PHYSIOLOGY 27: 187–199, 2012; doi:10.1152/physiol.00002.2012

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

Julie M. Miwa,[1] Henry A. Lester,[1] and Andreas Walz[2]

[1]California Institute of Technology, Pasadena, California; [2]Ophidion, Pasadena, California
miwaj@caltech.edu

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 (2, 8, 44, 48), executive function (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 $\alpha4_2\beta2_3$ stoichiometry exhibits less than at least 10-fold higher sensitivity than $\alpha4_3\beta2_2$ (85). The high sensitivity subtype ($\alpha4_2\beta2_3$) 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 CHRA5 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 α to β subunits) can influence receptor desensitization, ligand affinity profiles, and conductance. In addition, posttranslational mechanisms can alter receptor function. Processes including palmitylation of α4β2 and α7 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 underly disease. For instance, there have been reports of increased levels of α7 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: α4 and β2 in ADNFLE (11, 12, 59), α7 in schizophrenia (65, 79), and α5 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 nAChR to the cell surface. Ric-3 is required for maturation of α7 nAChRs (45), other homomeric receptors such as α9 and α10, as well as heteropentamers (60). The calcium binding protein VILIP-1 binds to the cytoplasmic loop of α4β2 nAChRs and increases surface expression and agonist sensitivity (67). The chaperone protein 14–3-3η aids in the assembly of α4β2 nAChRs, enhancing 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).

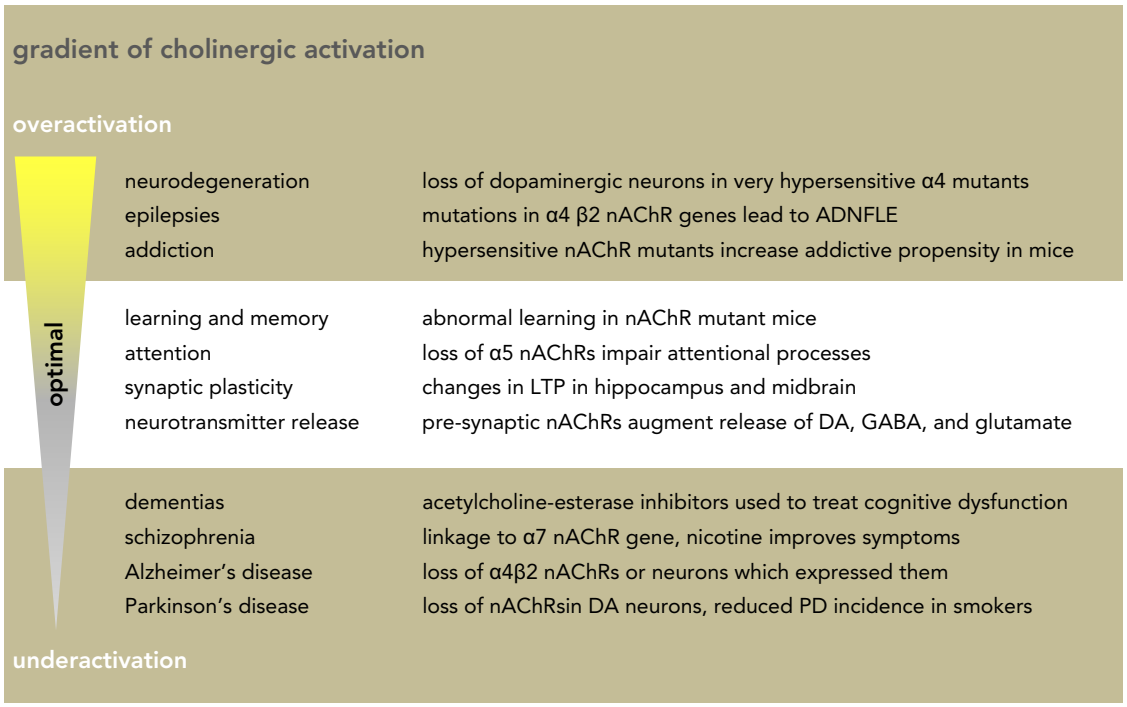

FIGURE 1. **Gradient of cholinergic activation**
The cholinergic system exists on a gradient of activation. Underactivation is associated with dementias that can occur in some neurological disorders (PD or AD) (48, 96, 102). Treatments for cognitive dysfunction and memory impairments in AD attempt to raise cholinergic tone by inhibiting the enzyme that breaks down acetylcholinesterase (ACh). The gene encoding α7 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-neurotoxin proteins, including α-btx (87), cobratoxin (125), κ-btx (15), etc., have sub-nM affinity for nAChRs (120) and other receptors and channels (7).

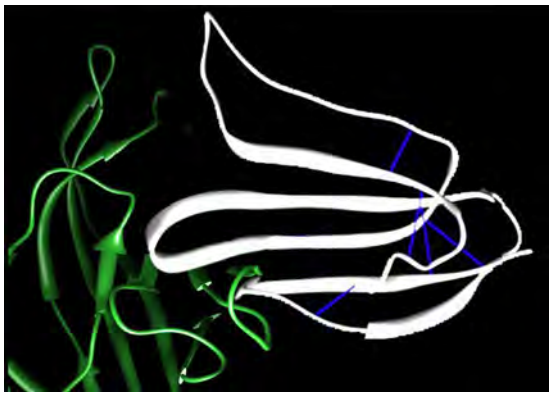

FIGURE 2. **Schematic representation of lynx1 and nAChRs**
Based on the crystal structure of α-Btx and nAChRs, and the NMR structure of lynx1 (69). nAChR is in green, and lynx1 is in white. The disulfide bonds between the conserved cysteine residues are depicted in blue. The disulfide bonds stabilize β sheets, from which three loops emerge.

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-colubritoxin, 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

lynx 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.

Stablizing 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 calcium levels, and enhancements in synaptic efficacy. Furthermore, lynx1KO mice exhibit alterations in synaptic plasticity and improved fear-conditioned learning. Normally, young developing brains undergo a period of robust plasticity–critical period plasticity–that is not available to adults. In the visual system, the critical period for plasticity closes after the *postnatal week 4* in mice. In lynx1KO mice, however, the robust plasticity of youth is demonstrated past the time window when the critical period is normally over (82). Previous studies in wild-type mice have demonstrated the role of the inhibitory network in critical period plasticity (33), indicating a possible alteration in inhibitory signaling in lynx1KO mice. Although the role of the cholinergic system during visual processing (30) and development has been recognized (9), it has been a mystery why the critical period closes in late postnatal development and remains closed despite heavy cholinergic innervation of the visual system. These findings indicate that suppression of the cholinergic system by lynx proteins stabilizes neural circuitry. In that vein, cholinergic enhancement (via cholinesterase inhibition) reopens the critical period for visual acuity in adult wild-type mice (82). This indicates that the cellular mechanisms for robust plasticity are maintained in adulthood through the cholinergic system but are normally suppressed by the action of lynx. Under

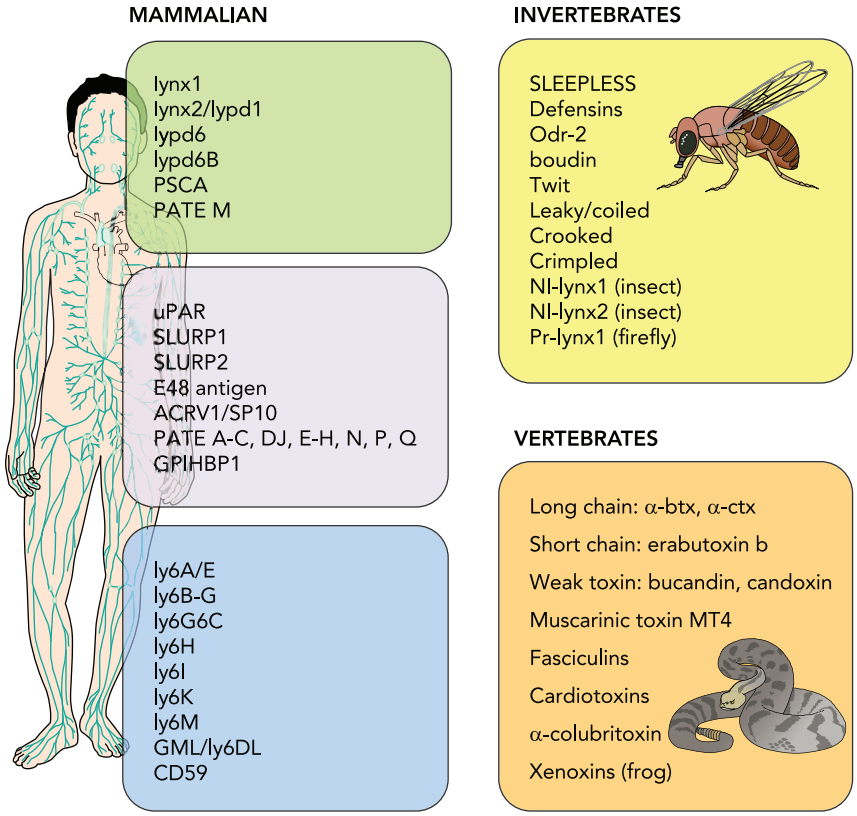

FIGURE 3. **Genes of the lynx superfamily**
Green: mammalian genes, brain enriched. Prostate stem cell antigen (PSCA) is expressed in the chicken and mouse telencephalon and peripheral ganglia (51), whereas lypd6 is highly expressed in some neurons in the cerebral cortex (24). Ectopic expression of a soluble version of lypd6 can lead to altered calcium permeability through nicotinic receptors and altered activity and antinociception (24). PATE-M is part of a cluster of genes expressed in the prostate, but one of its splice variants is also expressed in the brain (66). PATE-M is expressed at low levels in the cerebral cortex, among other areas, with relatively lower levels in the frontal cortex, and no expression in the amygdala, hippocampus, and thalamus (66). *Purple*: mammalian genes, skin and reproductive tissues (42). SLURP-1 is a secreted member of the family predominantly expressed in the periphery and is linked to the autoimmune disorder Mal de Maleda (6, 16). *Blue*: mammalian genes, immune system. CD59 is a complement inhibitor (25) and inhibits innate immunity, and ly6 antigens are expressed in immune tissues and are largely involved in acquired immunity. *Orange*: vertebrate genes. Vertebrate genes include a wide array of snake and other poisonous toxins. *Yellow*: invertebrate genes. Odr-2 in *C. elegans* is critical for chemotaxis (17), and the *Drosophila* (124) gene SLEEPLESS has been shown to bind to the potassium channel Shaker and control cholinergic neuron activity and sleep-wake patterns. Other *Drosophila* members of the family leaky/Coiled and crooked and crimpled (86) are important for forming tight junctions and blood-brain barrier integrity, possibly by forming a complex with neurexin IV, a central component of septal junctions, which form a diffusion barrier to the brain.

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

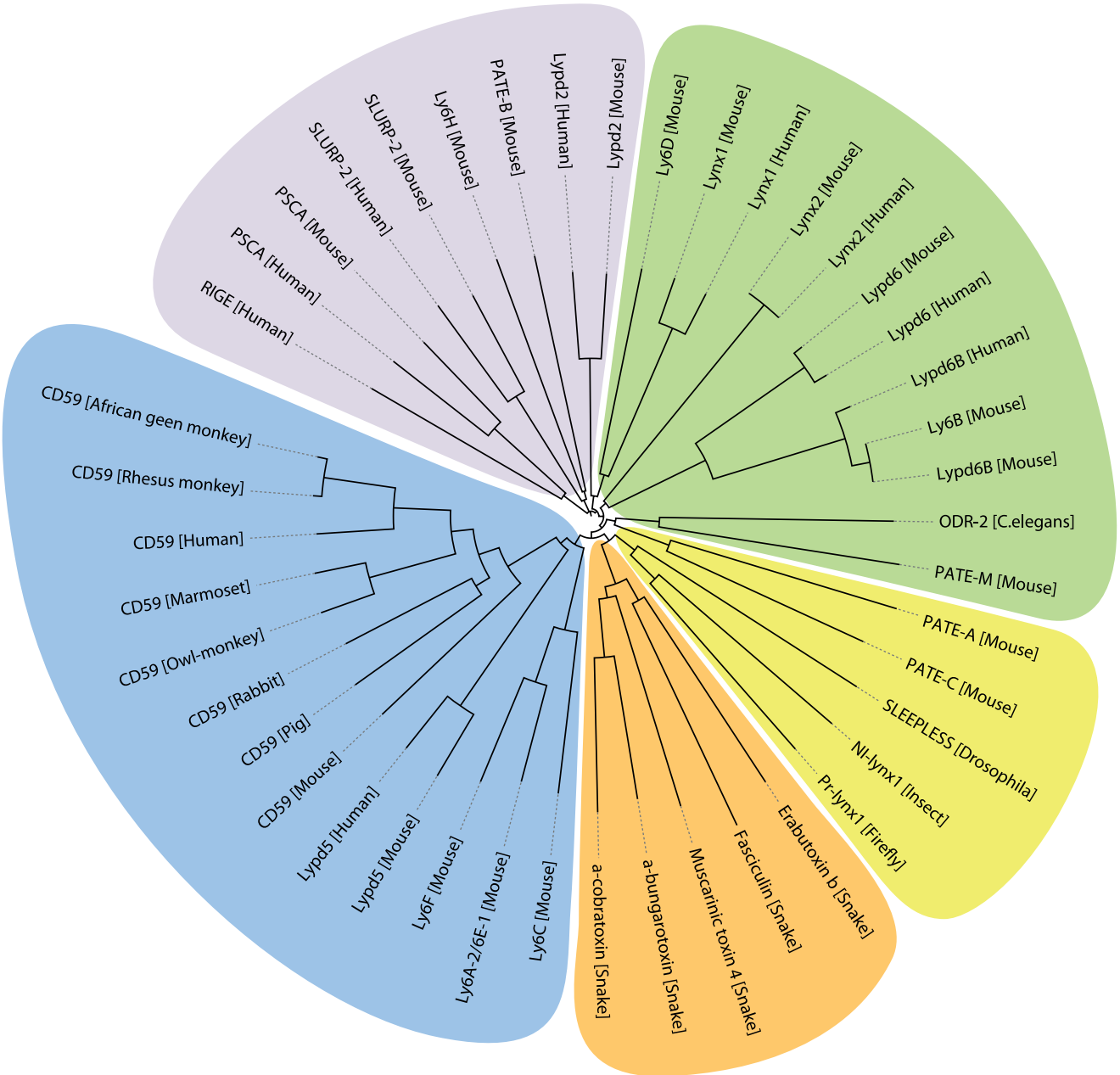

FIGURE 4. **Phylogenetic representation of the ly6/uPAR superfamily**
Mammalian brain genes (green), immunological genes (blue), skin and reproductive genes in mammals (purple), vertebrates genes (orange), and invertebrate genes (yellow). Shown is the phylogenetic relationship of the gene family through Clustal analysis on the amino acid sequence of the open-reading frame of the mature protein.

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 lynx1KO mice during fear-conditioning learning. The possibility of attentional differences in lynx1KO 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 lynx1KO 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. Furthfermore, pharmacological blockers of nAChRs abolished the enhanced critical period plasticity in lynx1KO 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 by 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 lynxpd6B 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

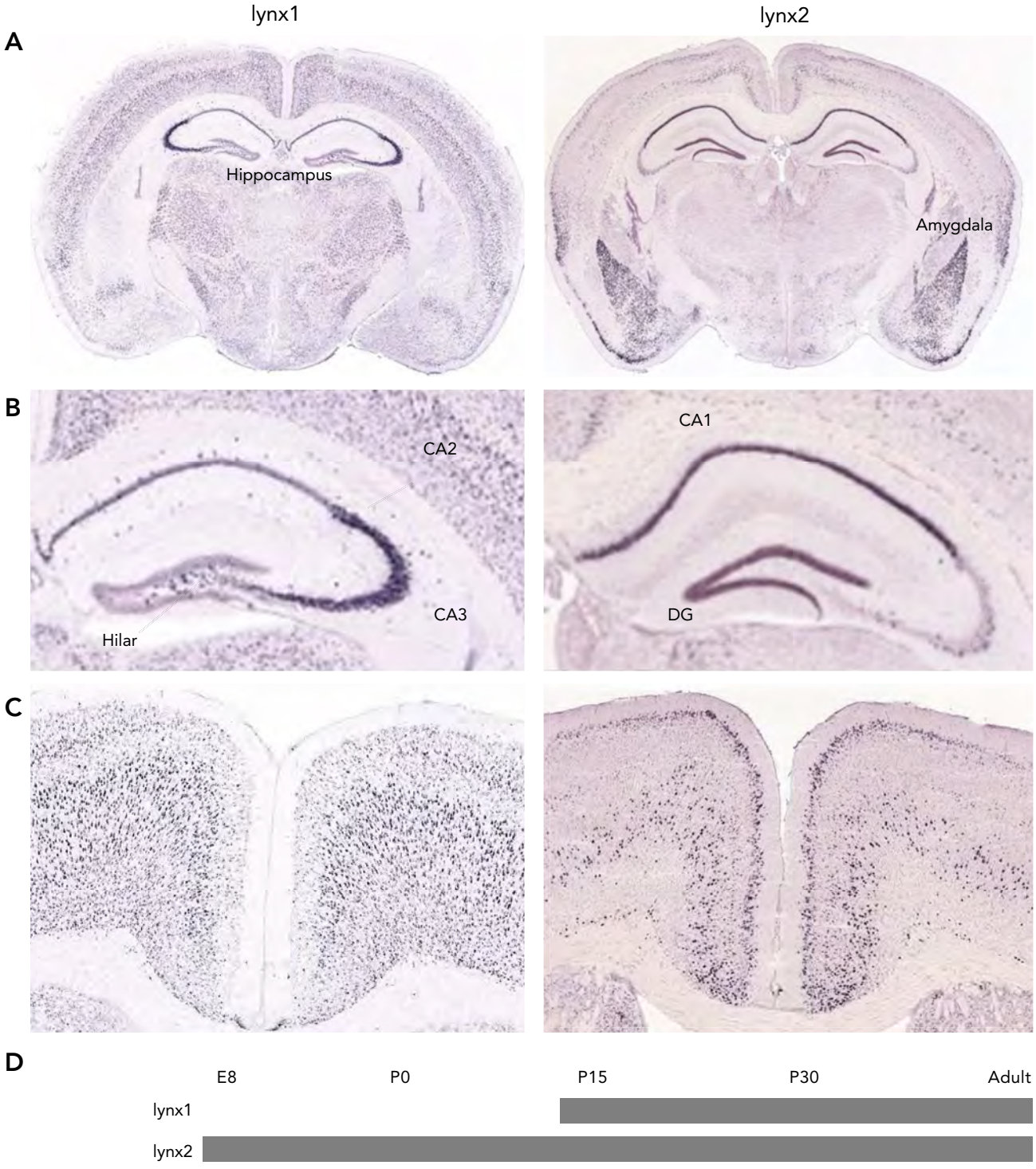

FIGURE 5. **Complementary expression patterns of lynx1 vs. lynx2 genes**
A: coronal mouse brain sections probed with in situ hybridization. Lynx1 has a widespread distribution with high levels in the hippocampus, whereas lynx2 is highly expressed in the amygdala. *B*: hippocampus. Complementary expression 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).

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

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

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.

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, another mutant that demonstrates altered fear-conditioned learning, rab3A KO mice, demonstrates downregulation of lynx1 in cortex and hippocampus (126).

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).

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 CHRNB2 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

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; 5R43MH083416-02 and 1R43MH094004-01 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 cognitive enhancement therapies related to the subject matter.

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. edited and revised manuscript; J.M.M., A.W., and H.A.L. approved final version of manuscript.

References

1. Adermann K, Wattler F, Wattler S, Heine G, Meyer M, Forssmann WG, Nehls M. Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily. *Protein Sci* 8: 810–819, 1999.
2. Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev* 89: 73–120, 2009.
3. Alexander JK, Govind AP, Drisdel RC, Blanton MP, Vallejo Y, Lam TT, Green WN. Palmitoylation of nicotinic acetylcholine receptors. *J Mol Neurosci* 40: 12–20, 2010.
4. Arredondo J, Chernyavsky AI, Grando SA. The nicotinic receptor antagonists abolish pathobiologic effects of tobacco-derived nitrosamines on BEP2D cells. *J Cancer Res Clin Oncol* 132: 653–663, 2006.
5. Arredondo J, Chernyavsky AI, Grando SA. SLURP-1 and -2 in normal, immortalized and malignant oral keratinocytes. *Life Sci* 80: 2243–2247, 2007.
6. Arredondo J, Chernyavsky AI, Webber RJ, Grando SA. Biological effects of SLURP-1 on human keratinocytes. *J Invest Dermatol* 125: 1236–1241, 2005.
7. Auer S, Sturzebecher AS, Juttner R, Santos-Torres J, Hanack C, Frahm S, Liehl B, Ibanez-Tallon I. Silencing neurotransmission with membrane-tethered toxins. *Nat Methods* 7: 229–236, 2010.
8. Bailey CD, De Biasi M, Fletcher PJ, Lambe EK. The nicotinic acetylcholine receptor alpha5 subunit plays a key role in attention circuitry and accuracy. *J Neurosci* 30: 9241–9252, 2010.
9. Bear MF, Singer W. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature* 320: 172–176, 1986.
10. Becanovic K, Pouladi MA, Lim RS, Kuhn A, Pavlidis P, Luthi-Carter R, Hayden MR, Leavitt BR. Transcriptional changes in Huntington disease identified using genome-wide expression profiling and cross-platform analysis. *Hum Mol Genet* 19: 1438–1452, 2010.
11. Bertrand D, Picard F, Le Hellard S, Weiland S, Favre I, Phillips H, Bertrand S, Berkovic SF, Malafosse A, Mulley J. How mutations in the nAChRs can cause ADNFLE epilepsy. *Epilepsia* 43, *Suppl* 5: 112–122, 2002.
12. Bertrand S, Weiland S, Berkovic SF, Steinlein OK, Bertrand D. Properties of neuronal nicotinic acetylcholine receptor mutants from humans suffering from autosomal dominant nocturnal frontal lobe epilepsy. *Br J Pharmacol* 125: 751–760, 1998.
13. Brakenhoff RH, Gerretsen M, Knippels EM, van Dijk M, van Essen H, Weghuis DO, Sinke RJ, Snow GB, van Dongen GA. The human E48 antigen, highly homologous to the murine Ly-6 antigen ThB, is a GPI-anchored molecule apparently involved in keratinocyte cell-cell adhesion. *J Cell Biol* 129: 1677–1689, 1995.
14. Buchmann AF, Blomeyer D, Jennen-Steinmetz C, Schmidt MH, Esser G, Banaschewski T, Laucht M. Early smoking onset may promise initial pleasurable sensations and later addiction. *Addict Biol*. In press.
15. Chiappinelli VA, Hue B, Mony L, Sattelle DB. Kappa-bungarotoxin blocks nicotinic transmission at an identified invertebrate central synapse. *J Exp Biol* 141: 61–71, 1989.
16. Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, Huber M, Bertrand D, Hohl D. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. *Hum Mol Genet* 12: 3017–3024, 2003.
17. Chou JH, Bargmann CI, Sengupta P. The *Caenorhabditis elegans* odr-2 gene encodes a novel Ly-6-related protein required for olfaction. *Genetics* 157: 211–224, 2001.
18. Chu LW, Ma ES, Lam KK, Chan MF, Lee DH. Increased alpha 7 nicotinic acetylcholine receptor protein levels in Alzheimer's disease patients. *Dement Geriatr Cogn Disord* 19: 106–112, 2005.
19. Chung BH, Mullegama S, Marshall CR, Lionel AC, Weksberg R, Dupuis L, Brick L, Li C, Scherer SW, Aradhya S, Stavropoulos DJ, Elsea SH, Mendoza-Londono R. Severe intellectual disability and autistic features associated with microduplication 2q231. *Eur J Hum Genet* 20: 398–403, 2012.
20. Conroy WG, Liu Z, Nai Q, Coggan JS, Berg DK. PDZ-containing proteins provide a functional postsynaptic scaffold for nicotinic receptors in neurons. *Neuron* 38: 759–771, 2003.
21. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* 127: 59–69, 2006.

22. Dalack GW, Becks L, Hill E, Pomerleau OF, Meador-Woodruff JH. Nicotine withdrawal and psychiatric symptoms in cigarette smokers with schizophrenia. *Neuropsychopharmacology* 21: 195–202, 1999.

23. Dani JA, Ji D, Zhou FM. Synaptic plasticity and nicotine addiction. *Neuron* 31: 349–352, 2001.

24. Darvas M, Morsch M, Racz I, Ahmadi S, Swandulla D, Zimmer A. Modulation of the Ca^{2+} conductance of nicotinic acetylcholine receptors by Lypd6. *Eur Neuropsychopharmacol* 19: 670–681, 2009.

25. Davies A, Simmons DL, Hale G, Harrison RA, Tighe H, Lachmann PJ, Waldmann H. CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med* 170: 637–654, 1989.

26. Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)* 184: 345–352, 2005.

27. Davis JA, Gould TJ. Hippocampal nAChRs mediate nicotine withdrawal-related learning deficits. *Eur Neuropsychopharmacol* 19: 551–561, 2009.

28. Davis JA, Kenney JW, Gould TJ. Hippocampal $\alpha4\beta2$ nicotinic acetylcholine receptor involvement in the enhancing effect of acute nicotine on contextual fear conditioning. *J Neurosci* 27: 10870–10877, 2007.

29. Dessaud E, Salaun D, Gayet O, Chabbert M, de-Lapeyriere O. Identification of lynx2, a novel member of the ly-6/neurotoxin superfamily, expressed in neuronal subpopulations during mouse development. *Mol Cell Neurosci* 31: 232–242, 2006.

30. Disney AA, Aoki C, Hawken MJ. Gain modulation by nicotine in macaque v1. *Neuron* 56: 701–713, 2007.

31. Drenan RM, Grady SR, Whiteaker P, McClure-Begley T, McKinney SR, Miwa JM, Bupp S, Heintz N, McIntosh JM, Bencherif M, Marks MA, Lester HA. In vivo activation of midbrain dopamine neurons via sensitized, high-affinity $\alpha6$* nicotinic acetylcholine receptors. *Neuron* 60: 123–136, 2008.

32. Eshel R, Zanin A, Kapon D, Sagi-Assif O, Brakenhoff R, van Dongen G, Witz IP. Human Ly-6 antigen E48 (Ly-6D) regulates important interaction parameters between endothelial cells and head-and-neck squamous carcinoma cells. *Int J Cancer* 98: 803–810, 2002.

33. Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK. Specific $GABA_A$ circuits for visual cortical plasticity. *Science* 303: 1681–1683, 2004.

34. Fenster CP, Beckman ML, Parker JC, Sheffield EB, Whitworth TL, Quick MW, Lester RA. Regulation of $\alpha4\beta2$ nicotinic receptor desensitization by calcium and protein kinase C. *Mol Pharmacol* 55: 432–443, 1999.

35. Fenster CP, Whitworth TL, Sheffield EB, Quick MW, Lester RA. Upregulation of surface $\alpha4\beta2$ nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. *J Neurosci* 19: 4804–4814, 1999.

36. Fernandes CC, Berg DK, Gomez-Varela D. Lateral mobility of nicotinic acetylcholine receptors on neurons is determined by receptor composition, local domain, and cell type. *J Neurosci* 30: 8841–8851, 2010.

37. Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. Habenular $\alpha5$ nicotinic receptor subunit signalling controls nicotine intake. *Nature* 471: 597–601, 2011.

38. Frahm S, Slimak MA, Ferrarese L, Santos-Torres J, Antolin-Fontes B, Auer S, Filkin S, Pons S, Fontaine JF, Tsetlin V, Maskos U, Ibanez-Tallon I. Aversion to nicotine is regulated by the balanced activity of beta4 and alpha5 nicotinic receptor subunits in the medial habenula. *Neuron* 70: 522–535, 2011.

39. Freedman R, Ross R, Leonard S, Myles-Worsley M, Adams CE, Waldo M, Tregellas J, Martin L, Olincy A, Tanabe J, Kisley MA, Hunter S, Stevens KE. Early biomarkers of psychosis. *Dialogues Clin Neurosci* 7: 17–29, 2005.

40. Fry BG, Lumsden NG, Wuster W, Wickramaratna JC, Hodgson WC, Kini RM. Isolation of a neurotoxin (alpha-colubritoxin) from a nonvenomous colubrid: evidence for early origin of venom in snakes. *J Mol Evol* 57: 446–452, 2003.

41. Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, Nevalainen TJ, Norman JA, Lewis RJ, Norton RS, Renjifo C, de la Vega RC. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. *Annu Rev Genomics Hum Genet* 10: 483–511, 2009.

42. Galat A. The three-fingered protein domain of the human genome. *Cell Mol Life Sci* 65: 3481–3493, 2008.

43. Grady SR, Salminen O, Laverty DC, Whiteaker P, McIntosh JM, Collins AC, Marks MJ. The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochem Pharmacol* 74: 1235–1246, 2007.

44. Guillem K, Bloem B, Poorthuis RB, Loos M, Smit AB, Maskos U, Spijker S, Mansvelder HD. Nicotinic acetylcholine receptor beta2 subunits in the medial prefrontal cortex control attention. *Science* 333: 888–891, 2011.

45. Halevi S, McKay J, Palfreyman M, Yassin L, Eshel M, Jorgensen E, Treinin M. The *C. elegans* ric-3 gene is required for maturation of nicotinic acetylcholine receptors. *EMBO J* 21: 1012–1020, 2002.

46. Hansen SB, Taylor P. Galanthamine and non-competitive inhibitor binding to ACh-binding protein: evidence for a binding site on non-alpha-subunit interfaces of heteromeric neuronal nicotinic receptors. *J Mol Biol* 369: 895–901, 2007.

47. Hasselmo ME. Neuromodulation: acetylcholine and memory consolidation. *Trends Cogn Sci* 3: 351–359, 1999.

48. Hasselmo ME, Sarter M. Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36: 52–73, 2010.

49. Hoffman PW, Ravindran A, Huganir RL. Role of phosphorylation in desensitization of acetylcholine receptors expressed in *Xenopus* oocytes. *J Neurosci* 14: 4185–4195, 1994.

50. Horie M, Okutomi K, Taniguchi Y, Ohbuchi Y, Suzuki M, Takahashi E. Isolation and characterization of a new member of the human Ly6 gene family (LY6H). *Genomics* 53: 365–368, 1998.

51. Hruska M, Keefe J, Wert D, Tekinay AB, Hulce JJ, Ibanez-Tallon I, Nishi R. Prostate stem cell antigen is an endogenous lynx1-like prototoxin that antagonizes $\alpha7$-containing nicotinic receptors and prevents programmed cell death of parasympathetic neurons. *J Neurosci* 29: 14847–14854, 2009.

52. Ibanez-Tallon I, Miwa JM, Wang HL, Adams NC, Crabtree GW, Sine SM, Heintz N. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. *Neuron* 33: 893–903, 2002.

53. Jeanclos EM, Lin L, Treuil MW, Rao J, DeCoster MA, Anand R. The chaperone protein 14–3-3η interacts with the nicotinic acetylcholine receptor α subunit. Evidence for a dynamic role in subunit stabilization. *J Biol Chem* 276: 28281–28290, 2001.

54. Jones IW, Westmacott A, Chan E, Jones RW, Dineley K, O'Neill MJ, Wonnacott S. Alpha7 nicotinic acetylcholine receptor expression in Alzheimer's disease: receptor densities in brain regions of the APP(SWE) mouse model and in human peripheral blood lymphocytes. *J Mol Neurosci* 30: 83–84, 2006.

55. Kawai H, Berg DK. Nicotinic acetylcholine receptors containing $\alpha7$ subunits on rat cortical neurons do not undergo long-lasting inactivation even when up-regulated by chronic nicotine exposure. *J Neurochem* 78: 1367–1378, 2001.

56. King SL, Marks MJ, Grady SR, Caldarone BJ, Koren AO, Mukhin AG, Collins AC, Picciotto MR. Conditional expression in corticothalamic efferents reveals a developmental role for nicotinic acetylcholine receptors in modulation of passive avoidance behavior. *J Neurosci* 23: 3837–3843, 2003.

57. Kordis D, Gubensek F. Adaptive evolution of animal toxin multigene families. *Gene* 261: 43–52, 2000.

58. Kumari S, Borroni V, Chaudhry A, Chanda B, Massol R, Mayor S, Barrantes FJ. Nicotinic acetylcholine receptor is internalized via a Rac-dependent, dynamin-independent endocytic pathway. *J Cell Biol* 181: 1179–1193, 2008.

59. Kuryatov A, Gerzanich V, Nelson M, Olale F, Lindstrom J. Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca^{2+} permeability, conductance, and gating of human $\alpha4\beta2$ nicotinic acetylcholine receptors. *J Neurosci* 17: 9035–9047, 1997.

60. Lansdell SJ, Gee VJ, Harkness PC, Doward AI, Baker ER, Gibb AJ, Millar NS. RIC-3 enhances functional expression of multiple nicotinic acetylcholine receptor subtypes in mammalian cells. *Mol Pharmacol* 68: 1431–1438, 2005.

60a. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, Desaki AL, Desta T, Diep E, Dolbeare TA, Donelan MJ, Dong HW, Dougherty JG, Duncan BJ, Ebbert AJ, Eichele G, Estin LK, Faber C, Face BA, Fields R, Fischer SR, Fliss TP, Frensley C, Gates SN, Glattfelder KJ, Halverson KR, Hart MR, Hohmann JG, Howell MP, Jeung DP, Johnson RA, Karr PT, Kawal R, Kidney JM, Knapik RH, Kuan CL, Lake JH, Laramee AR, Larsen KD, Lau C, Lemon TA, Liang AJ, Liu Y, Luong LT, Michaels J, Morgan JJ, Morgan RJ, Mortrud MT, Mosqueda NF, Ng LL, Ng R, Orta GJ, Overly CC, Pak TH, Parry SE, Pathak SD, Pearson OC, Puchalski RB, Riley ZL, Rockett HR, Rowland SA, Royall JJ, Ruiz MJ, Sarno NR, Schaffnit K, Shapovalova NV, Sivisay T, Slaughterbeck CR, Smith SC, Smith KA, Smith BI, Sodt AJ, Stewart NN, Stumpf KR, Sunkin SM, Sutram M, Tam A, Teemer CD, Thaller C, Thompson CL, Varnam LR, Visel A, Whitlock RM, Wohnoutka PE, Wolkey CK, Wong VY, Wood M, Yaylaoglu MB, Young RC, Youngstrom BL, Yuan XF, Zhang B, Zwingman TA, Jones AR. Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 445: 168–176, 2007.

61. Leonard S, Breese C, Adams C, Benhammou K, Gault J, Stevens K, Lee M, Adler L, Olincy A, Ross R, Freedman R. Smoking and schizophrenia: abnormal nicotinic receptor expression. *Eur J Pharmacol* 393: 237–242, 2000.

62. Lester HA, Xiao C, Srinivasan R, Son C, Miwa J, Pantoja R, Dougherty DA, Banghart MR, Goate AM, Wang JC. Nicotine is a Selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for drug discovery. *AAPS J* 11: 167–177, 2009.

63. Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, Luthi A. A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* 480: 331–335, 2011.

64. Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology (Berl)* 184: 523–539, 2005.

65. Levin ED, Rezvani AH. Nicotinic interactions with antipsychotic drugs, models of schizophrenia and impacts on cognitive function. *Biochem Pharmacol* 74: 1182–1191, 2007.

66. Levitin F, Weiss M, Hahn Y, Stern O, Papke RL, Matusik R, Nandana SR, Ziv R, Pichinuk E, Salame S, Bera T, Vincent J, Lee B, Pastan I, Wreschner DH. PATE gene clusters code for multiple, secreted TFP/Ly-6/uPAR proteins that are expressed in reproductive and neuron-rich tissues and possess neuromodulatory activity. *J Biol Chem* 283: 16928–16939, 2008.

67. Lin L, Jeanclos EM, Treuil M, Braunewell KH, Gundelfinger ED, Anand R. The calcium sensor protein visinin-like protein-1 modulates the surface expression and agonist sensitivity of the α-β nicotinic acetylcholine receptor. *J Biol Chem* 277: 41872–41878, 2002.

68. Liu Z, Neff RA, Berg DK. Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. *Science* 314: 1610–1613, 2006.

69. Lyukmanova EN, Shenkarev ZO, Shulepko MA, Mineev KS, D'Hoedt D, Kasheverov IE, Filkin SY, Krivolapova AP, Janickova H, Dolezal V, Dolgikh DA, Arseniev AS, Bertrand D, Tsetlin VI, Kirpichnikov MP. NMR structure and action on nicotinic acetylcholine receptors of water-soluble domain of human lynx1. *J Biol Chem* 286: 10618–10627, 2011.

70. Mallya M, Campbell RD, Aguado B. Characterization of the five novel Ly-6 superfamily members encoded in the MHC, and detection of cells expressing their potential ligands. *Protein Sci* 15: 2244–2256, 2006.

71. Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 27: 349–357, 2000.

72. Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J Pharmacol Exp Ther* 226: 817–825, 1983.

73. Marrosu F, Portas C, Mascia MS, Casu MA, Fa M, Giagheddu M, Imperato A, Gessa GL. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res* 671: 329–332, 1995.

74. Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, Huchet M, Damaj MI, and Changeux JP. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* 398: 805–810, 1999.

75. Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM, McIntosh JM, Rossi F, Champtiaux N, Zoli M, Changeux JP. Effects of nicotine in the dopaminergic system of mice lacking the α subunit of neuronal nicotinic acetylcholine receptors. *Eur J Neurosci* 17: 1329–1337, 2003.

76. McKinney M, Jacksonville MC. Brain cholinergic vulnerability: relevance to behavior and disease. *Biochem Pharmacol* 70: 1115–1124, 2005.

77. Miller BH, Schultz LE, Gulati A, Cameron MD, Pletcher MT. Genetic regulation of behavioral and neuronal responses to fluoxetine. *Neuropsychopharmacology* 33: 1312–1322, 2008.

78. Mineur YS, Abizaid A, Rao Y, Salas R, DiLeone RJ, Gundisch D, Diano S, De Biasi M, Horvath TL, Gao XB, Picciotto MR. Nicotine decreases food intake through activation of POMC neurons. *Science* 332: 1330–1332, 2011.

79. Miwa JM, Freedman R, Lester HA. Neural systems governed by nicotinic acetylcholine receptors: emerging hypotheses. *Neuron* 70: 20–33, 2011.

80. Miwa JM, Ibanez-Tallon I, Crabtree GW, Sanchez R, Sali A, Role LW, Heintz N. Lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. *Neuron* 23: 105–114, 1999.

81. Miwa JM, Stevens TR, King SL, Caldarone BJ, Ibanez-Tallon I, Xiao C, Fitzsimonds RM, Pavlides C, Lester HA, Picciotto MR, Heintz N. The prototoxin lynx1 acts on nicotinic acetylcholine receptors to balance neuronal activity and survival in vivo. *Neuron* 51: 587–600, 2006.

82. Morishita H, Miwa JM, Heintz N, Hensch T. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* 330: 1238–1240, 2010.

83. Nashmi R, Lester H. Cell autonomy, receptor autonomy, and thermodynamics in nicotine receptor up-regulation. *Biochem Pharmacol* 74: 1145–1154, 2007.

84. Nashmi R, Xiao C, Deshpande P, McKinney S, Grady SR, Whiteaker P, Huang Q, McClure-Begley T, Lindstrom JM, Labarca C, Collins AC, Marks MJ, Lester HA. Chronic nicotine cell specifically upregulates functional α4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. *J Neurosci* 27: 8202–8218, 2007.

85. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of α β nicotinic acetylcholine receptors. *Mol Pharmacol* 63: 332–341, 2003.

86. Nilton A, Oshima K, Zare F, Byri S, Nannmark U, Nyberg KG, Fehon RG, Uv AE. Crooked, coiled and crimpled are three Ly6-like proteins required for proper localization of septate junction components. *Development* 137: 2427–2437, 2010.

87. Nirthanan S, Gwee MC. Three-finger alpha-neurotoxins and the nicotinic acetylcholine receptor, forty years on. *J Pharm Sci* 94: 1–17, 2004.

88. Orr-Urtreger A, Broide RS, Kasten MR, Dang H, Dani JA, Beaudet AL, Patrick JW. Mice homozygous for the L250T mutation in the α7 nicotinic acetylcholine receptor show increased neuronal apoptosis and die within 1 day of birth. *J Neurochem* 74: 2154–2166, 2000.

89. Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW, Eichele G. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab* 4: 163–173, 2006.

90. Palfree RG. Ly-6-domain proteins: new insights and new members: a C-terminal Ly-6 domain in sperm acrosomal protein SP-10. *Tissue Antigens* 48: 71–79, 1996.

91. Perkins KA. Chronic tolerance to nicotine in humans and its relationship to tobacco dependence. *Nicotine Tob Res* 4: 405–422, 2002.

92. Pfeffer CK, Stein V, Keating DJ, Maier H, Rinke I, Rudhard Y, Hentschke M, Rune GM, Jentsch TJ, Hubner CA. NKCC1-dependent GABAergic excitation drives synaptic network maturation during early hippocampal development. *J Neurosci* 29: 3419–3430, 2009.

93. Picciotto MR. Nicotine as a modulator of behavior: beyond the inverted U. *Trends Pharmacol Sci* 24: 493–499, 2003.

94. Picciotto MR, Addy NA, Mineur YS, Brunzell DH. It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* 84: 329–342, 2008.

95. Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. *Neuroreport* 13: 1097–1106, 2002.

96. Picciotto MR, Zoli M. Neuroprotection via nAChRs: the role of nAChRs in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. *Front Biosci* 13: 492–504, 2008.

97. Plessy C, Fagiolini M, Wagatsuma A, Harasawa N, Kuji T, Asaka-Oba A, Kanzaki Y, Fujishima S, Waki K, Nakahara H, Hensch TK, Carninci P. A resource for transcriptomic analysis in the mouse brain. *PLos One* 3: e3012, 2008.

98. Ploug M, Ellis V. Structure-function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alpha-neurotoxins. *FEBS Lett* 349: 163–168, 1994.

99. Ponder CA, Kliethermes CL, Drew MR, Muller J, Das K, Risbrough VB, Crabbe JC, Gilliam TC, Palmer AA. Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. *Genes Brain Behav* 6: 736–749, 2007.

100. Porterfield VM, Piontkivska H, Mintz EM. Identification of novel light-induced genes in the suprachiasmatic nucleus. *BMC Neurosci* 8: 98, 2007.

101. Quick MW, Lester RA. Desensitization of neuronal nicotinic receptors. *J Neurobiol* 53: 457–478, 2002.

102. Quik M, Kulak JM. Nicotine and nicotinic receptors; relevance to Parkinson's disease. *Neurotoxicology* 23: 581–594, 2002.

103. Ramarao MK, Cohen JB. Mechanism of nicotinic acetylcholine receptor cluster formation by rapsyn. *Proc Natl Acad Sci USA* 95: 4007–4012, 1998.

104. Rattner A, Nathans J. The genomic response to retinal disease and injury: evidence for endothelin signaling from photoreceptors to glia. *J Neurosci* 25: 4540–4549, 2005.

105. Rezvani AH, Levin ED. Cognitive effects of nicotine. *Biol Psychiatry* 49: 258–267, 2001.

106. Rubin CM, van der List DA, Ballesteros JM, Goloshchapov AV, Chalupa LM, Chapman B. Mouse mutants for the nicotinic acetylcholine receptor ss2 subunit display changes in cell adhesion and neurodegeneration response genes. *PLos One* 6: e18626, 2011.

107. Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, Johnson EO, Madden PA, Swan GE, Wang JC, Goate AM, Rice JP, Bierut LJ. Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet* 150B: 453–466, 2009.

108. Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 16: 36–49, 2007.

109. Schwartz RD, Kellar KJ. Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. *Science* 220: 214–216, 1983.

110. Schwarz J, Schwarz S, Dorigo O, Stützer A, Wegner F, Labarca C, Deshpande P, Gil J, Berk A, Lester HA. Enhanced expression of hypersensitive α4* nAChR in adult mice increases the loss of midbrain dopaminergic neurons. *FASEB J* 20: 935–946, 2006.

111. Sekhon HS, Song P, Jia Y, Lindstrom J, Spindel ER. Expression of lynx1 in developing lung and its modulation by prenatal nicotine exposure. *Cell Tissue Res* 320: 287–297, 2005.

112. Small DH. Network dysfunction in Alzheimer's disease: does synaptic scaling drive disease progression? *Trends Mol Med* 14: 103–108, 2008.

113. Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, Wan X, Pavlidis P, Mills AA, Karayiorgou M, Gogos JA. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat Genet* 40: 751–760, 2008.

114. Stevens TR, Krueger SR, Fitzsimonds RM, Picciotto MR. Neuroprotection by nicotine in mouse primary cortical cultures involves activation of calcineurin and L-type calcium channel inactivation. *J Neurosci* 23: 10093–10099, 2003.

115. Tang J, Dani JA. Dopamine enables in vivo synaptic plasticity associated with the addictive drug nicotine. *Neuron* 63: 673–682, 2009.

116. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA. Nicotine activation of α4* receptors: sufficient for reward, tolerance and sensitization. *Science* 306: 1029–1032, 2004.

117. Tekinay AB, Nong Y, Miwa JM, Lieberam I, Ibanez-Tallon I, Greengard P, Heintz N. A role for LYNX2 in anxiety-related behavior. *Proc Natl Acad Sci USA* 106: 4477–4482, 2009.

118. Thorn CA, Graybiel AM. Pausing to regroup: thalamic gating of cortico-basal ganglia networks. *Neuron* 67: 175–178, 2010.

119. Tropea D, Kreiman G, Lyckman A, Mukherjee S, Yu H, Horng S, Sur M. Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex. *Nat Neurosci* 9: 660–668, 2006.

120. Tsetlin V, Utkin Y, Kasheverov I. Polypeptide and peptide toxins, magnifying lenses for binding sites in nicotinic acetylcholine receptors. *Biochem Pharmacol* 78: 720–731, 2009.

121. Tsuji H, Okamoto K, Matsuzaka Y, Iizuka H, Tamiya G, Inoko H. SLURP-2, a novel member of the human Ly-6 superfamily that is up-regulated in psoriasis vulgaris. *Genomics* 81: 26–33, 2003.

122. Wieczorek L, Maas JW Jr, Muglia LM, Vogt SK, Muglia LJ. Temporal and regional regulation of gene expression by calcium-stimulated adenylyl cyclase activity during fear memory. *PLos One* 5: e13385, 2010.

123. Wooltorton JR, Pidoplichko VI, Broide RS, Dani JA. Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* 23: 3176–3185, 2003.

124. Wu MN, Joiner WJ, Dean T, Yue Z, Smith CJ, Chen D, Hoshi T, Sehgal A, Koh K. SLEEPLESS, a Ly-6/neurotoxin family member, regulates the levels, localization and activity of Shaker. *Nat Neurosci* 13: 69–75, 2010.

125. Yang CC. Crystallization and properties of cobrotoxin from Formosan cobra venom. *J Biol Chem* 240: 1616–1618, 1965.

126. Yang S, Farias M, Kapfhamer D, Tobias J, Grant G, Abel T, Bucan M. Biochemical, molecular and behavioral phenotypes of Rab3A mutations in the mouse. *Genes Brain Behav* 6: 77–96, 2007.

127. Zhou D, Wang J, Zapala MA, Xue J, Schork NJ, Haddad GG. Gene expression in mouse brain following chronic hypoxia: role of sarcospan in glial cell death. *Physiol Genomics* 32: 370–379, 2008.