The cholinergic system is an essential modulatory neurotransmitter system in the mammalian brain responsible for the regulation of memory, attention, and cognitive processing. This dissertation investigates several key components of the cholinergic system that are important for human neurodevelopment and disease. First, I investigated the non-enzymatic role of the T30 neuropeptide, a 30-amino acid peptide endogenously produced as a cleavage product of the α7 nicotinic acetylcholine receptor (nAChR) activation of mTORC1. Second: The targeting of cholinergic nAChR, with small molecules or peptides, shows exciting promise in the development of drugs for treating human neurodegenerative and inflammatory disorders. Drug development however is often hampered by limitations in initial compound screening and target validation due to limitations in structural protein analysis and the computational limitations of crystalized modeling. To address this, I optimized the use of protein painting-based mass spectrometry to identify ligand interaction sites of the nAChR using the Aplysia californica acetylcholine-binding protein structural surrogate model. I show specific residues involved in the binding of nAChR ligands including α-bungarotoxin, choline, nicotine, and the pathogenic amyloid beta 1–42 peptide. Protein painting is thus presented as a novel strategy for drug development targeting the nAChR. Third: Human immunodeficiency virus (HIV) continues to be a major global health problem with infected individuals at a heightened risk for cognitive decline, dementia, and memory deficits. Moreover, almost half of all HIV-positive individuals also smoke tobacco products and studies have shown that smoking can worsen cognitive functions in HIV infected individuals. I examined interaction between the HIV structural glycoprotein gp120 and nicotine within human microglia (HMC3) cells. Microglia are a primary reservoir for HIV and have been recently shown to contribute to neuroinflammation during HIV infection. Mass spectrometry analysis of gp120 treated cells shows that HIV impacts the mitochondrial proteome of human microglia. Specifically, in the presence of nicotine, gp120 was found to increase mitochondrial fusion as well as network size, and to reduces fission. The effect of nicotine on gp120 correlated with an increase in amyloid peptide release as well as increase in autophagy within microglia. These results show that nicotine potentiates gp120 inflammatory effect on microglia by modulating both mitochondrial activity and amyloid peptide processing and release. Thus, these findings present a mechanism for HIV associated neurocognitive decline and heightened risk in the brain of smokers. Ultimately, this dissertation underscores the critical role of the cholinergic system in mediating neurodevelopmental processes, serving as a key target for therapeutic interventions, and contributing to the pathological consequences of neurodegenerative diseases.