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Modulation of cholinergic activity through lynx prototoxins: Implications for cognition and anxiety regulation

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1. The functional importance of the cholinergic system

The cholinergic system helps to transduce experiences through the modulation of functional circuits in the brain. It is a neurotransmitter system implicated in many complex cognitive processes; including attention, fear and anxiety, memory storage and cognitive flexibility. Cholinergic neurons fire in response to salient stimuli which contributes to heightened attentional states. The release of the excitatory neurotransmitter, acetylcholine, functions to amplify signal to noise ratios (Disney et al., 2007). For example, behaviorally driven cholinergic transients have been shown to aid in cue-detection (Sarter et al., 2014), maintaining information in the presence of distractors (Suzuki et al., 1997) and thus influencing attentional performance. In addition, cholinergic activity has been detected in response to unexpected reward (Hangya et al., 2015), and sustained attention (Gill et al., 2000; Himmelheber et al., 2000).

The cholinergic system aids in the encoding of experiences into long-term memories (Letzkus et al., 2011). The cholinergic signalling plays a role in the sleep-wake cycle and memory consolidation during sleep. Activation of cholinergic neurons is associated with sleep-wake cycles, and during wakefulness acetylcholine facilitates thalamocortical signaling by directly exciting thalamocortical relay neurons while reducing activity in the reticular nucleus of the thalamus (Yamakawa et al., 2016). In addition, blockade of cholinergic signalling during REM sleep impaires off-line consolidation motor skills (Rasch et al., 2009), and low levels of acetylcholine during slow wave sleep mediate critical declarative memory consolidation processes (Gais and Born, 2004). Due to the complex nature of the attentional and memory-driven functions mediated by acetylcholine, several regulation mechanisms of the cholinergic system exist.

2. Structural elements of the cholinergic system

Cholinergic neuronal cell bodies are relatively localized, with cholinergic neuronal cell bodies resident in the basal forebrain and brain-stem. Their terminals, however, radiate broadly throughout the central nervous system (Zoli et al., 1999; Dani, 2001; Zaborszky,

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2002; Dani and Bertrand, 2007; Guo et al., 2015). The release of acetylcholine is not confined within the synaptic cleft and thus extra-synaptic transmission to cholinergic receptors is possible. The widely radiating axonal projections of these discretely clustered cholinergic neurons might suggest a broad action for cholinergic signaling were it not for several layers of regulatory mechanisms. The connectivity of cholinergic neurons to their targets subserves functionally and spatially selective signaling (Zaborszky, 2002; Ballinger et al., 2016, Li et al., 2018). Previously, cholinergic signaling was thought to be carried largely through volume transmission with a slow diffuse release and spatially broad changes in ACh concentration over time and space (Runfeldt et al., 2014; Sarter et al., 2009). Emerging evidence from studies utilizing new technological advances such as optogenetics, however, suggests a role for temporally precise ACh release with acute cholinergic signaling (Ballinger et al., 2016). For instance, recent studies provide evidence for precise functional connectivity from cholinergic projections neurons to specific targets that results in a spatially selective transmission (Chandler and Waterhouse, 2012; Bloem et al., 2014; Unal et al., 2015; Zouridakis et al., 2019, Li et al., 2018). This precise functional connectivity may subserve distinct functioning to specific stimuli and represent targeted networks rather than the classical model of broad cholinergic signaling. Cholinergic projection neurons from the basal forebrain to the basolateral amygdala (BLA) can produce varying responses depending on the BLA cell type, which may result in state-dependent behaviors such as learning in fear conditioning only during specific patterns of unconditioned and conditioned stimuli presentations (Unal et al., 2015). In another study basal forebrain projections to the medial prefrontal cortex were demonstrated to display a frontocaudal organization with correlations between the rostral/caudal position in the basal forebrain with distribution in mPFC (Bloem et al., 2014). The role of cholinergic functional connectivity may facilitate control over precise stimuli or represent coordinated regulation between several brain regions for complex behaviors that is different from the regulation ensued by volume transmission. Acetylcholine signals through two families of neurotransmitter receptor classes, G-protein-coupled muscarinic receptors (mAChRs) and ion channel-containing nicotinic receptors (nAChRs), which bind muscarine and nicotine, respectively. Nicotinic receptors are present on both pre- and post-synaptic neuronal subdomains. Central cholinergic neurotransmission can therefore alter neuronal excitability by changing the presynaptic release of neurotransmitters, depolarizing neurons on which they are expressed, inducing secondary messenger cascades, and/or coordinating the firing of groups of neurons (Rice and Cragg, 2004; Kawai et al., 2007; Kutlu and Gould, 2015).

Both decreases and increases in cholinergic signaling can have deleterious or suboptimal effects (Picciotto, 2003; Dani and Bertrand, 2007; Picciotto et al., 2012). Optimal operation of the cholinergic system is dependent on several regulatory mechanisms that fine-tune the activity of the cholinergic system (Miwa et al., 2012). Factors include the number and activity of cholinergic neurons, the level of acetylcholine release, the presence of acetylcholinesterase, the state of calcium stores, or receptor composition. Acetylcholinesterase is highly efficient enzyme, breaking down the neurotransmitter acetylcholine, and shortening the duration of acetylcholine signal. Genetic abnormalities within the choline transporter is associated with attention deficit hyperactivity disorder

(ADHD) and result in higher than normal levels of ACh synthesis (English et al., 2009), and aberrant cholinergic signaling is associated with schizophrenia (Higley and Picciotto, 2014).

Nicotinic acetylcholine receptors (nAChRs) are nonselective ligand-gated cation channels that exist as pentamers composed of many variations of 15 possible subunits (Changeux et al., 1998; Picciotto et al., 2001). Nicotinic receptors typically exist as heteromeric combinations of α (2–10) and β (2–4) subunits (most commonly α4β2) or as α homopentamers (α7, α9, etc.) (Picciotto, 2003; Albuquerque et al., 2009) and are dispersed on the surface of neurons, including presynaptic terminals, cell bodies, and axons (Hill et al., 1993; McGehee et al., 1995; Wonnacott, 1997; Nashmi and Lester, 2006; Hurst et al., 2013). The most abundant subtypes in the brain are α4β2 and α7 nAChR subtypes. Receptor composition gives rise to specificity of the cholinergic response, as each combination displays distinct biophysical and pharmacological properties, such as agonist affinity and desensitization kinetics (Brown and Wonnacott, 2014). Differences in stoichiometry among heteromeric subunits results in differential response profiles and sensitivity to ligands, for instance the low sensitivity (LS) stoichiometry of α4β2 consists of three α and two β subunits $(\alpha4)_3(\beta2)_2$, whereas the high sensitivity (HS) stoichiometry consists of two α and three β subunits, $(\alpha4)_2(\beta2)_3$ (Marks et al., 1999; Gotti et al., 2007; Govind et al., 2012). The net sum of cholinergic activity and the effects on behavior will depend upon the integration of these multiple factors.

3. Lynx prototoxins, protein modulators of nicotinic receptors

Nicotinic receptor function is regulated by prototoxin protein modulators of the Ly6/uPAR superfamily. ly6/uPAR superfamily members adopt a three-loop β-rich fold structure stabilized by cysteine bonds (Lyukmanova et al., 2011; Tsetlin, 2015; Vasilyeva et al., 2017; Miwa et al., 2019) that is also observed for elapid snake venom α-neurotoxins that bind to nAChRs and other receptors with high affinity. Snake toxins evolved via functional mimicry of pathways operating in the prey, such as endogenous prototoxins (Fig. 1). The identification of Ly6/uPAR family members in the mammalian brain (Kuhar et al., 1993) and their homology to the cysteine-rich signature of α-neurotoxins, was suggestive that this family of prototoxins might bind to and regulate similar molecular targets (Miwa et al., 1999).

Members of the uPAR superfamily include CD59, lymphocyte antigen genes, ly6A-H, transforming growth factor β receptor ectodomains, and uPAR. In total, at least 2583 sequences within seven subfamilies have been identified (Kessler et al., 2017). Further, the human genome contains at least 45 genes encoding the three-fingered domain (Galat et al., 2008). Three-fingered proteins exert an influence over a wide-array of physiological processes, including cell proliferation, differentiation, inflammation, and neuromodulation. Within this large superfamily, prototoxin members with significant expression in the brain include lynx1 (Miwa et al., 1999), lynx2/lypd1 (Dessaud et al., 2006; Wu et al., 2015), lypd6 (Darvas et al., 2009; Zhang et al., 2009), lypd6B (Demars and Morishita, 2014), PSCA (Jensen et al., 2015), and Ly6H (Horie et al., 1998). These are considered to be peripheral membrane proteins, attached via a GPI-anchor embedded into the neuronal membrane. The

focus of this review will explore the *in vivo* role of prototoxins, members of this family expressed in the brain.

4. Biophysical mechanism of lynx1 action

The first characterized and most well-studied brain-expressed member of the prototoxin family is lynx1. Lynx1 exerts its modulatory effect on the cholinergic system by direct interactions with nAChRs. In pull-down experiments from rat cortical extracts, lynx1 has been shown to interact with all nAChR subunits tested (α3–7, β2, and β4) (Thomsen et al., 2016). lynx1 has been shown to functionally modulate α4β2 (Ibanez-Tallon et al., 2002; Nichols et al., 2014), α3β4, α5α3β4 (George et al., 2017), α6 (Parker et al., 2017), and α7 (Lyukmanova et al., 2011; 2013) nicotinic acetylcholine receptor subtypes. The influence of this interaction on heteromeric nAChR function can be multifactorial, influencing agonist affinity, desensitization kinetics, receptor number at the cell surface, and single-channel kinetics (Ibanez-Tallon et al., 2002; George et al., 2017; Nichols et al., 2014) and dependent on nAChR subtype (Parker et al., 2017), and isoform of lynx1, whether GPI-anchored or soluble (Lyukmanova et al., 2013; Thomsen et al., 2014).

Oocytes co-expressing α4β2 nicotinic receptors and lynx1 have a faster rate of desensitization to agonist, acetylcholine, and the agonist sensitivity is reduced, as assessed by a right-ward shift in the EC_{50} to acetylcholine (Ibanez-Tallon et al., 2002). These effects could be due to changes in receptor stoichiometry or the gating functions of lynx1. Removal of the GPI anchor by PI-PLC treatment did not alter the ACh dose-response properties of α4β2 nAChRs (Nichols et al., 2014), suggesting that influence on nAChR stoichiometry through receptor assembly could be the predominant effect of lynx1. A shift in the single channel species from faster inactivating, larger amplitude currents openings (Ibanez-Tallon et al., 2002), commonly thought to be correlated with LS nAChR stoichiometry, supports the idea the lynx1 can influence stoichiometry during nAChR assembly.

5. GPI-anchored vs secreted versions of prototoxins

Clear gating effects of lynx1 applied acutely as an engineered water-soluble version (ws-lynx1), have also been demonstrated, which are differentiated from the native GPI-anchored native membrane protein. Ws-lynx1 has an inhibitory effect on agonist sensitivity and peak amplitude and can inhibit α7, α4β2, and α3β2, although the functional effects were more pronounced with α4β2 nAChRs. The inhibitory effect is concentration specific and apparently can act in a non-competitive manner (Lyukmanova et al., 2011; 2013). Evidence supports lynx1 function on both gating and receptor assembly of α4β2 nAChRs. It should be noted that differential effects have been reported for lynx1 when co-expressed with nAChRs - and thus GPI-anchored membrane proteins - (Ibanez-Tallon et al., 2002) as opposed to when delivered as a soluble form of protein (Lyukmanova et al., 2013; Miwa and Walz, 2012). Thus *in vitro* studies on ws-lynx1 should be considered in this context. It is clear from the actions of elapid snake toxins and secreted mammalian family members such as SLURPs, that significant gating effects are capable when prototoxins are bound to the nAChR and not otherwise attached to the membrane (Vasilyeva et al., 2017; Durek et al., 2017; Chimienti et al., 2003; Lyukmanova et al., 2016). The functional effects of SLURPs

on nAChRs have been reported mostly outside the central nervous system (Adeyo et al., 2014; Chimienti et al., 2003).

6. Concatameric nAChR studies

To constrain the number of variables between multiple factors of stoichiometry, receptor number, and gating, concatemeric nAChRs have been used to fix nAChR receptor stoichiometry (George et al., 2017). In these studies, the five subunit cDNAs for α3β4 nAChRs were fused into a single polypeptide, inhibiting any potential heterogeneity in stoichiometry at the fifth position. In these studies, the effect of lynx1 on either the $(\alpha 3)_2(\beta 4)_3$ or $(\alpha 3)_2(\beta 4)_3$ nAChR stoichiometry can be carried out independently. The identity of the nAChR subunit in the fifth position is an important determinant of desensitization and agonist sensitivity (Wu and Lukas, 2011). In these studies, lynx1 reduced cell surface expression but not gating of $(\alpha 3)_2(\beta 4)_3$, whereas it had marked single-channel effects of the $(\alpha 3)_3(\beta 4)_2$ stoichiometry (decreased unitary conductance, altered burst proportions, and enhanced long closed dwell-times). The differential effect of lynx1 on stoichiometry lends support to the hypothesis, generated from data on α4β2 nAChRs (Nichols et al., 2014), that lynx1 could have preferential binding affinity for α:α over β:β interfaces. Reduced cell-surface expression and increased closed dwell times accounted for the reduction in $(\alpha 3)_2$ $(\beta 4)_2\alpha 5$ function mediated by lynx1. More defined structure-function studies will be required before a clearer understanding can be determined.

7. Structural information on lynx1 nAChR complexes

The lynx1 protein demonstrates some topological features of snake venom toxins, such as the the three loop toxin fold(Lyukmanova et al., 2011). α-btx is one of the most widely used snake toxins for the study of nAChRs. It exhibits nearly irreversible affinity for nicotinic receptors, while in contrast lynx1 is able to compete reversibly with other nAChR ligands. Residues on loop II and III are important for the interaction although the effects can be more diverse than that seen with toxin binding and function. There is limited structural information for prototoxins, relative to α-neurotoxins (Tsetlin, 2015), due to the lack of crystallographic data for lynx1. nAChR subunit structural information is emerging as more subunits (Kouvatsos et al., 2014; Zouridakis et al., 2019), or AChBP complexes (Shahsavar et al., 2012; Kaczanowska et al., 2014) are being reported (Giastas et al., 2018). The NMR solution structure of lynx1 has been solved, indicating the β-sheet rich three finger structure reminiscent of the toxin fold of α-neurotoxins (Lyukmanova et al., 2013; Tsetlin, 2015). This structure indicates the lynx1 contains multiple β-sheets forming the first and second loop structures and disulfide pairing similar to α-neurotoxins. This solution structure also indicates a flexible, relatively disordered third loop, a feature which could make crystallization efforts more difficult. Mutagenesis studies have indicated residues which are also important for lynx1 binding or function (Lyukmanova et al., 2013), particularly in the key second loop region which has been mapped as important for binding within the related α-neurotoxins (Tsetlin, 2015). Computational models of lynx1 with nAChRs have indicated possible interactions within important parts of the nAChR structure, for instance the cys-loop and C-loops (Lyukmanova et al., 2013; Nissen et al., 2018; Dong et al., in

press), which have been shown to be involved in the transduction of agonist binding to the receptor open state, and with ligand binding, respectively.

These computational models support interfacial binding of lynx1 to the nAChR, for instance with α7 or (Lyukmanova et al., 2011; 2013), or α4* (Nissen et al., 2018) nAChRs=. This helps to bolster vitro experimental evidence of interfacial binding in studies carried out in mammalian cells (Nichols et al., 2014) and oocytes (George et al., 2017).

8. Cortical plasticity influenced by lynx prototoxins

The overall effect of lynx1 on the major nAChR subtypes primarily acts as a negative allosteric modulator of nAChRs. The role of prototoxins *in vivo* has been addressed through transgenesis and knockout technologies. lynx1 null mutant (lynx1KO) mice have been a useful research tool for the understanding of the role of lynx1 in plasticity and learning and memory behaviors. The function of lynx1 through nAChRs has been shown to mediate plasticity in the adult visual cortex and auditory cortex (Morishita et al., 2010; Takesian et al., 2018). lynx1 mRNA and protein levels increase in the primary visual cortex (V1) during the time of closure of the critical period in primary visual cortex. Removal of lynx1 in lynx1KO mice lead to extended ocular dominance (OD) plasticity in adulthood (Morishita et al., 2010; Sadahiro et al., 2016). The mechanism of lynx1 action on ocular dominance plasticity is correlated with a functional association of lynx1 with tissue plasminogen activator (tPA), a molecule implicated in spine turnover (Morishita et al., 2010; Bukhari et al., 2015). The turnover of spines in V1 layers 5 and L2/3 pyramidal neuronal dendrites are doubled in lynx1KO mice, and there is a higher loss rate in layer 5 (Sajo et al., 2016). These observations indicate a role for lynx1 in the structural remodeling and spine dynamics required for plasticity in the visual cortex.

9. Multiple plasticity periods altered by lynx1 regulation

Within the auditory cortex, a nearly 2-fold developmental increase of lynx1 expression has been observed in the primary auditory cortex (A1) between postnatal days P11 and P20. This is consistent with the closing of the critical period in the primary auditory cortex. This was accompanied by a decrease in nAChR sensitivity in 5-HT3AR positive cells, as compared to plasticity in the visual cortex. lynx1 has also been linked to a reduction in auditory plasticity via association with the α4-containing nAChR in 5-HT3AR positive cells (Takesian et al., 2018). Heightened nicotine sensitivity was observed in A1 neurons of lynx1KO mice which was blocked using the α4 nAChR specific antagonist, DHβE (Takesian et al., 2018). There is an emerging concept that multiple critical periods associated with various brain areas and that critical periods for sensory processing are shorter and end earlier than critical periods for higher complex functions (Morrone, 2010). The reduction of α4 nAChR signaling across development by the expression of lynx1 within specific A1 cells may explain how lynx1 serves as a cortical plasticity brake in that region. It will be interesting to see if control of multiple critical periods can be explained by different temporal regulation of lynx1 within different cortical regions.

10. Removal of the lynx1 brake on nAChRs augments associative learning

Lynx1KO mice demonstrate augmented cued fear conditioned learning (Miwa et al., 2006), but no differences in basal anxiety or contextual conditioning, suggesting a specific role of lynx1 in associative learning. This type of learning involves fear and anxiety centers, such as the medial prefrontal cortex, amygdala, somatosensory and auditory cortices. The amygdala, auditory cortex, and medial prefrontal cortex receive cholinergic input (Woolf, 1991; Hill et al., 1993; Séguéla et al., 1993; Whalen et al., 1994; Mesulam, 1995; Mark et al., 1996; Passetti et al., 2000; Parikh et al., 2007; Mineur et al., 2007; Mansvelder et al., 2009; Poorthuis et al., 2014). Acetylcholine is released rapidly after aversive stimuli and is important for fear learning. A disinhibitory network is activated to regulate fear conditioning. Pairing of foot shocks with a sound causes activation of basal forebrain afferents to layer 1 interneurons in A1, which in turn inhibit layer 2/3 parvalbumin-positive interneurons (Letzkus et al., 2011). Reduction in intracolumar inhibition results in enhancement of excitatory output by layer IV pyramidal neurons. Such changes within A1 (Takesian et al., 2018) might be sufficient to account for all of the associate leaning augmentations, though other regions have not been explicitly addressed. Within other cortical regions, the mPFC expresses both α7* and α4β2* nAChRs which mediate cholinergic signaling in all layers (Poorthuis et al., 2013; Arroyo et al., 2014; Bloem et al., 2014; Verhoog et al., 2016). This fear conditioning lynx1KO phenotype does not appear to be due to alterations in pain processing, as nociception is not augmented in lynx1KO animals in a standard hot-plate assay (Nissen et al., 2018). Removal of lynx1, rather, has an augmented antinociceptive effect when lynx1KO animals are injected with nicotine relative to wild-type mice. This is consistent with reports that nAChR activation has an antinociceptive effect (Freitas et al., 2013) and hypersensitivity of nAChRs due to lynx1 removal (Miwas et al., 2006).

11. Subcortical effects of lynx1

In vitro studies indicate a positive functional effect of lynx1 on α6* nAChR activity in nicotine-evoked flux assays in striatal synaptosomes. This is somewhat at odds with the inhibitory role of lynx1 on most other nAChR subtypes studied thus far. Consistent with the restricted expression of α6* nAChRs in dopaminergic neurons, lynx1 KO mice demonstrate reduced levels of nicotine-evoked dopamine release from striatal synaptosomes. On the other hand, dopaminergic neurons from lynx1KO mice do not demonstrate changes in dopaminergic firing, nicotine-elicited responses in dopaminergic neurons, or dopamine measurements in fast-scan voltammetry studies (Parker et al., 2017). The lack of clear dopaminergic phenotype could be due to compensation due to the multiplicity of nAChR subtypes expressed within dopaminergic neurons. The composition of nAChRs expressed in the VTA dopaminergic projections to the NAc include α3, α4, α5, α6, α7 and β2, β3, and β4 subunits in various combinations (Klink et al., 2001; Mansvelder et al., 2009). Most VTA GABAergic neurons express α4 and β2 subunits while most VTA pre-synaptic glutamatergic terminals express α7* nAChRs (McGehee et al., 1995; Mansvelder et al., 2009). Cholinergic regulation of dopamine has been widely studied in the context of nicotine addiction. Nicotine can stimulate dopamine release in the NAc (Picciotto et al., 1998).

Similarly, stimulation of VTA nAChRs increases excitability of dopamine neurons (Corrigall et al., 2002).

12. Complementary expression patterns of prototoxins in the brain

Spatial control over nicotinic receptor responses can also be achieved because prototoxins exhibit mostly non-overlapping expression patterns in the CNS (Miwa et al., 2012). For instance, within the hippocampus, lynx1 is expressed in the CA3 subfield and select cells in the hilar region, whereas lynx2 is expressed in the CA1 subfield and dentate gyrus. In general, however, the expression patterns of prototoxins are widespread. For instance, lynx1 has highest levels in the hippocampus and cerebellum (Miwa et al., 1999), but is also found extensively throughout other brain regions, and can be found in the retina (Maneu et al., 2010), the lung (Fu et al., 2012), and the spinal cord (Meyer, 2014). In addition to differential spatial expression, lynx1 has an interesting temporal expression profile with expression beginning at around postnatal week 2 or 3 in a mouse model (Miwa et al., 1999; Thomsen et al., 2014) correlating with the close of the critical period in the visual system. Other prototoxins, such as lypd6, are expressed in more limiting patterns, for instance, to defined GABAergic subpopulations (Demars and Morishita, 2014). The cell type and region specific expression can influence an array of distinct functions, in addition to the nAChR subtype binding specificity, and gating function on those receptors.

13. Orchestrating cholinergic responsiveness through opposing lynx gene expression: lypd6 and lynx1

Prototoxins exist within a larger family of genes (Tsetlin, 2015). Some evidence suggests that lynx1 opposed some of the effects of another prototoxin family member, lypd6. One piece of evidence lies in responsiveness to somatosensory processing. Overexpression of lypd6 (e.g. Synapsin-driven lypd6 transgenic mice) produced a reduced sensitivity to painful stimuli. In these studies, antinociception was also assessed by the writhing test, measuring the response to acetic acid (Darvas et al., 2006). These animals showed decreased responsiveness in this test. Nicotine-evoked nAChR currents in trigeminal neurons displayed higher calcium fluxes in transgenic mice as compared to those of wild-type mice. While the studies differed in the types of assays, genetic removal of lynx1 in lynx1 KO mice or addition of lypd6 in transgenic mice seems to produce similar cellular effects (e.g. elevated calcium levels), suggesting that they act differently at the biophysical level on nAChRs (Parker et al., 2017). These differences appear to manifest in apposite at the behavioral level too with respect to analgesia (Nissen et al., 2018; Darvas et al., 2006).

The pattern of expression of prototoxin family members within distinct interneurons places them in a position to potentially sculpt the activity patterns of circuits in the visual cortex. The two prototoxins, lynx1 and lypd6, demonstrate complementary temporal and spatial expression patterns within inhibitory subpopulations in the visual cortex (Fig. 2). Lynx1 is found in parvalbumin positive interneurons, whereas lypd6 is found in somatostatin-positive interneurons localized to deep cortical layers, a cell type in which lynx1 was not detected (Demars and Morishita, 2014). Somatostatin-positive interneurons synapse robustly onto parvalbumin-positive interneurons. Considering that lypd6 can augment the

calcium component of nicotine-evoked currents, the release of acetylcholine could increase the inhibitory drive more robustly in somatostatin-positive interneurons, as compared to parvalbumin ones due to the differential expression of these two prototoxins and lypd6 is highly expressed in other brain regions and spinal cord of mice and humans (Darvas et al., 2009; Zhang et al., 2009). Lypd6 has been shown to form complexes with α3, α4, α5, α6, α7, β2, and β4 nAChR subunits and competes with α-btx for binding α7 subunits (Arvaniti et al., 2016). Previous studies have also reported, however, that blockade of α7 with α-btx and methyllycaconitine does not affect the modulation of nicotine-induced currents by lypd6 (Darvas et al., 2009). The function of lypd6 has been demonstrated in several model systems. For example, in PC12 cells, a soluble version of lypd6 completely inhibits nicotine-induced phosphorylation of ERK, which is an important pathway activated during plasticity induction. Furthermore, lypd6 KO mice exhibit decreased baseline levels of anxiety-like behavior in two-independent behavioral assessments (i.e., elevated plus maze and marble burying tests) (Arvaniti et al., 2018). Lypd6, however, also contains a Nxl motif, which allows it to bind LRP5/6, a member of the Wnt signaling pathway (Zhao et al., 2018), and therefore some of the phenotypes may be mediated by the Wnt coreceptor, low density lipoprotein receptor-related protein 6 LRP5/6.

14. Lynx1 and disease relevance

Alzheimer's Disease (AD) pathology is associated with an increase in soluble β-amyloid (Aβ), a peptide cleaved from the amyloid precursor protein. Aβ been shown to gain entry through nAChRs and to elicit toxic effects (Thomsen et al., 2016; Inestrosa et al., 2013). There is evidence that lynx1 and Aβ1–42 compete for binding to nAChRs (Thomsen et al., 2016). In pull-down experiments from rat cortical extracts, water soluble lynx1 (ws-lynx1) pulled down all nAChR subunits tested (α3–7, β2, and β4), but the only subunits in which Aβ1–42 led to reduced lynx1/nAChR interactions were the α3, α4, α5, and α7 nAChR subunits. In contrast, the α6, β2, and β4 nAChR subunits were not sensitive to Aβ1–42 competition (Thomsen et al., 2016). Although the authors speculated that the interactions occurred at the cell membrane these interactions were insensitive to β subunits suggesting a significant association of lynx1 with individual α subunits or non-pentameric receptors. Interactions of a nAChR lacking a β subunit are likely to occur beneath the membrane surface, consistent with the reported interaction of lynx1 with nAChR dimers in the endoplasmic reticulum prior to receptor maturation (Nichols et al., 2014). Conversely, when Aβ1–42 was used to pull-down nAChR complexes, it also pulled down all nAChRs tested, and lynx1 could compete at α7 and β2 subunits. Such findings are in accordance with the results of previous studies, which reported that Aβ1–42 can bind α7, α4β2, and α4α5β2 receptors (Dougherty et al., 2003; Lamb et al., 2005; Wu et al., 2004). These results indicate that lynx1 and Aβ1–42 bind at similar sites on nAChRs. Aβ1–42 is thought to bind at the orthosteric binding site, whereas the lynx1 binding site on nAChR has yet to be mapped.

If the lynx1 and Aβ1–42 interactions are significant *in vivo*, lynx1 may exert protective effects against the pathological progression of AD (Thomsen et al., 2016; Thomsen and Mikkelsen, 2012). Supporting this, a small but significant (e.g. 10%) reduction in lynx1 messagerRNA is associated with Alzheimer's pathology (Thomsen et al., 2016), and wslynx1 has been shown to block the inhibitory effect of Aβ1–42 on long-term potentiation

(Bychkov et al., 2018). A protective role for lynx is supported by *in vitro* (Miwa et al., 2006) and *in vivo* (Miwa et al., 2006; Kobayashi et al., 2014) studies in lynx1KO mice. Thus, further studies are required to elucidate the role of Aβ1–42 in normal and disease states (Kroker et al., 2013).

Although the role of lynx1 on disease states has focused primarily on Alzheimer's, recently (Artoni et al., 2019) utilized the enhanced cholinergic tone of lynx1 KO mice to investigate if alterations in cholinergic circuit alter arousal dynamics similar to those observed in mouse models of Autism Spectrum Disorder (ASM). These studies determined lynx1 KO mice exhibited a shifted distribution toward maximal pupil size similar to ASD model mice (Artoni et al., 2019). lynx1 KO mice were also used to screen for genes differentially regulated and linked to genes in patients with risk for neurodevelopmental disorders such as epilepsy and schizophrenia (Smith et al., 2018).

15. Lynx2 prototoxin and anxiety regulation

Another member of the lynx family, lynx2, is expressed within key regions of the anxiety response circuitry, namely the amygdala and medial prefrontal cortex. Characteristic of the three-looped structure of the ly6/uPAR super family (Dessaud et al., 2006), the lynx2 protein binds to and suppresses the activity of nAChRs within these regions (Tekinay et al., 2009; Wu et al., 2015). *In vitro* immunoprecipitation experiments have demonstrated that lynx2 forms stable complexes with α7, α4β2, and α4β4 nAChRs (Tekinay et al., 2009; Wu et al., 2015). Co-expression of lynx2 and α4β2 leads to faster desensitization kinetics in response to acetylcholine (Tekinay et al., 2009) and a shift in the EC_{50} for acetylcholine (Tekinay et al., 2009), nicotine, and epibatidine (Wu et al., 2015). The presence of lynx2 also decreases the expression of α4β2 at the cell surface, suggesting an additional potential mechanism for the decreased response to agonists (Wu et al., 2015). There is also evidence that the lynx2 protein can blunt nicotine-induced upregulation of α4β2 (Wu et al., 2015).

Key regions associated with anxiety, the amygdala and medial prefrontal cortex, not only express lynx2 but also receive cholinergic input (Woolf, 1991; Hill et al., 1993; Séguéla et al., 1993; Whalen et al., 1994; Mesulam, 1995; Mark et al., 1996; Passetti et al., 2000; Parikh et al., 2007; Mineur et al., 2007; Mansvelder et al., 2009). Nicotinic receptors have been implicated in the regulation of anxiety responses (Picciotto, 2003; Klein and Yakel, 2006; Gozzi et al., 2010; Mineur et al., 2016; Jiang et al., 2016; Wilson and Fadel, 2017). Anecdotal evidence comes from smokers wherein individuals have reported using nicotine to ameliorate anxiety symptoms (Moylan et al., 2012). nAChRs have been shown to regulate activity in anxiety/fear circuits and have been linked to fear and anxiety-related behaviors in animal studies, particularly the amygdala and substructures of the amygdala such as the basolateral amygdala (BLA)(Picciotto, 2003; Klein and Yakel, 2006; Gozzi et al., 2010; Mineur et al., 2016; Jiang et al., 2016; Wilson and Fadel, 2017).

Endogenous acetylcholine modulates excitability of the BLA, as well as stimulating cortical-BLA inputs. Activation of nAChRs with nicotine increases glutamatergic transmission in the BLA and post-synaptic glutamatergic currents from cortical inputs into the BLA, whereas blockade of nAChRs decreases activity in the BLA (Mineur et al., 2007; Jiang and Role,

2008; Jiang et al., 2016). Behavioral consequences of altered nAChR signaling is highly dependent upon the activity of the amygdala and its inputs. For example, α7 nAChRs can modulate the activity of both excitatory and inhibitory neurons, and the outcome depends upon the starting conditions (Jiang and Role, 2008; Pidoplichko et al., 2013). Both of the most common nAChR subtypes are involved in anxiety-like behavior as focal knockdown of α7* and β2* (*indicates containing that subunit) within the amygdala have anxiolytic-like effects (Mineur et al., 2016). Only β2* nAChRs were shown to be involved in social defeat (Mineur et al., 2016). In fear extinction, activation of cholinergic terminals in the BLA hinders the acquisition of extinction and maintains the fear memory by increasing the firing of BLA principal neurons (Jiang et al., 2016).

Behaviorally, Lynx2 null mutant mice (lynx2 KO) exhibit increased sensitivity to nicotine in pyramidal neurons in the medial prefrontal cortex as compared to wild-type controls (Tekinay et al., 2009). These data suggest that ligand sensitivity is altered in the presence of lynx2, and that lynx2 also acts to inhibit the activity of nAChRs. The functional consequences of lynx2 deletion include increased anxiety-like behaviors across several paradigms (e.g. light-dark box, thigmotaxis, and elevated plus maze), as well as reduced social interaction in a standard social interaction test (Tekinay et al., 2009). Thus, lynx2 may play an important role in limiting or regulating the function of its cognate receptors to respond adaptively in circuits mediating anxiety-like behavior, and deletion of lynx2 in mice can serve as a robust model of excessive anxiety.

16. Prototoxin family members

Several members of the ly6/uPAR/neurotoxin superfamily have been shown to bind to and differentially modulate the function of multiple nAChRs (Wu et al., 2015; Arvaniti et al., 2016; Puddifoot et al., 2015) (Table 1). One example, LYPD6 interacts with α3, α4, α5, α6, α7, β2, and β4 nAChR subunits and is expressed in somatostatin interneurons of the V1 layers 5 and 6 (Darvas et al., 2009). Other prototoxins, however, are known to show decided preferences specific nAChR partners. For example, LYPD6B has been shown to modulate α3β4-containing but not α7-containing nAChRs (Ochoa et al., 2016), Ly6g6e interacts with potentiated α4β2 nAChRs (Wu et al., 2015), and Ly6h interacts with α7 nAChRs (Puddifoot et al., 2015) Prostate stem cell antigen (PSCA), another prototoxin modulator of nAChR function, has a preferential modulatory effect on α7-containing but not α3β4-containing nAChRs (Hruska et al., 2009). Recent work demonstrates that prototoxins can also have significant preferences for interacting with particular subunit interfaces within nAChR complexes.

In addition to the proteins discussed prior, several members of the prototoxins family are expressed and function peripherally to inhibit signalling of the nicotinic system (e.g., SLURP1, SLURP2, lypdg6e, PATE-M, PATE-B, etc.). Although each of them plays an important role in signalling regulation and are therefore are worth mentioning, their distribution and targets remain generally peripheral and ultimately fall outside of the scope of this review.

17. Therapeutic potential of nicotinic receptors

Considerable efforts have been made to develop agonists against nAChRs for a number of indications. Nicotinic receptors suffer from some challenges as a therapeutic target. They are part of a large gene family with very similar sequences, making them difficult to target selectively. Secondly, nicotinic receptors are wide-spread throughout the brain, so a drug could have multiple functional consequences (Mineur and Picciotto, 2008). Nicotinic receptors also desensitize quickly and down-regulate, making selective targeting a short-term proposition in some cases (Quik and Wonnacott, 2011), thus subject to tachyphylaxis. Lastly, neurons usually express more than one subtype generally, and the potential for receptor compensation is a complexity that requires more exploration. Therefore combinatorial targeting of multiple subtypes within a neuron, which might be an ideal strategy, is a challenge. Also the inverted U-shaped curve of nicotinic receptor based therapeutics means that efficacy and safety are a hurdle higher than other targets (Colquhoun and Patrick, 1997). The ability to successfully create new nicotinic therapeutics has largely been unsuccessful with several failed clinical trials pointing to a translational gap (Bertrand and Terry, 2018; Vieta et al., 2013).

Several features make lynx prototoxins intriguing as possible therapeutic targets for the cholinergic system, and could possibly circumvent some of the issues associated with direct targeting of nicotinic receptors. Lynx genes have a degree of regional selectivity, some more restricted than others (Dessaud et al., 2006; Miwa et al., 1999; Thomsen et al., 2014). The advantage of multiple lynx family members is the possibility of exerting better spatial or temporal control over the cholinergic system by selectively acting on a single lynx family member which is associated with a specific function or region. Thus, targeting the lynx-receptor interface, could increase the possible specificity of action over the receptor alone. This has the potential of lowering unwanted side-effects. Removal of lynx1 leads to reduced levels of desensitization (Ibanez-Tallon et al., 2002), suggesting that lynx-based drugs could be less sensitive to either desensitization or tachyphylaxis. The reported effects on the number, stoichiometry, and desensitization kinetics of nicotinic receptors at the neuronal cell surface due to lynx interactions (George et al., 2017; Wu et al., 2015), suggest changes in nicotinic receptor number could also be a beneficial outcome of lynx targeting, and consideration of possible intracellular effects of a lynx-based therapeutics is warranted (Lester et al., 2012).

18. Overview

Shaping the responsiveness of acetylcholine through modulation of nAChRs has significant influence on a number of complex brain functions adaptive to the organism. Different prototoxins have been shown to be selectively expressed in different cell types within circuits and have differential binding capacity on nAChR subtypes. At present, the majority of reports of prototoxin function have been inhibitory on α4β2, α3β4, α5α3β4 (e.g. lowering agonist response property, lowering receptor number, or accelerating desensitization of nAChRs, etc.). Exceptions to this are the reports of lypd6, as well as the positive effects of lynx1 for α6β2, and α6* nAChR function. As of yet, no *in vivo* role of a prototoxin has been reported on mAChRs, so the nicotinic selectivity of prototoxins could

also influence the relative degree of muscarinic drive over the nicotinic one in response to acetylcholine release. The detailed understanding of the relative weighting of nicotinic function imparted through nAChR subtypes in complexes with specific prototoxins will require further in vitro and circuit-based investigations. Lynx prototoxin regulation of the cholinergic system holds promise as a therapeutic target for a wide range of disordered states by imparting both spatial and subtype specific regulation of a widespread neurotransmitter system.

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References

Adeyo O, Allan BB, Barnes RH 2nd, Goulbourne CN, Tatar A, Tu Y, Young LC, Weinstein MM, Tontonoz P, Fong LG, Beigneux AP, Young SG, 2014. Palmoplantar keratoderma along with neuromuscular and metabolic phenotypes in Slurp1-deficient mice. J. Invest. Aermatol 134 (6), 1589–1598. 10.1038/jid.2014.19. PMID: 24499735.

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW, 2009. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol. Rev 89, 73–120 PMID:19126755. [PubMed: 19126755]

Arroyo S, Bennett C, Hestrin S, 2014. Nicotinic modulation of cortical circuits. Front. Neural Circ 8, 30. 10.3389/fncir.2014.00030. PMID: 24734005.

Artoni P, Piffer A, Vinci V, Leblanc J, Nelson CA, Hensch TK, Fagiolini M, 2019. Deep learning of spontaneous arousal fluctuations detects early cholinergic defects across neurodevelopmental mouse models and patients. 201820847. Proc. Natl. Acad. SCi 10.1073/pnas.1820847116. PMID: 31332003.

Arvaniti M, Jensen MM, Soni N, Wang H, Klein AB, Thiriet N, et al. , 2016. Functional interaction between Lypd6 and nicotinic acetylcholine receptors. J. Neurochem 138 (6), 806–820. 10.1111/jnc.13718. PMID: 27344019. [PubMed: 27344019]

Arvaniti M, Polli FS, Kohlmeier KA, Thomsen MS, Andreasen JT, 2018. Loss of Lypd6 leads to reduced anxiety-like behaviour and enhanced responses to nicotine. Prog. Neuro-Psychopharmacol. Bio 82, 86–94. 10.1016/j.pnpbp.2017.11.025. PMID: 29195920.

Ballinger EC, Ananth M, Talmage DA, Role LW, 2016. Basal forebrain cholinergic circuits and signaling in cognition and cognitive decline. Neuron 91 (6), 1199–1218. 10.1016/j.neuron.2016.09.006. PMID: 27657448. [PubMed: 27657448]

Bertrand D, Terry A, 2018. The wonderland of neuronal nicotinic acetylcholine receptors. Biochem. Pharmacol 151, 214–225. 10.1016/j.bcp.2017.12.008. PMID: 29248596. [PubMed: 29248596]

Bloem B, Poorthuis RB, Mansvelder HD, 2014. Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. Front. Nerual Circuits 8, 17. 10.3389/fncir.2014.00017. PMID: 24653678.

Brown JL, Wonnacott S, 2014. Sazetidine-A activates and desensitizes native α7 nicotinic acetylcholine receptors. Neurochem. Res 40 (10), 2047–2054. 10.1007/s11064-014-1302-6. PMID: 24728867. [PubMed: 24728867]

Bukhari N, Burman PN, Hussein A, Demars MP, Sadahiro M, Brady DM, et al. , 2015. Unmasking proteolytic activity for adult visual cortex plasticity by the removal of Lynx1. J. Neurosci 35 (37), 12693–12702. 10.1523/JNEUROSCI.4315-14.2015. PMID: 26377459. [PubMed: 26377459]

Bychkov ML, Vasilyeva NA, Shulepko MA, Balaban PM, Kirpichnikov MP, Lyukmanova EN, 2018. Lynx1 prevents long-term potentiation blockade and reduction of neuromodulator expression caused by aβ1–42 and JNK activation. Acta Naturae 10 (3), 57–61 PMID: 30397527. [PubMed: 30397527]

Chandler D, Waterhouse BD, 2012. Evidence for broad versus segregated projections from cholinergic and noradrenergic nuclei to functionally and anatomically discrete subregions of prefrontal cortex. Front. in behav. neurosci 6, 20. 10.3389/fnbeh.2012.00020. PMID: 22661934.

Changeux J-P, Bertrand D, Corringer P-J, Dehaene S, Edelstein S, Léna C, et al. , 1998. Brain nicotinic receptors: structure and regulation, role in learning and reinforcement1Published on the World Wide Web on 24 October 1997.1. Brain Res. Rev 26 (2–3), 198–216. 10.1016/s0165-0173(97)00040-4. PMID: 9651527. [PubMed: 9651527]

Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, et al. , 2003. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. Hum. Mol. Genet 12, 3017–3024. 10.1093/hmg/ddg320. PMID: 14506129. [PubMed: 14506129]

Colquhoun LM, Patrick JW, 1997. Pharmacology of neuronal nicotinic acetylcholine receptor subtypes. Adv. Pharmacol 39, 191–220. 10.1016/s1054-3589(08)60072-1. PMID: 9160116. [PubMed: 9160116]

Corrigall W, Coen K, Zhang J, Adamson L, 2002. Pharmacological manipulations of the pedunculopontine tegmental nucleus in the rat reduce self-administration of both nicotine and cocaine. Psychopharmacol 160 (2), 198–205. 10.1007/s00213-001-0965-2. PMID: 11875638.

Dani JA, 2001. Overview of nicotinic receptors and their roles in the central nervous system. Biol. Psychiatr 49 (3), 166–174. 10.1016/s0006-3223(00)01011-8. PMID: 11230867.

Dani JA, Bertrand D, 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu. Rev. Pharmacol. Toxicol 47 (1), 699–729. 10.1146/annurev.pharmtox.47.120505.105214. PMID: 17009926. [PubMed: 17009926]

Darvas F, Ermer JJ, Mosher JC, Leahy RM, 2006. Generic head models for atlas-based EEG source analysis. Hum. Brain Mapp 27 (2), 129–143. 10.1002/hbm.20171. PMID: 16037984. [PubMed: 16037984]

Darvas M, Morsch M, Racz I, Ahmadi S, Swandulla D, Zimmer A, 2009. Modulation of the Ca2+ conductance of nicotinic acetylcholine receptors by Lypd6. Eur. Neuropsychopharmacol 19 (9), 670–681. 10.1016/j.euroneuro.2009.03.007. PMID: 19403274. [PubMed: 19403274]

Demars MP, Morishita H, 2014. Cortical parvalbumin and somatostatin GABA neurons express distinct endogenous modulators of nicotinic acetylcholine receptors. Mol. Brain 7, 75. 10.1186/s13041-014-0075-9. PMID: 25359633. [PubMed: 25359633]

Dessaud E, Salaun D, Gayet O, Chabbert M, deLapeyriere O, 2006. 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 PMID:16236524. [PubMed: 16236524]

Disney AA, Aoki C, Hawken MJ, 2007. Gain modulation by nicotine in macaque v1. Neuron 56 (4), 701–713. 10.1016/j.neuron.2007.09.034. PMID: 18031686. [PubMed: 18031686]

Dong Chuqiao, Kern Nathan R., Anderson Kristin R., Zhang X. Frank, Miwa Julie M., Wonpil Im, 2020. Dynamics and Interactions of GPI-Linked lynx1 Protein with/without Nicotinic Acetylcholine Receptor in Membrane Bilayers. Journal of Physical Chemistry 8. https://pubs.acs.org/doi/pdf/10.1021/acs.jpcb.0c00159 In press.

Dougherty JJ, Wu J, Nichols RA, 2003. Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex. J. Neurosci 23 (17), 6740–6747. 10.1523/JNEUROSCI.23-17-06740.2003. PMID: 12890766. [PubMed: 12890766]

Durek T, Shelukhina IV, Tae HS, Thongyoo P, Spirova EN, Kudryavtsev DS, Kasheverov IE, Faure G, Corringer PJ, Craik DJ, Adams DJ, Tsetlin VI, 2017. Interaction of synthetic human SLURP-1 with the nicotinic acetylcholine receptors. Sci. Rep 7 (1), 16606. 10.1038/s41598-017-16809-0. PMID: 29192197. [PubMed: 29192197]

English BA, Hahn MK, Gizer IR, Mazei-Robison M, Steele A, Kurnik DM, et al. , 2009. Choline transporter gene variation is associated with attention-deficit hyper-activity disorder. J. Neurodev. Disord 1 (4), 252–263. 10.1007/s11689-009-9033-8. PMID: 21547719. [PubMed: 21547719]

Freitas K, Carroll FI, Damaj MI, 2013. The antinociceptive effects of nicotinic receptors α7-positive allosteric modulators in murine acute and tonic pain models. 2013. J. Pharmacol. Exp. Therapeut 344 (1), 264–275. 10.1124/jpet.112.197871. PMID: 23115222.

Fu XW, Rekow SS, Spindel ER, 2012. The ly-6 protein, lynx1, is an endogenous inhibitor of nicotinic signaling in airway epithelium. Am. J. Physiol. Lung Cell Mol. Physiol 303, L661–L668 PMID: 22923641. [PubMed: 22923641]

Galat A, Gross G, Drevet P, Sato A, Ménez A, 2008. Conserved structural determinants in three-fingered protein domains. FEBS J 275 (12), 3207–3225. 10.1111/j.1742-4658.2008.06473.x. PMID: 18485004. [PubMed: 18485004]

George S, Wang SM, Bi Y, Treidlinger M, Barber KR, Shaw GS, Odonoghue P, 2017. Ubiquitin phosphorylated at Ser57 hyper-activates parkin. Biochimica. Et. Biophysica. Acta 1861 (11), 3038–3046. 10.1016/j.bbagen.2017.06.023. PMID: 28689991. [PubMed: 28689991]

Giastas P, Zouridakis M, Tzartos SJ, 2018. Understanding structure-function relationships of the human neuronal acetylcholine receptor: insights from the first crystal structures of neuronal subunits. Br. J. Pharmacol 175 (11), 1880–1891. 10.1111/bph.13838. PMID: 28452148. [PubMed: 28452148]

Gais S, Born J, 2004. Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. Proc. Natl. Acad. Sci. U.S.A 101 (7), 2140–2144. 10.1073/pnas.0305404101. PMID: 14766981. [PubMed: 14766981]

Gill TM, Sarter M, Givens B, 2000. Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. J. Neurosci 20 (12), 4745–4757. 10.1523/JNEUROSCI.20-12-04745.2000. PMID: 10844044. [PubMed: 10844044]

Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M, 2007. Heterogeneity and complexity of native brain nicotinic receptors. Biochem. Pharmacol 74 (8), 1102–1111. 10.1016/j.bcp.2007.05.023. PMID: 17597586. [PubMed: 17597586]

Govind AP, Walsh H, Green WN, 2012. Nicotine-induced upregulation of native neuronal nicotinic receptors is caused by multiple mechanisms. J. Neurosci 32 (6), 2227–2238. 10.1523/JNEUROSCI.5438-11.2012. PMID: 22323734. [PubMed: 22323734]

Gozzi A, Jain A, Giovanelli A, Bertollini C, Crestan V, Schwarz AJ, et al. , 2010. A neural switch for active and passive fear. Neuron 67 (4), 656–666. 10.1016/j.neuron.2010.07.008. PMID: 20797541. [PubMed: 20797541]

Guo Q, Wang D, He X, Feng Q, Lin R, Xu F, Fu L, Luo M, 2015. Whole-brain mapping of inputs to projection neurons and cholinergic interneurons in the dorsal striatum. PloS One 10, e0123381 PMID: 25830919. [PubMed: 25830919]

Higley MJ, Picciotto MR, 2014. Neuromodulation by acetylcholine: examples from schizophrenia and depression. Curr. Opin. Neurobiol 29, 88–95. 10.1016/j.conb.2014.06.004. PMID: 24983212. [PubMed: 24983212]

Hangya B, Ranade SP, Lorenc M, Kepecs A, 2015. Central cholinergic neurons are rapidly recruited by reinforcement feedback. Cell 162 (5), 1155–1168. 10.1016/j.cell.2015.07.057. PMID: 26317475. [PubMed: 26317475]

Hill CE, Powis DA, Hendry IA, 1993. Involvement of pertussis toxin-sensitive and -insensitive mechanisms in alpha-adrenoceptor modulation of noradrenaline release from rat sympathetic neurones in tissue culture. Br. J. Pharmacol 110 (1), 281–288. 10.1111/j.1476-5381.1993.tb13806.x. PMID: 8106104. [PubMed: 8106104]

Himmelheber AM, Bruno JP, Sarter M, 2000. Effects of intra-accumbens infusions of amphetamine or cis-flupenthixol on sustained attention performance in rats. Behav. Brain Res 116 (2), 123–133. 10.1016/s0166-4328(00)00266-7. PMID: 11080543. [PubMed: 11080543]

Hoffman KM, Eisen MR, Chandler JK, Nelson MR, Johnson EA, Mcnutt PM, 2019. Retrograde activation of CB1R by muscarinic receptors protects against central organophosphorus toxicity. Neuropharmacology 155, 113–120. 10.1016/j.neuropharm.2019.05.028. PMID: 31132436. [PubMed: 31132436]

Horie M, Okutomi K, Taniguchi Y, Ohbuchi Y, Suzuki M, Takahashi E-I, 1998. Isolation and characterization of a new member of the HumanLy6Gene family (LY6H). Genomics 53 (3), 365–368. 10.1006/geno.1998.5462. PMID: 9799603. [PubMed: 9799603]

Hruska M, Keefe J, Wert D, Tekinay AB, Hulce JJ, Ibañez-Tallon I, Nishi R, 2009. 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 (47), 14847–14854. 10.1523/JNEUROSCI.2271-09.2009. PMID: 19940180. [PubMed: 19940180]

Hurst R, Rollema H, Bertrand D, 2013. Nicotinic acetylcholine receptors: from basic science to therapeutics. Pharmacol. Ther 137 (1), 22–54. 10.1016/j.pharmthera.2012.08.012. PMID: 22925690. [PubMed: 22925690]

Ibanez-Tallon I, Miwa JM, Wang HL, Adams NC, Crabtree GW, Sine SM, Heintz N, 2002. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. Neuron 33, 893–903 PMID: 11906696. [PubMed: 11906696]

Inestrosa NC, Godoy JA, Vargas JY, Arrazola MS, Rios JA, Carvajal FJ, et al. , 2013. Nicotine prevents synaptic impairment induced by amyloid-β oligomers through α7-nicotinic acetylcholine receptor activation. NeuroMolecular Med 15 (3), 549–569. 10.1007/s12017-013-8242-1. PMID: 23842742. [PubMed: 23842742]

Jensen MM, Arvaniti M, Mikkelsen JD, Michalski D, Pinborg LH, Härtig W, Thomsen MS, 2015. Prostate stem cell antigen interacts with nicotinic acetylcholine receptors and is affected in Alzheimer's disease. Neurobiol. Aging 36 (4), 1629–1638. 10.1016/j.neurobiolaging.2015.01.001. PMID: 25680266. [PubMed: 25680266]

Jiang L, Role LW, 2008. Facilitation of cortico-amygdala synapses by nicotine: activity-dependent modulation of glutamatergic transmission. J. Neurophysiol 99 (4), 1988–1999. 10.1152/jn.00933.2007. PMID: 18272879. [PubMed: 18272879]

Jiang L, Kundu S, Lederman JD, López-Hernández GY, Ballinger EC, Wang S, Talmage DA, Role LW, 2016. Cholinergic signaling controls conditioned fear behaviors and enhances plasticity of cortical-amygdala circuits. Neuron 90 (5), 1057–1070. 10.1016/j.neuron.2016.04.028. PMID: 27161525. [PubMed: 27161525]

Kaczanowska K, Harel M, Radić Z, Changeux JP, Finn MG, Taylor P, 2014. Structural basis for cooperative interactions of substituted 2-aminopyrimidines with the acetylcholine binding protein. Proc. Natl. Acad. Sci. Unit. States Am 111 (29), 10749–10754. 10.1073/pnas.1410992111. PMID: 25006260.

Kawai H, Lazar R, Metherate R, 2007. Nicotinic control of axon excitability regulates thalamocortical transmission. Nat. Neurosci 10, 1168–1175. 10.1038/nn1956. PMID. 17704774. [PubMed: 17704774]

Kessler P, Marchot P, Silva M, Servent D, 2017. The three-finger toxin fold: a multifunctional structural scaffold able to modulate cholinergic functions. J. Neurochem 142, 7–18. 10.1111/jnc.13975. PMID: 28326549. [PubMed: 28326549]

Klein RC, Yakel JL, 2006. Functional somato-dendritic alpha7-containing nicotinic acetylcholine receptors in the rat basolateral amygdala complex. J. Physiol 576 (Pt 3), 865–872. 10.1113/jphysiol.2006.118232. PMID: 16931547. [PubMed: 16931547]

Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP, 2001. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J. Neurosci 21 (5), 1452–1463. 10.1523/JNEUROSCI.21-05-01452.2001. PMID: 11222635. [PubMed: 11222635]

Kobayashi A, Parker RL, Wright AP, Brahem H, Ku P, Oliver KM, et al. , 2014. Lynx1 supports neuronal health in the mouse dorsal striatum during aging: an ultrastructural investigation. J. Mol. Neurosci.: M. Inc 53 (3), 525–536. 10.1007/s12031-014-0352-1. PMID: 25027556.

Kouvatsos N, Niarchos A, Zisimopoulou P, Eliopoulos E, Poulas K, Tzartos S, 2014. Purification and functional characterization of a truncated human α4nβic2otinic acetylcholine receptor. Int. J. Biol. Macromol 70, 320–326. 10.1016/j.ijbiomac.2014.06.058. PMID: 25014634. [PubMed: 25014634]

Kroker KS, Moreth J, Kussmaul L, Rast G, Rosenbrock H, 2013. Restoring long-term potentiation impaired by amyloid-beta oligomers: comparison of an acetylcholinesterase inhibitior and selective neuronal nicotinic receptor agonists. Brain Res. Bull 96, 28–38 PMID:23639920. [PubMed: 23639920]

Kuhar SG, Fang L, Viden S, Ross ME, Hatten ME, Heintz N, 1993. Changing patterns of gene expression define four stages of cerebellar granule neuron differentiation. Development 117 (1), 97–104 PMID: 8223263. [PubMed: 8223263]

Kutlu MG, Gould TJ, 2015. Nicotine modulation of fear memories and anxiety: implications for learning and anxiety disorders. Biochem. Pharmacol 97 (4), 498–511. 10.1016/j.bcp.2015.07.029. PMID: 26231942. [PubMed: 26231942]

Lamb PW, Melton MA, Yakel JL, 2005. Inhibition of neuronal nicotinic acetylcholine receptor channels expressed in *Xenopus*Oocytes by β-amyloid1–42Peptide. J. Mol. Neurosci 27 (1), 13–22. 10.1385/jmn:27:1:013. PMID: 16055943. [PubMed: 16055943]

Lester HA, Miwa JM, Srinivasan R, 2012. Psychiatric drugs bind to classical targets within early exocytotic pathways: therapeutic effects. Biol. Psychiatr 72, 907–915 PMID:22771239.

Letzkus JJ, Wolff SBE, Meyer EMM, Tovote P, Courtin J, Herry C, Lüthi A, 2011. A disinhibitory microcircuit for associative fear learning in the auditory cortex. Nature 480 (7377), 331–335. 10.1038/nature10674. PMID: 22158104. [PubMed: 22158104]

Li X, Yu B, Sun Q, Zhang Y, Ren M, Zhang X, Li A, Yuan J, Madisen L, Luo Q, Zeng H, Gong H, Qiu Z, 2018. Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. Proc. Nal. Acad. Sci. U. S. A 115 (2), 415–420. 10.1073/pnas.1703601115. PMID: 29259118.

Lyukmanova EN, Shulepko MA, Kudryavtsev D, Bychkov ML, Kulbatskii DS, Kasheverov IE, Astapova MV, Feofanov AV, Thomsen MS, Mikkelsen JD, Shenkarev ZO, Tsetlin VI, Dolgikh DA, Kirpichnikov MP, 2016. Human secreted ly-6/uPAR related protein-1 (SLURP-1) is a selective allosteric antagonist of α7 nicotinic acetylcholine receptor. PloS One 11 (2), e0149733. 10.1371/journal.pone.0149733. [PubMed: 26905431]

Lyukmanova EN, Shenkarev ZO, Shulepko MA, Mineev KS, D'Hoedt D, Kasheverov IE, Filkin SY, Krivolapova AP, Janickova H, Dolezal V, et al. , 2011. NMR structure and action on nicotinic acetylcholine receptors of water-soluble domain of human LYNX1. J. Biol. Chem 286, 10618–10627 PMID:21252236. [PubMed: 21252236]

Lyukmanova EN, Shulepko MA, Buldakova SL, Kasheverov IE, Shenkarev ZO, Reshetnikov RV, et al. , 2013. Water-soluble LYNX1 residues important for interaction with muscle-type and/or neuronal nicotinic receptors. J. Biol. Chem 288 (22), 15888–15899. 10.1074/jbc.M112.436576. PMID: 23585571. [PubMed: 23585571]

Maneu V, Gerona G, Fernandez L, Cuenca N, Lax P, 2010. Evidence of alpha 7 nicotinic acetylcholine receptor expression in retinal pigment epithelial cells. Vis. Neurosci 27, 139–147 PMID:20932358. [PubMed: 20932358]

Mansvelder HD, Mertz M, Role LW, 2009. Nicotinic modulation of synaptic transmission and plasticity in cortico-limbic circuits. Semin. Cell Dev. Biol 20 (4), 432–440. 10.1016/j.semcdb.2009.01.007. PMID: 19560048. [PubMed: 19560048]

Mark G, Rada P, Shors T, 1996. Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. Neuroscience 74 (3), 767–774. 10.1016/0306-4522(96)00211-4. PMID: 8884772. [PubMed: 8884772]

Marks MJ, Whiteaker P, Calcaterra J, Stitzel JA, Bullock AE, Grady SR, et al. , 1999. Two pharmacologically distinct components of nicotinic receptor-mediated rubidium effux in mouse brain require the beta 2 subunit. J. Pharmacol. Exp. Therapeut 289 (2), 1090–1103 PMID: 10215692.

McGehee D, Heath M, Gelber S, Devay P, Role L, 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269 (5231), 1692–1696. 10.1126/science.7569895. PMID: 7569895. [PubMed: 7569895]

Mesulam MM, 1995. Cholinergic pathways and the ascending reticular activating system of the human brain. Ann. N. Y. Acad. Sci 757 (1), 169–179. 10.1111/j.1749-6632.1995.tb17472.x. PMID: 7611672. [PubMed: 7611672]

Meyer MA, 2014. Identification of 17 highly expressed genes within mouse lumbar spinal cord anterior horn region from an in-situ hybridization atlas of 3430 genes: implications for motor neuron disease. Neurol. Int 6, 5367 PMID:24987504. [PubMed: 24987504]

Mineur YS, Fote GM, Blakeman S, Cahuzac EL, Newbold SA, Picciotto MR, 2016. Multiple nicotinic acetylcholine receptor subtypes in the mouse amygdala regulate affective behaviors and response to social stress. Neuropsychopharmacology 41 (6), 1579–1587. 10.1038/npp.2015.316. PMID: 26471256. [PubMed: 26471256]

Mineur YS, Picciotto MR, 2008. Genetics of nicotinic acetylcholine receptors: relevance to nicotine addiction. Biochem. Pharmacol 75 (1), 323–333. 10.1016/j.bcp.2007.06.010. PMID: 17632086. [PubMed: 17632086]

Mineur YS, Somenzi O, Picciotto MR, 2007. Cytisine, a partial agonist of high-affinity nicotinic acetylcholine receptors, has antidepressant-like properties in male C57BL/6Jmice. Neuropharmacology 52 (5), 1256–1262. 10.1016/j.neuropharm.2007.01.006. PMID: 17320916. [PubMed: 17320916]

Miwa JM, Anderson KR, Hoffman KM, 2019. Lynx prototoxins: roles of endogenous mammalian neurotoxin-like proteins in modulating nicotinic acetylcholine receptor function to influence complex biological processes. Front. Pharmacol 10, 343. 10.3389/fphar.2019.00343. PMID: 31114495. [PubMed: 31114495]

Miwa JM, Walz A, 2012. Enhancement in motor learning through genetic manipulationoftheLynx1gene. PloS One 7 (11), e43302. 10.1371/journal.pone.0043302. PMID: 23139735. [PubMed: 23139735]

Miwa JM, Ibanez-Tallon I, Crabtree GW, Sanchez R, Sali A, Role LW, Heintz N, 1999. lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. Neuron 23, 105–114 PMID:10402197. [PubMed: 10402197]

Miwa JM, Lester HA, Walz A, 2012. Optimizing cholinergic tone through lynx modulators of nicotinic receptors: implications for plasticity and nicotine addiction. Physiology 27, 187–199 PMID:22875450. [PubMed: 22875450]

Miwa JM, Stevens TR, King SL, Caldarone BJ, Ibanez-Tallon I, Xiao C, Fitzsimonds RM, Pavlides C, Lester HA, Picciotto MR, et al. , 2006. The prototoxin lynx1 acts on nicotinic acetylcholine receptors to balance neuronal activity and survival in vivo. Neuron 51, 587–600 PMID:16950157. [PubMed: 16950157]

Morishita H, Miwa JM, Heintz N, Hensch TK, 2010. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. Science 330 (6008), 1238–1240. 10.1126/science.1195320. PMID: 21071629. [PubMed: 21071629]

Morrone MC, 2010. Brain development: critical periods for cross-sensory plasticity. Curr. Biol 20 (21). 10.1016/j.cub.2010.09.052. PMID: 21056835.

Moylan S, Jacka FN, Pasco JA, Berk M, 2012. Cigarette smoking, nicotine dependence and anxiety disorders: a systematic review of population-based, epidemiological studies. BMC Med 10, 123. 10.1186/1741-7015-10-123. PMID: 23083451. [PubMed: 23083451]

Nashmi R, Lester HA, 2006. CNS localization of neuronal nicotinic receptors. J. Mol. Neurosci 30 (1–2), 181–184. 10.1385/jmn:30:1:181. PMID: 17192671. [PubMed: 17192671]

Nichols WA, Henderson BJ, Yu C, Parker RL, Richards CI, Lester HA, Miwa JM, 2014. Lynx1 shifts alpha4beta2 nicotinic receptor subunit stoichiometry by affecting assembly in the endoplasmic reticulum. J. Biol. Chem 289, 31423–31432 PMID:25193667. [PubMed: 25193667]

Nissen NI, Anderson KR, Wang H, Lee HS, Garrison C, Eichelberger SA, et al. , 2018. Augmenting the antinociceptive effects of nicotinic acetylcholine receptor activity through lynx1 modulation. PloS One 13 (7), e0199643. 10.1371/journal.pone.0199643. PMID: 29969495. [PubMed: 29969495]

Ochoa V, George AA, Nishi R, Whiteaker P, 2016. The prototoxin LYPD6B modulates heteromeric α3β4-containing nicotinic acetylcholine receptors, but not α7 homomers. Faseb. J 30 (3), 1109–1119. 10.1096/fj.15-274548. PMID: 26586467. [PubMed: 26586467]

Passetti F, Dalley JW, Oconnell MT, Everitt BJ, Robbins TW, 2000. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. Eur. J. Neurosci 12 (8), 3051–3058. 10.1046/j.1460-9568.2000.00183.x. PMID: 10971646. [PubMed: 10971646]

Parikh V, Kozak R, Martinez V, Sarter M, 2007. Prefrontal acetylcholine release controls cue detection on multiple timescales. Neuron 56 (1), 141–154. 10.1016/j.neuron.2007.08.025. PMID: 17920021. [PubMed: 17920021]

Parker RL, O'Neill HC, Henley BM, Wageman CR, Drenan RM, Marks MJ, et al. , 2017. Deletion of lynx1 reduces the function of α6* nicotinic receptors. PloS One 12 (12), e0188715. 10.1371/journal.pone.0188715. PMID: 29206881. [PubMed: 29206881]

Picciotto MR, 2003. Nicotine as a modulator of behavior: beyond the inverted U. Trends Pharmacol. Sci 24, 493–499 PMID: 12967775. [PubMed: 12967775]

Picciotto MR, Caldarone BJ, Brunzell DH, Zachariou V, Stevens TR, King SL, 2001. Neuronal nicotinic acetylcholine receptor subunit knockout mice: physiological and behavioral phenotypes and possible clinical implications. Pharmacol. Ther 92 (2–3), 89–108. 10.1016/s0163-7258(01)00161-9. PMID: 11916531. [PubMed: 11916531]

Picciotto MR, Higley MJ, Mineur YS, 2012. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 76 (1), 116–129. 10.1016/j.neuron.2012.08.036. PMID: 23040810. [PubMed: 23040810]

Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, et al. , 1998. Acetylcholine receptors containing the β2 subunit are involved in the reinforcing properties of nicotine. Nature 391 (6663), 173–177. 10.1038/34413. PMID: 9428762. [PubMed: 9428762]

Pidoplichko VI, Prager EM, Aroniadou-Anderjaska V, Braga MF, 2013. α7-Containing nicotinic acetylcholine receptors on interneurons of the basolateral amygdala and their role in the regulation of the network excitability. J. Neurophysiol 110 (10), 2358–2369. 10.1152/jn.01030.2012. PMID: 24004528. [PubMed: 24004528]

Poorthuis RB, Bloem B, Schak B, Wester J, de Kock CP, Mansvelder HD, 2013. Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors. Cerebr. Cortex 23 (1), 148–161. 10.1093/cercor/bhr390. PMID: 22291029.

Poorthuis RB, Enke L, Letzkus JJ, 2014. Cholinergic circuit modulation through differential recruitment of neocortical interneuron types during behaviour. J. Physiol 592 (19), 4155–4164. 10.1113/jphysiol.2014.273862. PMID: 24879871. [PubMed: 24879871]

Puddifoot CA, Wu M, Sung RJ, Joiner WJ, 2015. Ly6h regulates trafficking of alpha7 nicotinic acetylcholine receptors and nicotine-induced potentiation of glutamatergic signaling. J. Neurosci 35 (8), 3420–3430. 10.1523/JNEUROSCI.3630-14.2015. PMID: 25716842. [PubMed: 25716842]

Quik M, Wonnacott S, 2011. α6β2* and α4β2* nicotinic acetylcholine receptors as drug targets for Parkinson's disease. Pharmacol. Rev 63 (4), 938–966. 10.1124/pr.110.003269. PMID: 21969327. [PubMed: 21969327]

Rasch B, Gais S, Born J, 2009. Impaired off-line consolidation of motor memories after combined blockade of cholinergic receptors during REM sleep-rich sleep. Neuropsychopharmacology 34 (7), 1843–1853. 10.1038/npp.2009.6.Epub.2009.Feb.4. [PubMed: 19194375]

Rice M, Cragg S, 2004. Nicotine amplifies reward-related dopamine signals in striatum. Nat. Neurosci 7, 583–584. 10.1038/nn1244. PMID: 15146188. [PubMed: 15146188]

Runfeldt MJ, Sadovsky AJ, MacLean JN, 2014. Acetylcholine functionally reorganizes neocortical microcircuits. J. Neurophysiol 112 (5), 1205–1216. 10.1152/jn.00071.2014. PMID: 24872527. [PubMed: 24872527]

Sadahiro M, Sajo M, Morishita H, 2016. Nicotinic regulation of experience-dependent plasticity in visual cortex. J. Physiol. Paris 110 (1–2), 29–36. 10.1016/j.jphysparis.2016.11.003. PMID: 27840212. [PubMed: 27840212]

Sajo M, Ellis-Davies G, Morishita H, 2016. Lynx1 limits dendritic spine turnover in the adult visual cortex. J. Neurosci 36 (36), 9472–9478. 10.1523/JNEUROSCI.0580-16.2016. PMID: 27605620. [PubMed: 27605620]

Sarter M, Albin RL, Kucinski A, Lustig C, 2014. Where attention falls: increased risk of falls from the converging impact of cortical cholinergic and midbrain dopamine loss on striatal function. Exp. Neurol 257, 120–129. 10.1016/j.expneurol.2014.04.032. PMID: 24805070. [PubMed: 24805070]

Sarter M, Parikh V, Howe WM, 2009. nAChR agonist-induced cognition enhancement: integration of cognitive and neuronal mechanisms. Biochem. Pharmacol 78 (7), 658–667. 10.1016/j.bcp.2009.04.019. PMID: 19406107. [PubMed: 19406107]

Séguéla P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW, 1993. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J. Neurosci 13 (2), 596–604. 10.1523/JNEUROSCI.13-02-00596.1993. PMID: 7678857. [PubMed: 7678857]

Shahsavar A, Kastrup JS, Nielsen EØ, Kristensen JL, Gajhede M, Balle T, 2012. Crystal structure of Lymnaea stagnalis AChBP complexed with the potent nAChR antagonist DHβE suggests a unique mode of antagonism. PloS One 7 (8), e40757. 10.1371/journal.pone.0040757. PMID: 22927902. [PubMed: 22927902]

Smith MR, Glicksberg BS, Li L, Chen R, Morishita H, Dudley JT, 2018. Loss-offunction of neuroplasticity-related genes confers risk for human neurodevelopmental disorders. Pac. Symp. Biocom 23, 68–79 PMID: 29218870.

Suzuki WA, Miller EK, Desimone R, 1997. Object and place memory in the macaque entorhinal cortex. J. Neurophysiol 78 (2), 1062–1081. 10.1152/jn.1997.78.2.1062. PMID: 9307135. [PubMed: 9307135]

Takesian AE, Bogart LJ, Lichtman JW, Hensch TK, 2018. Inhibitory circuit gating of auditory critical-period plasticity. Nat. Neurosci 21 (2), 218–227. 10.1038/s41593-017-0064-2. PMID: 29358666. [PubMed: 29358666]

Tekinay AB, Nong Y, Miwa JM, Lieberam I, Ibanez-Tallon I, Greengard P, Heintz N, 2009. A role for LYNX2 in anxiety-related behavior. Proc. Nal. Acad. Sci. U. S. A 106, 4477–4482 PMID:19246390.

Thomsen MS, Arvaniti M, Jensen MM, Shulepko MA, Dolgikh DA, Pinborg LH, et al. , 2016. Lynx1 and Aβ1–42 bind competitively to multiple nicotinic acetylcholine receptor subtypes. Neurobiol. Aging 46, 13–21. 10.1016/j.neurobiolaging.2016.06.009. PMID: 27460145. [PubMed: 27460145]

Thomsen MS, Cinar B, Jensen MM, Lyukmanova EN, Shulepko MA, Tsetlin V, Klein AB, Mikkelsen JD, 2014. Expression of the Ly-6 family proteins Lynx1 and Ly6H in the rat brain is compartmentalized, cell-type specific, and developmentally regulated. Brain Struct. Funct 219, 1923–1934 PMID:23860910. [PubMed: 23860910]

Thomsen MS, Mikkelsen JD, 2012. The a7 nicotinic acetylcholine receptor complex: one, two or multiple drug targets? Curr. Drug Targets 13 (5), 707–720. 10.2174/138945012800399035. PMID: 22300038. [PubMed: 22300038]

Tsetlin VI, 2015. Three-finger snake neurotoxins and Ly6 proteins targeting nicotinic acetylcholine receptors: pharmacological tools and endogenous modulators. Trends Pharmacol. Sci 36 (2), 109–123. 10.1016/j.tips.2014.11.003. PMID: 25528970. [PubMed: 25528970]

Unal CT, Pare D, Zaborszky L, 2015. Impact of basal forebrain cholinergic inputs on basolateral amygdala neurons. J. Neruoscience 35 (2), 853–863. 10.1523/JNEUROSCI.2706-14.2015. PMID: 25589777.

Vasilyeva NA, Loktyushov EV, Bychkov ML, Shenkarev ZO, Lyukmanova EN, 2017. Three-finger proteins from the Ly6/uPAR family: functional diversity within one structural motif. Biochemistry (Mosc.) 82 (13), 1702–1715. 10.1134/s0006297917130090. PMID: 29523067. [PubMed: 29523067]

Verhoog MB, Obermayer J, Kortleven CA, Wilbers R, Wester J, Baayen JC, et al. , 2016. Layer-specific cholinergic control of human and mouse cortical synaptic plasticity. Nat. Commun 7, 12826. 10.1038/ncomms12826. PMID: 27604129. [PubMed: 27604129]

Vieta E, Popovic D, Rosa A, Solé B, Grande I, Frey B, et al. , 2013. The clinical implications of cognitive impairment and allostatic load in bipolar disorder. Eur. Psychiatr 28 (1), 21–29. 10.1016/j.eurpsy.2011.11.007. PMID: 22534552.

Whalen PJ, Kapp BS, Pascoe JP, 1994. Neuronal activity within the nucleus basalis and conditioned neocortical electroencephalographic activation. J. Neurosci 14 (3 Pt 2), 1623–1633. 10.1523/JNEUROSCI.14-03-01623.1994. PMID: 8126559. [PubMed: 8126559]

Wilson MA, Fadel JR, 2017. Cholinergic regulation of fear learning and extinction. J. Neurosci. Res 95 (3), 836–852. 10.1002/jnr.23840. PMID: 27704595. [PubMed: 27704595]

Wonnacott S, 1997. Presynaptic nicotinic ACh receptors. Trends Neurosci 20 (2), 92–98. 10.1016/s0166-2236(96)10073-4. PMID: 9023878. [PubMed: 9023878]

Woolf N, 1991. Cholinergic systems in mammalian brain and spinal cord. Prog. Neurobiol 37 (6), 475–524. 10.1016/0301-0082(91)90006-m. PMID: 1763188. [PubMed: 1763188]

Wu J, George AA, Schroeder KM, Xu L, Marxer-Miller S, Lucero L, Lukas RJ, 2004. Electrophysiological, pharmacological, and molecular evidence for α7-nicotinic acetylcholine

receptors in rat midbrain dopamine neurons. J. Pharmacol. Exp. Therapeut 311 (1), 80–91. 10.1124/jpet.104.070417. PMID: 15178698.

Wu J, Lukas RJ, 2011. Naturally-expressed nicotinic acetylcholine receptor subtypes. Biochem. Pharmacol 82 (8), 800–807. 10.1016/j.bcp.2011.07.067. PMID: 23139735. [PubMed: 21787755]

Wu M, Puddifoot CA, Taylor P, Joiner WJ, 2015. Mechanisms of inhibition and potentiation of nαi4cβo2tinic acetylcholine receptors by members of the Ly6 protein family. J. Biol. Chem 290 (40), 24509–24518. 10.1074/jbc.M115.647248. PMID: 26276394. [PubMed: 26276394]

Yamakawa GR, Basu P, Cortese F, MacDonnell J, Whalley D, Smith VM, Antle MC, 2016. The cholinergic forebrain arousal system acts directly on the circadian pacemaker. Proc. Natl. Acad. Sci. U.S.A 113 (47), 13498–13503. 10.1073/pnas.1610342113. PMID: 27821764. [PubMed: 27821764]

Zaborszky L, 2002. Chapter 28 the modular organization of brain systems. Basal forebrain: the last frontier. Prog. Brain Res. Changing Views Cajals Neuron 136, 359–372. 10.1016/s0079-6123(02)36030-8. PMID: 12143394.

Zhang Y, Lang Q, Li J, Xie F, Wan B, Yu L, 2009. Identification and characterization of human LYPD6, a new member of the Ly-6 superfamily. Mol. Biol. Rep 37 (4), 2055–2062. 10.1007/s11033-009-9663-7. PMID: 19653121. [PubMed: 19653121]

Zhao Y, Ren J, Lu W, Harlos K, Jones YE, 2018. Structure of the Wnt signaling enhancer LYPD6 and its interactions with the Wnt coreceptor LRP6. FEBS Lett 592 (18), 3152–3162. 10.1002/1873-3468.13212. PMID: 30069874. [PubMed: 30069874]

Zoli M, Picciotto MR, Ferrari R, Cocchi D, Changeux JP, 1999. Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. EMBO J 18 (5), 1235–1244. 10.1093/emboj/18.5.1235. PMID: 10064590. [PubMed: 10064590]

Zouridakis M, Papakyriakou A, Ivanov IA, Kasheverov IE, Tsetlin V, Tzartos S, Giastas P, 2019. Crystal structure of the monomeric extracellular domain of α9 nicotinic receptor subunit in complex with α-conotoxin RgIA: molecular dynamics insights into RgIA binding to α9α10 nicotinic receptors. Front. Pharmacol 10, 474. 10.3389/fphar.2019.00474.eCollection.2019. PMID: 31118896. [PubMed: 31118896]

HIGHLIGHTS

- Cholinergic signaling is broad due to widespread cholinergic axonal radiation.
- Prototoxins exert spatial selectivity by binding to specific nAChR subtypes.
- Different prototoxins up- and down-regulate nAChR function.
- Cell-type specific prototoxins effect nuanced circuit responses to acetylcholine.
- Circuit modulation alters plasticity, learning, anxiety, etc.

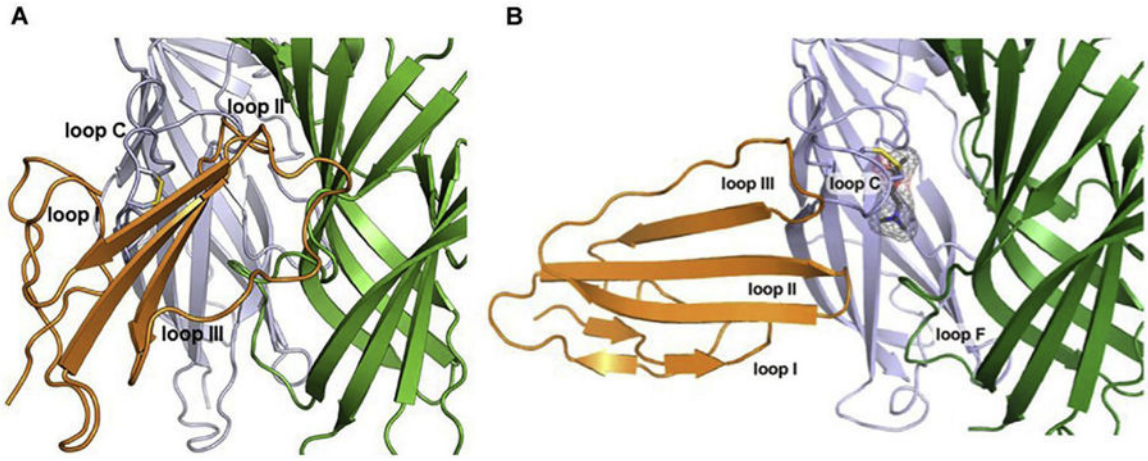

Fig. 1.

Schematic of lynx1 interaction with nAChRs.

A model of lynx1 (orange) is depicted interacting with A. two a4 nAChR subunits (from Nissen et al., 2018) and B. two a7 nAChR subunits (Lyukmanova et al., 2011). From Hoffman et al., 2019.)

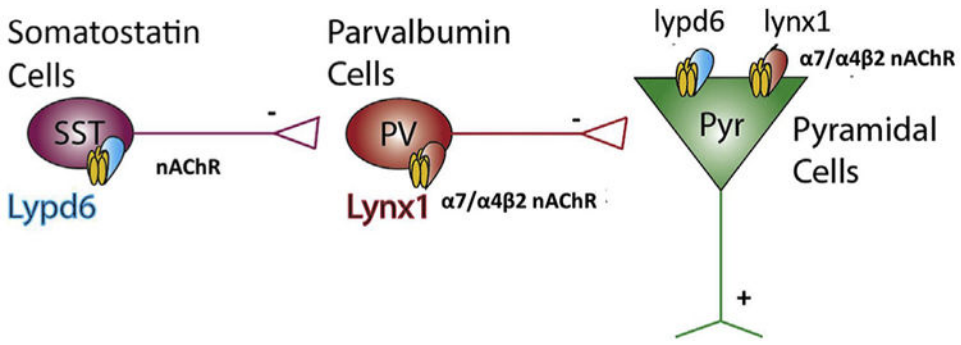

Fig. 2.

Selective expression of lynx1 and lypd6 prototoxins within circuits. From Demars and Morishita (2014).

Table 1

Spatial Control Over the Central Nervous System. Overview of select Ly6 family members with known expression within the rodent central nervous system.

Spatial Control Over the Central Nervous System				
Gene	**Expression patterns within rodent brain**	**Behavioral Consequences**	**nAChR Interactions**	**References**
Lynx1	Substantia nigra, dorsal raphe nucleus, hippocampus, cortex, cerebellum, Isocortex, olfactory areas, cortical subplate, straitum, pallidum, thalamus, midbrain, hypothalamus, pons, and medulla; temporally, expression in mice is upregulated around postnatal week.	The loss of lynx1 in a knockout mouse results in enhanced associative learning, enhanced antinociception to nicotine or epibatidine, and can enhance motor activity	Interacts with α7, α4β2, α6, and α3β2. Lynx1 presence shifts Ach EC50 to the right and results in increased agonist desensitization	Miwa et al., 1999; Ibanez-Tallon et al., 2002; Lyukmanova et al., 2013; Thomsen et al., 2014; Parker et al., 2017; Nissen et al., 2018; Allen Brain Map
Lynx2/ lypd1	Basolateral amygdala, medial prefrontal cortex, Isocortex, olfactory areas, hippocampal formation, cortical subplate, straitum, pallidum, thalamus, hypothalamus, midbrain, pons, and medulla.	Loss of lynx2 leads to increased fear- and anxiety-like behavior in a knockout mouse	Interact with a7 and α4β2 nAChRs; enhances receptor desensitization and decreases ACh sensitivity	Dessaud et al., 2006; Tekinay et al., 2009; Wu et al., 2015; Allen Brain Map
Lypd6	Known expression in somatostatin interneurons of V1 layers 5 and 6	Lypd6 overexpression results in increased locomotion. hypoalgesia, and pre-pulse inhibition of the acoustic startle response while the loss of lypd6 in knockout mice decreases anxiety-like behavior. In humans, mutations are reported to result in developmental delay, hypotonia, and autistic features.	Interacts with α3, α4, α5, α6, α7, β2, and β4 nAChR subunits, Lypd6 presence enhances the Ca^{2+}-component of nicotine-evoked currents	Darvas et al., 2009; Chandler and Waterhouse, 2012 (22085900); Zhang et al., 2009; Arvaniti et al., 2018; Allen Brain Map
PSCA	Isocortex, olfactory areas, hippocampal formation, cortical subplate, striatum, pallidum, thalamus, midbrain, hypothalamus, pons, medulla, ciliary ganglion, neural and choroid plexus	PSCA is up-regulated in the cortex of AD patients	Interacts with α4 nAChRs, decreases nicotine-induced kinase phosphorylation, interacts with α7-containing chick nAChRs	Hruska et al., 2009; Jensen et al., 2015; Ochoa et al., 2016; Allen Brain Map
Ly6g6e	Olfactory areas, hippocampus, cortical areas, and midbrain	No *in vivo* rodent behavioral studies; hypothesized to regulate nicotine addiction	Interacts with potentiates α4β2 nAChRs and slows desensitization but effects persist in absence of extracellular calcium	Wu et al., 2015; Allen Brain Map
Ly6h	Frontal cortex, Isocortex, olfactory areas, hippocampal formation, cortical subplate, straitum, pallidum, thalamus, hypothalamus, midbrain, pons, and medulla; a temporal pattern exists: expression is first observed around postnatal week 1–2 in mouse models	Ly6h enhances nicotine-induced potentiation of hippocampal pyramidal neurons; hypothesized role in nicotine addiction	Interacts with α7 nAChRs and causes a right-ward shift of nicotine or epibatidine-evoked responses, demonstrated to both influence and not interact with α4β2 nAChRs in different assays	Horie et al., 1998 (9799603); Artoni et al., 2019; Tekinay et al., 2009; Thomsen et al., 2016; Puddifoot et al., 2015; Wu et al., 2015; Allen Brain Map
Lypd6B	Visual cortex, Isocortex, olfactory areas, hippocampal formation, cortical subplate, pallidum, hypothalamus, midbrain, pons, and medulla	Mutations in humans are linked to intellectual disability and developmental delay; no *in vivo* rodent behavioral studies	Inhibits α3β4-, not α7-mediated nAChR currents; specifically for α3β4: Lypd6B presence reduces EC_{50} for ACh in nAChRs containing 3 (not 2) α3 subunits	Chandler and Waterhouse, 2012 (22085900); Demars and Morishita 2014 (25359633); Ochoa et al., 2016 and 2019; Allen Brain Map