Optimizing Cholinergic Tone Through Lynx Modulators of Nicotinic Receptors: Implications for Plasticity and Nicotine Addiction

Julie M. Miwa, Henry A. Lester and Andreas Walz


doi: 10.1152/physiol.00002.2012

You might find this additional info useful...

This article cites 127 articles, 44 of which you can access for free at:

http://physiologyonline.physiology.org/content/27/4/187.full#ref-list-1

This article has been cited by 4 other HighWire-hosted articles:

http://physiologyonline.physiology.org/content/27/4/187#cited-by

Updated information and services including high resolution figures, can be found at:

http://physiologyonline.physiology.org/content/27/4/187.full

Additional material and information about *Physiology* can be found at:

http://www.the-aps.org/publications/physiol

This information is current as of October 9, 2016.
Optimizing Cholinergic Tone Through Lynx Modulators of Nicotinic Receptors: Implications for Plasticity and Nicotine Addiction

The cholinergic system underlies both adaptive (learning and memory) and nonadaptive (addiction and dependency) behavioral changes through its ability to shape and regulate plasticity. Protein modulators such as lynx family members can fine tune the activity of the cholinergic system and contribute to the graded response of the cholinergic system, stabilizing neural circuitry through direct interaction with nicotinic receptors. Release of this molecular brake can unmask cholinergic-dependent mechanisms in the brain. Lynx proteins have the potential to provide top-down control over plasticity mechanisms, including addictive propensity. If this is indeed the case, then, what regulates the regulator? Transcriptional changes of lynx genes in response to pharmacological, physiological, and pathological alterations are explored in this review.

The cholinergic system is a modulatory neurotransmitter system with a widespread reach throughout the central and peripheral nervous systems. Nicotinic receptors have been implicated in a growing number of complex brain processes (including learning and memory), attentional processes (e.g., ADHD), arousal, reward (reviewed in Refs. 94, 105), mood (e.g., depression, anxiety) (95), pain, and even the peripheral functions such as parasympathetic signaling, muscle function, lymphocyte proliferation, etc. Several mechanisms of regulation over the cholinergic system moderate the contribution of such a widespread neurotransmitter system. Key points of sensitivity exist that are particularly subject to regulation and can lead to both adaptive and nonadaptive behavioral changes. Nicotinic receptors involved in learning and memory can contribute to addiction processes since mechanisms underlying addiction share many commonalities with the synaptic plasticity mechanisms implicated in learning and memory (23, 71, 115).

The Structural Elements of the Cholinergic System

The cholinergic system is largely modulatory in nature; it is not typically engaged in classical fast synaptic transmission. The structural elements that make up the cholinergic system lend themselves to this modulatory function. The majority of the neurotransmitter of the cholinergic system [acetylcholine (ACh)] in the brain is released from small clusters of cholinergic neurons resident in the basal forebrain and brain stem. Terminals of cholinergic neurons radiate widely throughout the brain and release neurotransmitter diffusely (76) rather than being confined within the synaptic cleft. Coupled with an extrasynaptic localization of its receptors, these elements can exert broad modulatory effects on the activity of neurons.

The cholinergic system can be influenced on a global level by regulating the levels of its neurotransmitter ACh (76) or it can be regulated in a more restricted fashion through its receptors. ACh levels fluctuate based on activity levels of the organism and correlate with the times of sleep-wake cycles (47, 73), with highest levels being reached just before waking. A decrease of ACh levels has been hypothesized to underlie the transition from sensory processing to information storage. Furthermore, tonic basal firing activity of cholinergic interneurons in the striatum can contribute to cholinergic tone in this region through the short range release of ACh. Differential firing of tonically active striatal cholinergic interneurons is thought to regulate output of dopaminergic and/or target neurons, and can pause in expectation of a reward (118).

The receptors of the cholinergic system come in two main classes, the muscarinic and nicotinic classes of ACh receptors. Nicotinic receptors have
been localized presynaptically on terminals where they modulate neurotransmitter release of other neurotransmitters such as glutamate, GABA, and dopamine. In addition, somatodendritic receptors localized extrasynaptically govern general excitability of the neuron. Muscarinic and nicotinic classes comprise 5 and 15 subunits, respectively. Nicotinic receptors are pentamers. Brain nicotinic receptors can exist as heteromeric combinations of α (2–10) and β (2–4) subunits, and as α7 homopentamers (in muscle-type receptors, the non-α subunits are β1, γ or ε, and δ).

The number of subunit genes and the combinatorial complexity of their multimeric assembly provide an opportunity for specificity, since each receptor subtype has unique biophysical characteristics. There is a nearly limitless array of potential combinations of receptor subtypes, but the majority of nAChRs are either α4β2 or α7 nAChRs, which some estimate to provide 70 and 16%, respectively, of receptor binding in the brain (43, 74). Even the ratio of subunits within a pentamer or stoichiometry of α- and β-subunits in the pentamer imposes differential response profiles. For α4β2 subunits, the α4,β2 stoichiometry exhibits less than at least 10-fold higher sensitivity than α4β2 (85). The high sensitivity subtype (α4β2) is activated at nicotine concentrations of in the range of 0.1–1 μM, which is within the range produced by tobacco use.

Nicotinic Receptor Involvement in Nicotine Dependency

Most nicotinic receptor subtypes have been shown to have some involvement in nicotine dependency or to respond to the actions of nicotine exposure. Upregulation of α4β2 subunits is a well-documented response to nicotine exposure and may underlie nicotine addiction (35, 72, 84, 109), and α4 nAChR subunits have been implicated in nicotine dependence in animal models (75, 116). α7 nAChR subunits have been linked to schizophrenia (61) and psychosis (39) in humans. Smoking has been shown to normalize auditory physiology in schizophrenia patients, and the prevalence of smoking in the schizophrenic population can be as high as 90% (22, 79). Less abundant subtypes are increasingly appreciated for their specific roles in a number of important brain functions. For instance, both the α5+ nAChRs and α6+ nAChRs have been linked to susceptibility in nicotine dependence (107), apparently through separate mechanisms. α5+ Subunits mediate nicotine through aversion to nicotine in the medial habenula (37, 38), whereas α6+ in dopaminergic neurons are involved in positive reward in the VTA. The aversive reaction mediated by the medial habenula may balance the positive signals from the VTA to control the intake of nicotine under normal circumstances. This balance may go awry for those individuals harboring one of the risk alleles in the CHRNA5 gene. Interestingly, it has been recently shown that nicotine decreases food intake through α3β4 nAChRs (78). Having long been considered to be a ganglionic subtype only, α3β4 nAChRs expressed in POMC neurons in the hypothalamus have been recently shown to suppress appetite due to nicotine consumption. Reduction in α3β4 nAChR function after quitting smoking, then, may underlie weight gain for recent quitters. Since weight gain may confound quit attempts, α3β4-specific compounds may be an effective aid for smoking cessation and could be a viable strategy as an appetite suppressant.

The Inverted U-Shaped Response Curve of Cholinergic Activation

A body of literature on the cholinergic system and nicotinic receptors suggests that this neurotransmitter system functions along a gradient of activation, and the cholinergic system follows an inverted U-shaped curve of activation (26, 48, 64, 93–94, 96, 114, 116). Neural dysfunction can occur at either extreme of this range. Hypoactivation of the cholinergic system is associated with lower cognitive performance and dementias (48). Furthermore, loss of cholinergic neurons or the neurons that express nicotinic ACh receptors are associated with Alzheimer’s disease and its concomitant memory loss and cognitive decline (48). Treatments for such cognitive impairment attempt to raise cholinergic activity in the brain by inhibiting enzymes that break down ACh. At the other extreme, overactivation of the cholinergic system may be linked to some forms of epilepsy (11) and, in even more extreme cases, to synaptic loss (112) and neurodegeneration (88, 110) (FIGURE 1). Tight control over cholinergic systems, operating at several levels, appears to act as a counter-balancer to prevent the brain from reaching such extremes. Within moderate levels of activation, referred to here as optimized cholinergic tone, modest nicotinic receptor activation can be procognitive, enhancing neurotransmitter release and aiding in synaptic plasticity, leading to improvements in attention and some types of learning and memory.

The Hierarchy of Controls Over the Cholinergic System

Control over the cholinergic system is tightly balanced through a multilayered set of mechanisms. ACh-esterase is highly efficient at breaking down
ACh once it is released, turning off cholinergic signaling and reducing the likelihood of receptor desensitization. Changes in subunit composition and stoichiometry (the ratio of $\alpha$ to $\beta$ subunits) can influence receptor desensitization, ligand affinity profiles, and conductance. In addition, posttranslational mechanisms can alter receptor function. Processes including palmitoylation of $\alpha_4$ and $\alpha_7$ nAChRs (3), myristoylation (103), glycosylation (55), phosphorylation (34, 49), upregulation, etc., can all play a part in modifying the response properties of nAChRs. Furthermore, changes in the transcriptional levels of nicotinic receptor subunits may underlie disease. For instance, there have been reports of increased levels of $\alpha_7$ nicotinic receptors in Alzheimer’s disease (18, 54). Hyperactivating mutations introduced into nAChR subunit sequences have uncovered new cholinergic mechanisms in the brain (31, 83), which were previously underappreciated. Furthermore, mutations in nicotinic receptor subunits have been linked to human disease: $\alpha_4$ and $\beta_2$ in ADNFLE (11, 12, 59), $\alpha_7$ in schizophrenia (65, 79), and $\alpha_5$ in nicotine addiction (108), and each mutation ultimately manifests itself as an imbalance in the properties of neuronal circuits.

### Nicotinic Receptor Regulation Through Interacting Proteins

In addition to regulation at the receptor level, the environmental context surrounding the receptors has been increasingly appreciated. Interacting proteins exist in complexes with nAChRs and aid in the assembly and trafficking of nAChRs to the cell surface. Ric-3 is required for maturation of $\alpha_4\beta_2$ nAChRs (45), other homomeric receptors such as $\alpha_9$ and $\alpha_{10}$, as well as heteropentamers (60). The calcium binding protein VILIP-1 binds to the cytoplasmic loop of $\alpha_4\beta_2$ nAChRs and increases surface expression and agonist sensitivity (67). The chaperone protein 14-3-3 binds to the cytoplasmic loop of $\alpha_4\beta_2$ nAChRs and increases surface expression twofold (53). These intracellular proteins bind to intracellular domains of nAChRs particularly important for interacting with trafficking proteins (i.e., PDZ-domain-containing proteins such as PSD-95), which forms a functional scaffold for nAChRs (20). These interactions can stabilize receptor mobility at specialized domains at synaptic or extrasynaptic domains (36). In addition, receptor interactions at the cell surface and at intracellular sites may trigger an acute down-modulation of the receptor (58).

### FIGURE 1. Gradient of cholinergic activation

The cholinergic system exists on a gradient of activation. Underactivation is associated with dementias that can occur in some neurological disorders (PD or AD) (48, 96, 102). Treatments for cognitive dysfunction and memory impairments in AD attempt to raise cholinergic tone by inhibiting the enzyme that breaks down acetylcholinesterase (ACh). The gene encoding $\alpha_7$ nAChRs is associated with schizophrenia (39). On the other extreme of this gradient, overactivation can lead to some forms of epilepsy (11) and neurodegeneration (110). Within an optimal window of activation/optimized cholinergic tone (white box), moderate activation of the cholinergic system can lead to augmentations in neurotransmitter release and enhanced synaptic plasticity (23). nAChR activation can lead to improved attention (44) and learning and memory functions (26). ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; PD, Parkinson’s disease; DA, dopamine.
Therefore, analyzing the function in the contextual milieu of the receptor as it would exist in situ is warranted. Furthermore, a special class of modulators, lynx proteins, binds to nAChRs with important implications with respect to cholinergic processes, which will be detailed in the following sections.

**Protein Modulators of the Cholinergic System: Lynx Genes**

Unlike the interacting proteins detailed, above, which bind at the intracellular domain of the receptors, a class of protein modulators, lynx modulators, binds on the extracellular face of the nicotinic receptor (FIGURE 2). Lynx genes belong to the ly-6/uPAR superfamily, which adopts a three-looped folding structure, termed the toxin fold (42). At the amino acid level, a signature consensus motif, termed the Ly6 motif, codes for 8–10 cysteine residues that participate in a stereotyped disulfide bonding pattern, critical in inducing its tertiary structure. This is a highly evolved receptor binding structure, termed the toxin fold or, alternatively, the three-fingered fold. This results in a three-looped structure stabilized by rigid beta sheets that can form an almost limitless array of receptor and/or channel binding conformations.

For the purposes of this review, we will divide the superfamily into mammalian genes enriched in the brain and outside the brain (FIGURE 3). Brain-enriched genes include members such as lynx1 (80), lynx2/lypd1 (29, 117), lypd6 (24), lypd6b (19), PSCA (51), and PATE-M (66). One group of peripheral genes is expressed in the immune system and include genes such as the complement inhibitor CD59 (25) and ly6 antigens A-I (13, 42, 50, 70). Other peripheral genes expressed in non-immunological cells are expressed in skin, uPAR (98), SLURP-1 (1, 16), SLURP-2 (5, 121), E48 antigen (13, 32), and reproductive tissues, i.e., ACRV1 (90), and genes of the PATE cluster (66) (FIGURES 3 AND 4). The superfamily includes membrane-bound, GPI-linked proteins or secreted versions. Each lynx paralog has a relative binding specificity and modulatory capability on α4β2 (52, 66, 80), α3 (4), and α7 (16, 51) nAChR subtypes. Some of the PATE molecules increase activity of net charge through α7 nAChRs (66). Venomous α-neurotoxins, including α-btx (87), cobratoxin (125), κ-btx (15), etc., have sub-nM affinity for nAChRs (120) and other receptors and channels (7).

The extensive investigation of the three-fingered fold class of proteins can be highly informative for an understanding of their mammalian counterparts. α-neurotoxins interact on the extracellular face of the nAChR near ligand binding sites in contrast to most other nAChR-interacting proteins thus far identified, which bind to the intracellular portion between the third and fourth transmembrane regions (M3-M4 loop). The structurally similar lynx proteins may bind at such sites as well (FIGURE 2) (69). Five interfaces occur in each nAChR pentamer; the interfaces that form the binding sites for various lynx paralogs have yet to be mapped (46, 62).

**Snake Toxins Identify Critical Control Points and the Prototoxin Hypothesis**

On first glance, it may appear counterintuitive that toxin-like proteins, with virulent antireceptor activities, would be present in the brain. Studies on the origin of snake toxins, however, indicate that venomous species often employ functional mimicry of cellular proteins operating in normal physiological processes to create toxic variants. During toxin recruitment, cellular genes are expressed in the venom glands of venomous species, and are altered through gene duplication and mutation (41, 57) or posttranslational modifications. Three-finger toxin proteins, such as α-cobratoxin, have been isolated from an older snake species long considered to be nonvenomous (40), lending support for this idea. Although numerous toxins have arisen through convergent evolution, amino acid sequence similarity and conserved exon-intron break points support a common ancestry between the elapid snake toxin proteins and lynx genes (81). The high level of conservation with toxins indicates the potential for lynx genes to be evolutionary antecedents to α-neurotoxins or prototoxins. Often, toxic variants target endogenous pathways at rate-limiting steps (41), since this would be the step most sensitive to manipulation in their prey. Therefore, the evolutionary relationship between...
Lynx1 modulators and the α-neurotoxins indicates that lynx modulators govern critical control points in the pathway of nicotinic receptor signaling.

**Functional Modulation of Nicotinic Receptors by Lynx1 Prototoxins**

Lynx1, the first discovered member of this family expressed in the brain (80), has an overall inhibitory effect on nAChR function. In an α4β2* nAChR-expressing cell, co-expression of lynx1 results in reduced agonist sensitivity, manifested as a rightward shift in the dose-response relationship to ACh and reduced agonist sensitivity. nAChRs also have accelerated desensitization rate and slower recovery from desensitization (52). Single-channel studies on α4β2 nAChRs demonstrate a shift in the proportion of one class of channel openings toward higher conductance, faster inactivating species when complexed with lynx. These studies indicate that lynx proteins exert a global modulatory effect over nAChR channel function. The blunting effect of lynx proteins could be responsible for the paucity of synaptically driven nicotinic responses recorded in brain tissue despite the rich cholinergic innervation in the brain. Interestingly, it has long been noted that different response properties characterize nicotinic responses in brain tissue compared with heterologous expression systems (101). Therefore, the suppressing actions of lynx modulators may mask latent cholinergic mechanisms in vivo.

**Stabilizing Plasticity Through Lynx Modulators**

Relieved of the molecular brake down-playing nicotinic receptor responses in the brains of lynx1KO mice, nicotinic receptor-dependent processes can be detected more readily. Lynx1KO mice demonstrate features of elevated cholinergic tone: nicotinic responses are hypersensitive with slower desensitization kinetics, larger nicotine stimulated nicotinic responses are hypersensitive with slower recovery from desensitization (52). Single-channel studies on α4β2 nAChRs demonstrate a shift in the proportion of one class of channel openings toward higher conductance, faster inactivating species when complexed with lynx. These studies indicate that lynx proteins exert a global modulatory effect over nAChR channel function. The blunting effect of lynx proteins could be responsible for the paucity of synaptically driven nicotinic responses recorded in brain tissue despite the rich cholinergic innervation in the brain. Interestingly, it has long been noted that different response properties characterize nicotinic responses in brain tissue compared with heterologous expression systems (101). Therefore, the suppressing actions of lynx modulators may mask latent cholinergic mechanisms in vivo.

![Figure 3: Genes of the lynx superfamily](http://physiologyonline.physiology.org/)
normal circumstances, losing synaptic lability once patterned activity of early visual experience takes place would provide an adaptive advantage to a complex organism. Coherence of information over time is important for creating a stable internal representation of our environment and could allow for pattern recognition to proceed efficiently. This representation provides a backdrop against which salient information can more readily reach our attention. In some cases, however, reopening the critical period and thus recapturing youthful plasticity may be beneficial. This may be particularly relevant in cases of imbalances in circuitry, which occur during development but its manifestation may not present itself until later in life, such as in some neuropsychiatric disorders.

Cholinergic-Dependent Learning and Memory Processing

The synaptic lability observed in lynx1KO mice manifests itself at the behavioral level. We have observed enhanced associative learning ability in the fear-conditioning paradigm. This is a classical sound-based associative learning paradigm that seeks to assess the ability of the animal to associate
innocuous stimulus (tone) with a noxious one (mild foot shock) when paired during training sessions. Animals with better associative learning will react in a fearful way to the innocuous tone in subsequent tests. Recent studies have indicated that learning opens a prolonged time window of reduced inhibition or disinhibition in the auditory cortex (63). Foot shock induces cholinergic activation in the upper layers of the cerebral cortex that mediates this disinhibition. Although nicotinic involvement in the contextual component of this task has been previously reported (28), there has been little understanding of the role that the cholinergic system had on the associative fear conditioning. It has been appreciated, however, that sensory input can trigger the cholinergic system, and this could help to align attention with a source of sensory input, therefore aiding cue detection (48). Also, specific nicotinic receptor subunits have been implicated in attention (8, 44), and the model suggests an interplay between the activation of cholinergic neurons and specific receptor systems and circuitry within the cortex. At this point in time, it is not possible to discriminate between attentional and learning mechanisms acting in lynxKO mice during fear-conditioning learning. The possibility of attentional differences in lynxKO mice awaits direct measurements.

**Top-Down Control Over the Cholinergic System Through Lynx Function**

Our evidence suggests that lynx acts as a gain of function control or upstream modulator over cholinergic function. For instance, lynx phenotypes are ameliorated by crossing lynxKO mice to null mutations in nicotinic receptor subunits α7 and β2. This indicates that nicotinic receptors are necessary for the expression of some lynx phenotypes. Furthermore, pharmacological blockers of nAChRs abolished the enhanced critical period plasticity in lynxKO mice. Together, our model of lynx action indicates that lynx proteins act as upstream modulators of nicotinic receptor function. As mentioned above, excessive activation of nAChRs can be detrimental in the brain, leading to overactivation of the cholinergic system. This could explain part of the teleological consequence of lynx gene expression in the nervous system: the brain has a clear need to restrict the degree of nAChR activation, yet specific enhancement of cholinergic activity in functional circuits would benefit many processes, as described above. Therefore, subtle shifts in lynx function that are correlated with the demands of the organism should allow the sensitivity of the cholinergic system to respond adaptively to environmental conditions. Transient, partial, or local reductions in lynx function may produce an optimal balance, raising cholinergic tone in the brain to aid synaptic plasticity mechanisms at specific times or locations while preventing overactivation of the cholinergic system that can encourage susceptibility of neurons to excitotoxic damage.

Orchestrating the cholinergic response through different lynx modulators could yield differential functional effects, which is likely to be determined by binding affinity and expression pattern. For instance, lynx1 has the widest expression profile and is the most permissive for binding a wide range of nicotinic receptor subtypes. Lynx2, however, has a more restricted although complementary expression to lynx1 (FIGURE 5), with a high level of expression in the amygdala (29). Consistent with this expression, lynx2KO mice exhibit a marked alteration in anxiety levels and socialization skills (117). In addition, lynx2KO mice demonstrate enhanced sensitivity of the EPSC frequency in the cortex to nicotine and also exhibit elevated cued learning in fear conditioning tests, although the demonstrable anxiety phenotypes confound the interpretation of these results. Lynx2 is expressed early in development and is present at the tips of growing axons (29). Lypd6, on the other hand, is expressed in more scattered cells within the cortex and hippocampus. Mice with specific knockdown of lypd6 demonstrate altered prepulse inhibition and locomotor activity (24). Therefore, although some family members may share some biophysical properties in common, their expression profiles are strong determinants of function. The implication of these findings is the potential to control specific functional domains through selectively regulated lynx genes (i.e., cognition for lynx1 and anxiety for lynx2).

**What Regulates the Regulator?**

Through functionally driven regulation of lynx expression, cholinergic systems have the ability to exert top-down influences on circuits underlying relevant behavior via coordinated regulation of nicotinic receptors subsets. What, then, regulates the regulator? Mounting evidence indicates that lynx can be regulated at the transcriptional level. Lynx1 expression fluctuates in response to complex perturbations, downregulating in NKCC1 knockout mice (92) and α7 nAChR blockade (51), whereas it is upregulated at the close of the critical period in the visual cortex and by nicotine in the lung (111) (Table 1). Lynx1 has been shown to be upregulated in dark-reared animals and by monocular deprivation (119). As mentioned above, lynx1 is upregulated at the close of the critical period for visual plasticity (97). Lynx expression is
also suppressed by light pulses after a period of darkness (100). Furthermore, mice disrupted in normal circadian rhythms, per mutant mice, demonstrate downregulation of lynx1 (89). Lynx family member lynxp6D6B has been linked to autism (19), and evidence for cholinergic misregulation has been linked to nonneuronal human disease (16). These studies indicate that selective regulation is possible to achieve through a variety of genetic and/or pharmacological manipulations. Manipulating lynx

![Diagram of brain sections showing expression patterns of lynx1 and lynx2 genes.](image)

**FIGURE 5. Complementary expression patterns of lynx1 vs. lynx2 genes**

A: coronal mouse brain sections probed with in situ hybridization. Lynx1 has a widespread distribution with high levels in the hippocampus, whereas lynx2 is highly expressed in the amygdala. B: hippocampus. Complementary expression pattern with lynx1 expressed in CA2 and CA3 of the hippocampus and the hilar region of the dentate gyrus, whereas lynx2 is expressed in CA1 of the hippocampus and dentate gyrus granule neurons. C: frontal cortex. Lynx1 is expressed throughout the cortex, with highest levels in the deep layers, whereas lynx2 is mainly expressed in upper cortical layers. D: temporal expression profile. Lynx1 is upregulated between postnatal week 2 and 3 (P15 on), whereas lynx2 is found as early as embryonic day 8.5 (E8.5) (60a).
dosage may be a useful therapeutic strategy for ameliorating cognitive decline associated with neurological disorders.

### Transcriptional Regulation of Lynx1 in Learning Models

Gene expression studies have indicated that lynx is differentially regulated in learning and memory models. Alterations in lynx1 levels associated with learning and memory deficits are found in a few animal models. Lynx1 is downregulated in double-null mutant mice for Ca\(^{2+}\)-stimulated adenylyl cyclase genes AC1 and AC8 in the hippocampus (122). These mutant mice show deficits in long-term consolidation of the task that can be rescued by reexpression of AC8. This reexpression did not completely rescue lynx1 levels to the level of wild-type mice, suggesting that lynx1 was not causative for the learning deficits in these mice. These results are most suggestive of a compensatory change in lynx1 levels as a downstream consequence of the loss of the two adenylyl cyclase genes. Interestingly, lynx1 and lynx2 have opposite responses in regulation to the same perturbations. The antidepressant fluoxetine, which regulates the proliferation of neurons in the hippocampus, leads to upregulation of lynx1 and downregulation of lynx2 in the hippocampus (77). Furthermore, in microdeletion syndrome in 22q1, lynx1 is downregulated in prefrontal cortex and hippocampus, whereas lynx2 is upregulated (113, 119). One of the implications of the differential response is control over different overall neural functions. Lynx2 is more highly expressed in the amygdala of animals expressing a higher level of fear behavior (99). A family member to lynx1, lypd2 is downregulated in the LGN of null mutant mice for the CHRNA2 gene (β2 KO mice) (106). In this mouse line, retinal ganglion cells axons have been shown to have more diffuse projections into the lateral geniculate nucleus.

### Possible Role of Lynx in Nonadaptive Plasticity Processes: Implications for Nicotine Addiction and Disease

Taken together, it appears possible for lynx family members to be regulated by environmental, genetic, or pathological states. This indicates the potential for the brain to adjust cholinergic tone to prevailing conditions. This has implications for disease amelioration. Both aversive-based learning (fear conditioning) and reward-based learning (drug dependency) are considered associative learning processes (27, 115, 123). Environmental stimuli during smoking can become reinforced due to dopamine release that occurs with nicotine intake. It has been proposed that lynx molecules can act against the actions of nicotine through their inhibitory effect on nicotinic receptors (38, 52). The suppressing

### Table 1. Expression profiling of lynx1 and lynx2 genes

<table>
<thead>
<tr>
<th>Model/Treatment</th>
<th>Brain Region</th>
<th>Gene</th>
<th>Change</th>
<th>Description of the Model</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia risk</td>
<td>pFC</td>
<td>lynx1</td>
<td>Down</td>
<td>Microdeletion syntenic to human 22q in mice</td>
<td>PMID:18469815</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lynx2</td>
<td>Up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Hippocampi</td>
<td>lynx1</td>
<td>Down</td>
<td>Fluoxetine treatment in DBA/2J mice</td>
<td>PMID:17609676</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lynx2</td>
<td>Up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed maturation</td>
<td>Hippocampi</td>
<td>lynx1</td>
<td>Down</td>
<td>NKCC1 KO mice, poor synaptic development</td>
<td>PMID:19295148</td>
</tr>
<tr>
<td>Inhibitor1 KO mice</td>
<td>Hippocampi</td>
<td>lynx1</td>
<td>Down</td>
<td>PPI1 inhibitor KO mice, learning</td>
<td>GSE4040</td>
</tr>
<tr>
<td>Anticonvulsant diet</td>
<td>Hippocampi</td>
<td>lynx1</td>
<td>Down</td>
<td>Rat fed ketogenic, anticonvulsant diet</td>
<td>GSE1155</td>
</tr>
<tr>
<td>Neurodegeneration</td>
<td>Striatum</td>
<td>lynx1</td>
<td>Down</td>
<td>PGC-1 alpha KO mice, implicated in HD</td>
<td>PMID:17018277</td>
</tr>
<tr>
<td>Huntington model</td>
<td>Striatum</td>
<td>lynx1</td>
<td>Down</td>
<td>YAC128 transgenic, HD model</td>
<td>PMID:20089533</td>
</tr>
<tr>
<td>Light pulse</td>
<td>SCN</td>
<td>lynx1</td>
<td>Down</td>
<td>Mice, 30 minute light pulse &gt; lights off</td>
<td>PMID:18021443</td>
</tr>
<tr>
<td>Retinal dysfunction</td>
<td>Retina</td>
<td>lynx1</td>
<td>Down</td>
<td>Retinochisin (RS1 h) KO mice degeneration</td>
<td>PMID:20709948</td>
</tr>
</tbody>
</table>

Table of expression data indicating changes in gene expression for the lynx1 and lynx2 genes in different regions of the brain. pFC, prefrontal cortex; SCN, suprachiasmatic nucleus; NKCC1, sodium, potassium chloride co-transporter; PPI1, protein phosphatase inhibitor 1; PGC, peroxisome proliferator-activated receptor γ coactivator 1α.

Lynx1 Levels in Neurodegenerative and Neuropsychiatric Disorders

Alterations in lynx1 transcript levels have also been associated with neurodegenerative disorders, such as in the Huntington’s mouse model, PGC1αKO (21), which causes mitochondrial dysfunction and neurodegeneration. In addition, lynx1 regulation has been associated with retinal disease (104) and hypoxia in the frontal cortex (127). Finally, the R6/1 transgenic animal, which expresses a mutant human huntingtin gene that contains multiple CAG trinucleotide repeats—a model for early pathogenesis of HD–downregulates lynx1 (10).
action of lynx modulators on neural plasticity mechanisms, then, could play a role in nicotine addiction. This may be particularly relevant for adolescent smoking because evidence suggests that people who initiate smoking early have more pronounced nicotine dependence (14, 91), and animal studies indicate a developmental role for nicotinic receptors in circuits implicated in learning (56, 68). The late upregulation of lynx1 later in development could underlie greater susceptibility of younger smokers to the reinforcing actions of nicotine. Influencing lynx regulation is a possible strategy for disrupting the association of contextual cues that can accrue with prolonged nicotine intake. Furthermore, once imbalances occur, reopening a window of synaptic lability may allow for a therapeutic rebalancing of circuitry.

Summary

Nicotinic receptors must operate in a window of activation for optimal efficiency. Proteins that engage nAChRs within stable complexes, such as lynx proteins, provide a dampening effect on nicotinic receptors. Release of the brake on the cholinergic system can allow greater manifestation of plasticity mechanisms inherent in the brain, which has the potential to lead to both adaptive (learning) and nonadaptive (addiction) behavioral changes. Control over lynx levels can have important implications for learning and memory and plasticity mechanisms. Therefore, the restricted expression of lynx family members is a mechanism by which top-down control over specific circuitry subserving specific complex brain functions can be achieved.

We thank Sharon Grady and Rell L. Parker for helpful discussions and Pauline Ku and Wesley Chen for help with figures. This work was supported by funds from Tobacco-Related Disease Research Program of the University of California, Grant No. TRDRP19KT-0032 for J. M. Miwa; SR43MH083416-02 and 1R43MH083416-02 for A. Walz; and R01 AG-033954-01 for H. A. Lester and J. M. Miwa. J. M. Miwa holds shares in Ophidion. A. Walz holds shares in Ophidion. Ophidion pursues the development of Ophidion to control over specific circuitry subserving specific complex brain functions can be achieved.

Author contributions: J.M.M. analyzed data; J.M.M. interpreted results of experiments; J.M.M. and A.W. prepared figures; J.M.M. drafted manuscript; J.M.M., A.W., and H.A.L. approved final version of manuscript.

References


