

**Chemical-Scale Studies of the Nicotinic
and Muscarinic Acetylcholine Receptors**

Thesis by

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In memory of my grandparents:

Margaret and Ronald McCann

Virginia and Carl Torrice

Acknowledgments

*The struggle itself towards the heights
is enough to fill a man's heart. One
must imagine Sisyphus happy.*

- Albert Camus

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Abstract

This dissertation describes three chemical-scale studies of neuroreceptor structure and function. Incorporation of unnatural amino acids into two acetylcholine receptors—the nicotinic acetylcholine receptor (nAChR) and the M₂ muscarinic acetylcholine receptor (M₂AChR)—and an electrophysiology assay of receptor function were performed in each of the studies. The nAChR is a ligand-gated ion channel (LGIC) and the M₂AChR is a G-protein-coupled receptor (GPCR).

In Chapter 2, a highly conserved aspartate residue (D89) that is near the agonist binding site of the nAChR was probed for its role in agonist binding. We found that the side chain of D89 establishes a redundant network of hydrogen bonds and preorganizes the agonist binding site by positioning a critical agonist-binding residue, tryptophan 149 (W149). Previous studies of a D89N mutant led to the proposal that a negative charge at D89 was essential for receptor function. However, our studies show that neutral side chains at position 89 function well, only if an unfavorable electrostatic clash is avoided.

Chapter 3 describes our attempts to incorporate unnatural amino acids into the M₂AChR, a GPCR. GPCR activity is assayed through second messenger signaling pathways, unlike the direct readout assays of LGICs. These second messenger pathways require significant amounts of optimization to create assays that produce reliable and robust data. In our experiments, variability of dose-response relationship data between batches of cells was the most significant concern. Several factors were investigated to reduce this batch-to-batch variability. After a reliable means to assay M₂AChR function

was found, we performed a preliminary search for tryptophan residues in the agonist binding site that form a cation- π interaction with acetylcholine.

Finally, in Chapter 4, we discuss the use of hydroxy acids to scan the α M1 transmembrane helix of the nAChR for residues that undergo structural rearrangements during gating. Hydroxy acids disrupt hydrogen bonding in protein backbones and thus provide a means to detect backbone interactions that form or break during gating. The hydroxy acid analog of valine, valic acid (Vah), was incorporated at ten positions along the α M1 helix. Backbone mutations at five residues on the intracellular side of a conserved proline (P221) produced shifts in dose-response relationships.

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