly binds to nAChRs (*9*), such as the major central subunits $\alpha_4\beta_2$ heteromers or α_7 homomers, to reduce their sensitivity to acetylcholine. We directly assessed the response to systemic nicotine injection in Lynx1 knockout mice by measuring visual evoked potential (VEP) response in anesthetized V1. Enhancement of the VEP response was only observed in Lynx1 knockout mice (Fig. 2, B and C). To test whether nAChR signaling mediates adult plasticity in Lynx1 knockout mice, we applied the broad-spectrum antagonist mecamylamine concurrent with short-term MD. Either systemic injection or restricted infusion directly into V1 by osmotic minipump was sufficient to prevent adult plasticity. These results were corroborated by systemic treatment with a mixture of α_4- and α_7-subunit–selective nAChR antagonists (*10*), dihydro-β-erythroidine (DHβE) plus methyllycaconitine (MLA) (Fig. 2A).

To establish the clinical relevance of these findings, we directly measured recovery from amblyopia in adulthood. In wild-type mice, long-term MD spanning the entire critical period results in a significant reduction of visual acuity as measured directly in V1 by VEP (*3*). Notably, this reduction persisted into adulthood even if the closed eye was

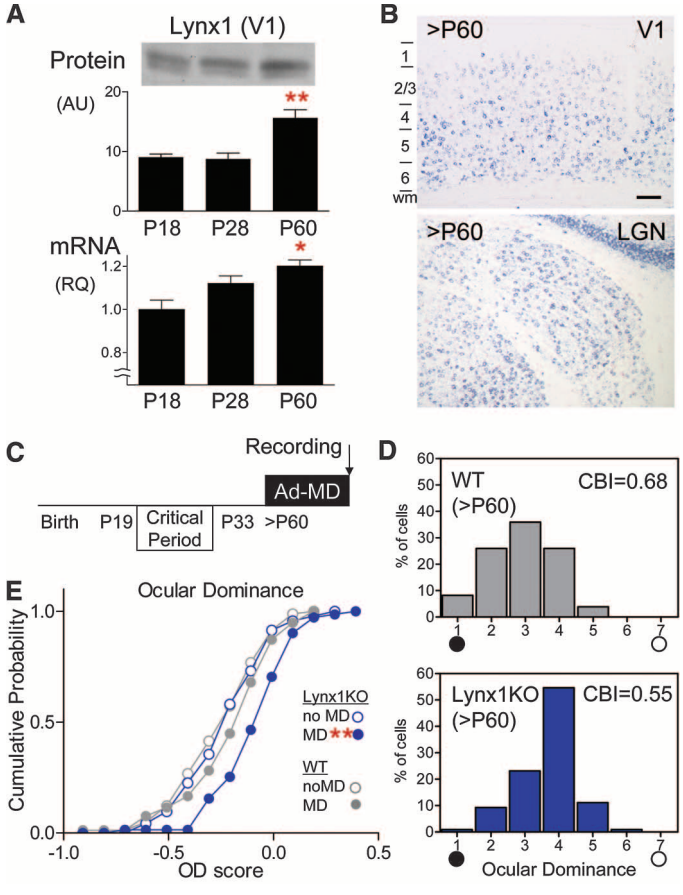

Fig. 1. Lynx1 expression increases in adulthood to limit visual plasticity. (**A**) Expression of Lynx1 protein (top) and mRNA (bottom) across the critical period (CP) (pre-CP: P18; CP: P28; post-CP: P60). $^{**}P < 0.01$, $^{*}P < 0.05$, one-way analysis of variance. AU, arbitrary units; RQ, relative quantification. Data are shown as the mean ± SEM. (**B**) In situ hybridization of *Lynx1* in adult V1 (top) and LGN (bottom). Scale bar, 100 μm. (**C**) Adult V1 plasticity paradigm by short-term MD (Ad-MD). (**D**) Ad-MD shifts the ocular dominance distribution of Lynx1 knockout (KO) mice [bottom; contralateral bias index (CBI) = 0.55, 216 cells, 8 mice], but not in wild-type (WT) mice (top; CBI = 0.68, 231 cells, 9 mice). KO versus WT: $P < 0.0001$, χ^2 test. (**E**) Cumulative probability of quantified spike response after Ad-MD confirms shifted ocular dominance scores for Lynx1 KO (blue filled circles), compared to WT (gray filled circles) ($^{**}P < 0.005$, Kolmogorov-Smirnov test) or no MD (blue open circles, KO, 93 cells; gray open circles, WT, 82 cells; $P = 0.75$, Kolmogorov-Smirnov test).

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reopened for more than 1 month after the critical period (Fig. 3, A and C). Lynx1 knockout mice spontaneously recovered visual acuity to normal levels simply by reopening the closed eye (Fig. 3, A and C), exhibiting VEPs even at higher spatial frequencies (Fig. 3B). Given the cholinergic basis of this plasticity, we further attempted to induce recovery even in adult wild-type mice by enhancing endogenous ACh signaling. Injection of an acetylcholinesterase inhibitor, physostigmine, during the period of eye reopening similarly restored vision to wild-type mice initially rendered amblyopic (Fig. 3C).

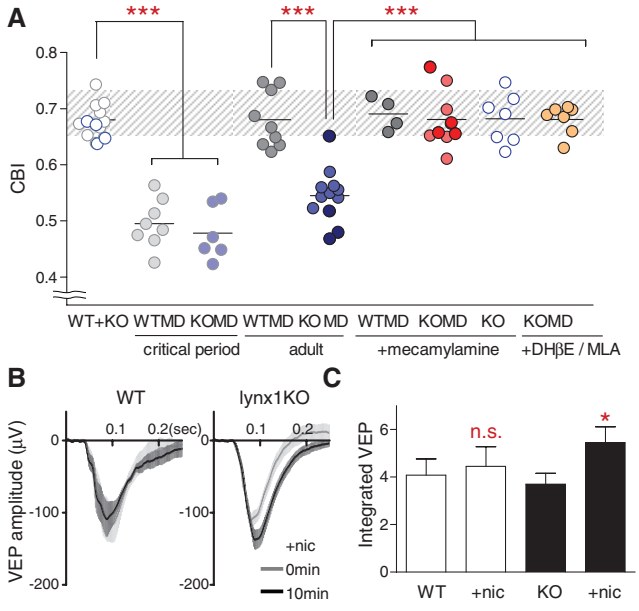

Fig. 2. Nicotinic receptors mediate adult plasticity in Lynx1 KO mice. (**A**) Mice without MD (open circles, gray WT/blue KO) shift equally after MD during the CP (light blue, KO: mean CBI = 0.48, 6 mice; light gray, WT: CBI = 0.50, 8 mice; $P > 0.5$, *t* test). Adult plasticity (blue, KOMD: CBI = 0.55, 12 mice versus gray, WTMD: CBI = 0.68, 9 mice; ***$P < 0.0001$, *t* test) is abolished by concurrent nAChR antagonists (red, KOMD + mecamylamine: CBI = 0.68, 9 mice versus KOMD, ***$P < 0.0001$; versus gray, WTMD + mecamylamine: CBI = 0.69, 4 mice, $P > 0.7$; versus no MD KO + mecamylamine: CBI = 0.68, 7 mice, $P > 0.9$, *t* test; orange, KOMD + DHβE/MLA: CBI = 0.68, 7 mice versus KOMD, ***$P < 0.0001$, *t* test). Darker circles represent cortical minipump infusion. (**B**) Enhanced nicotine response in Lynx1 KO mice. Averaged VEP traces (mean ± SEM) before (light gray) and 10 min after (black) subcutaneous nicotine injection (+nic) in WT (left) and Lynx1 KO mice (right). (**C**) Integrated VEP (area of first negative peak) for WT (empty bars, 6 mice) and Lynx1 KO mice (filled bars, 11 mice). *$P < 0.05$, *t* test; n.s., not significant.

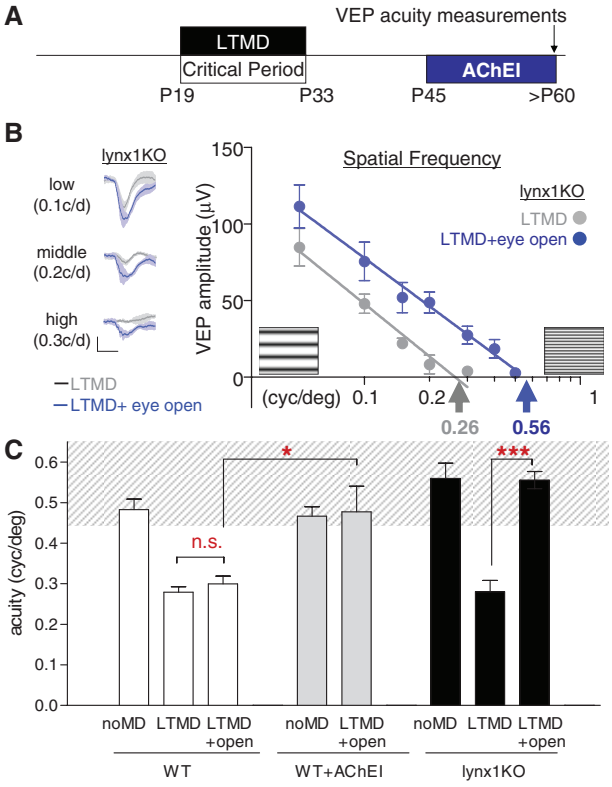

Fig. 3. Recovery from amblyopia in Lynx1 KO mice. (**A**) After long-term MD (LTMD) spanning the critical period (P19 to P33), the deprived eye was reopened (>1 month) until VEP acuity was measured in V1 (>P60). For AChEI experiments, physostigmine was injected daily starting at P45. (**B**) Averaged VEP traces (left, mean ± SEM; scale: 20 μV, 0.1 s) and amplitudes (right) of first negative peak (mean ± SEM) reveal acuity recovery after reopening an eye (blue, 6 mice) initially deprived during the critical period (gray, 5 mice). (**C**) Visual acuity in WT mice (white bars) without deprivation [no MD: 0.48 ± 0.03 cycles per degree (cyc/deg), 6 mice] decreases after LTMD spanning the critical period (LTMD: 0.28 ± 0.01 cyc/deg, 3 mice) and endures (+eye open: 0.30 ± 0.02 cyc/deg, 5 mice; versus LTMD, $P > 0.45$; versus no MD, $P < 0.0005$, *t* test). In contrast, reopening the deprived eye together with cholinesterase inhibitor restores vision (gray bar, AChEI: 0.48 ± 0.06 cyc/deg, 4 mice; versus WT + eye open, *$P < 0.05$; versus WT no MD + AChEI: 0.47 ± 0.02 cyc/deg, 6 mice, $P > 0.8$, *t* test). Lynx1 KO mice (black bars) spontaneously recover from LTMD (0.28 ± 0.03 cyc/deg, 5 mice) simply by reopening the deprived eye (0.56 ± 0.02 cyc/deg, 6 mice; ***$P < 0.0001$, *t* test) to reach normal levels (no MD: 0.56 ± 0.04 cyc/deg, 3 mice).

Recovery of function in Lynx1 knockout mice is likely due to an enhanced visual responsiveness during arousal. We did not observe structural changes at the level of perineuronal nets (*4*) or myelination (*5*) in Lynx1 knockout mice (fig. S2). Aging-related neurodegeneration reported previously in these animals (*11*) was confirmed to occur only past 9 months of age. Instead, local excitatory-inhibitory circuit balance might have been affected earlier (*3*) (Fig. 4A). Previous reports across various species have localized nAChRs to thalamocortical terminals presynaptic to principal cells (*12*–*16*), facilitating excitation in V1 (*17*, *18*). Activation of nAChRs upon specific inhibitory neurons could further modulate excitatory-inhibitory balance by disinhibition (*19*, *20*), as in the case of congenital nAChR mutation that disrupts GABA-mediated transmission (*21*).

Indeed, Lynx1 and nAChR mRNAs were coexpressed not only in the LGN (fig. S3) but also in a subpopulation of GABA cells, primarily parvalbumin-positive interneurons (Fig. 4B and fig. S3B). Activation of nAChRs may also exert long-term epigenetic effects on GABA synthesis (*22*). To probe whether excitatory-inhibitory imbalance may contribute to adult plasticity in Lynx1 knockout mice, we directly restored intracortical inhibition by focal benzodiazepine infusion from osmotic minipumps. Diazepam treatment of V1 abolished adult plasticity in Lynx1 knockout mice (Fig. 4C), as did nAChR blockade (Fig. 2A). Thus, Lynx1 reduces adult plasticity through cholinergic signaling mechanisms that may adjust excitatory-inhibitory balance later in life (*3*).

Taken together, Lynx1 provides both a valuable endogenous tool with which to probe critical-period closure and offers novel therapeutic and conceptual insight. In contrast to muscarinic receptors engaged during the critical period (*23*), our results highlight a nicotinic component for adult V1 plasticity. Although we do not rule out a role for muscarinic receptors (*24*), deletion of Lynx1 alone is sufficient to rescue visual acuity. Recovery strategies aimed at the Lynx1-nAChR interaction (*8*, *9*) could be fruitful in conjunction with attentional tasks that stimulate cholinergic release (e.g., perceptual learning, video-game training) (*25*–*28*). Clinically approved cholinesterase inhibitors that boost the afferent response in human visual cortex (*29*) may be useful for treating some amblyopes (Fig. 3C), including those with subcortical changes (*30*). Amblyopia might further serve as a diagnostic measure to identify tobacco exposure (*31*) or schizophrenia (*32*).

Although a permissive role for cholinergic input has long been appreciated during the critical period (*33*), it has remained a mystery why V1 plasticity is severely restricted in adulthood even in the presence of massive innervation from the basal forebrain. Lynx1 expression not only

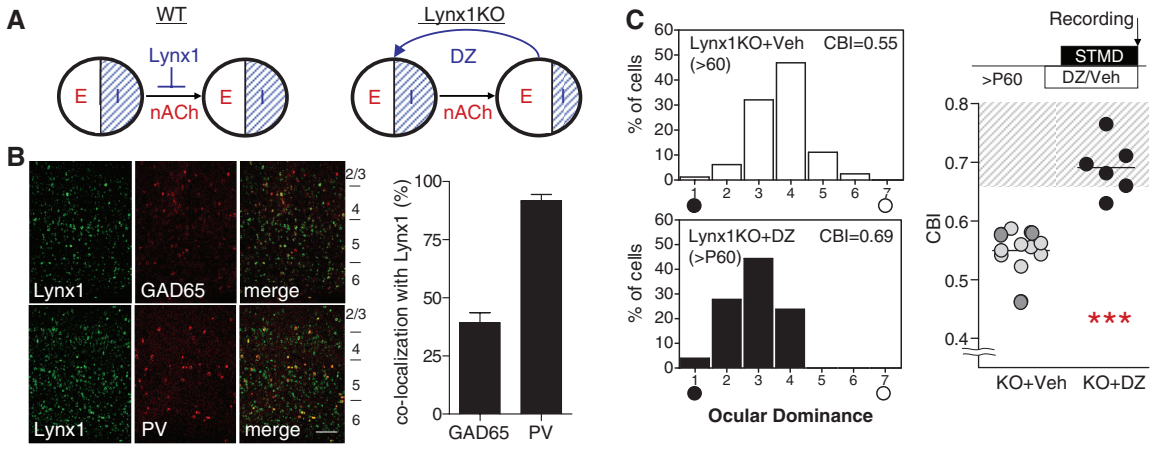

Fig. 4. Lynx1 may adjust cortical excitatory-inhibitory balance to regulate adult plasticity. (**A**) In WT animals (left), mature excitatory-inhibitory balance is maintained by Lynx1 that limits nAChR response. In Lynx1 KO mice (right), enhanced nAChR signaling may lead to excitatory-inhibitory imbalance and adult plasticity, which could be sensitive to acute restoration of inhibition with diazepam (DZ). (**B**) Double in situ hybridization of Lynx1 (green) with GAD65 (red, top) or parvalbumin (PV, bottom) in adult V1 (left). Scale bar, 100 μm. Quantification of overlapping pixels (right) indicates selective expression of Lynx1 in a subset (40%) of GAD65-positive interneurons, most likely PV-positive cells (>90% colocalization). (**C**) Focal diazepam infusion during adult MD in Lynx1 KO mice abolishes ocular dominance plasticity (black, DZ: CBI = 0.67, 6 mice versus gray, vehicle (Veh): CBI = 0.54, 14 mice; ***$P < 0.001$, *t* test). Dark circles represent cortical minipump infusion.

contributes to nAChR agonist binding and desensitization kinetics (*8*), but also may respond to changes in network activity (*34*). Local regulation of Lynx1 levels may allow cholinergic activation to induce islands of plasticity while maintaining overall circuit stability. Visual attention tasks in fact preferentially modulate fast-spiking inhibitory neurons (*35*, *36*), consistent with a convergence of top-down influences upon local excitatory-inhibitory circuit balance.

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Materials and Methods
Figs. S1 to S3
References

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Motor Control by Sensory Cortex

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Classical studies of mammalian movement control define a prominent role for the primary motor cortex. Investigating the mouse whisker system, we found an additional and equally direct pathway for cortical motor control driven by the primary somatosensory cortex. Whereas activity in primary motor cortex directly evokes exploratory whisker protraction, primary somatosensory cortex directly drives whisker retraction, providing a rapid negative feedback signal for sensorimotor integration. Motor control by sensory cortex suggests the need to reevaluate the functional organization of cortical maps.

The remarkable findings of Penfield and Boldrey (*1*), which have been supported by many subsequent studies (*2*–*11*), emphasize a key role for motor cortex in mammalian movement control. Investigating the mouse whisker system (*12*–*14*), we found that primary somatosensory barrel cortex forms an equally direct and equally prominent motor control pathway, compared with that originating from the classical motor cortex.

We first functionally mapped the sensory activity evoked by a single brief deflection of the C2 whisker through wide-field voltage-sensitive dye (VSD) imaging of the contralateral sensorimotor cortex in awake head-restrained mice (*15*, *16*). The earliest cortical VSD response to C2 whisker deflection occurred at 7.4 ± 0.5 ms ($n = 5$ mice, mean ± SD) and was specifically localized to the C2 barrel column of primary somatosensory neocortex ($S1_{C2}$) (Fig. 1A). Over the subsequent milliseconds, nearby cortical columns depolarized, with activity propagating in a wavelike manner.

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