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**Allosteric modulation of neuronal nicotinic acetylcholine receptors requires inter-subunit movement**

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Allosteric modulation of neuronal nicotinic acetylcholine receptors (nAChRs) is an increasingly important avenue of drug development for CNS cholinergic systems. We have been studying a series of compounds that modulate  $\alpha 3\beta 2$  receptors from a site, homologous to the canonical agonist site, at the  $\beta(+)/\alpha(-)$  interface. Having established that potentiation in the system arises from enhanced gating efficacy, we have asked whether allosteric modulation requires inter-subunit movement, as is the case with stereotypical agonist activation of the channel. To answer this question, we studied point mutant  $\alpha 3\beta 2$  receptors, as well as  $\alpha/\beta$  paired mutants for a series of residues we predicted could make contact across the interface, based on a homology model of the receptor extracellular domain. For example,  $\alpha 3S125$ , a hot spot along the ligand binding-gating transduction pathway, when substituted with tyrosine, converts the modulator and partial agonist morantel into a full agonist. This agonist activity is dramatically reduced when  $\alpha 3S125Y$  is co-expressed with  $\beta 2W149A$ , presumably because that highly conserved tryptophan is involved in binding morantel. When the mutant  $\alpha 3S125C$  is co-expressed with  $\beta 2Q39C$ , where this pair of residues is predicted to be adjacent across the  $\alpha(+)/\beta(-)$  interface, an oxidation treatment reduces morantel potentiation of ACh-evoked currents. Similar studies on other positions in the  $\alpha(+)/\beta(-)$  [agonist] and  $\beta(+)/\alpha(-)$  [modulator] interfaces shed light on the extent of inter-subunit movement and receptor symmetry required for allosteric modulation of nAChRs.

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**Positive and negative cooperativity of agonist and allosteric modulator binding in  $\alpha 7$  nAChR: Looking for the therapeutic window**Roger L. Papke<sup>1,\*</sup>, Dustin K. Williams<sup>1</sup>, Jingyi Wang<sup>2</sup>, Nicole A. Horenstein<sup>2</sup><sup>1</sup> *Dept. of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL, USA*<sup>2</sup> *Dept. of Chemistry, University of Florida, Gainesville, FL, USA*

The intrinsic open probability of  $\alpha 7$  nAChR is very low, even under the most fully optimized conditions. Average single channel open times are on the order of 70  $\mu$ s and bursts of openings are not observed. Studies of both macroscopic and single currents suggest that rapid saturation of the  $\alpha 7$  agonist binding sites may produce synchronized channel opening, but little increase in the time-averaged P-open of individual channels. In contrast, while heteromeric nAChR activate to a brief open state ( $O^*$ ) in the presence of low concentrations of agonist, at higher concentrations of agonist bursts of longer openings are observed, suggesting a positive co-operativity between binding two agonist molecules and the stabilization of a long-lived open state ( $O'$ ). We hypothesize that in native  $\alpha 7$  receptors, the cooperative effect of multiple agonist binding events is to promote the conversion of receptors to a unique non-conducting state ( $D_s$ ) that is associated with conformational change within the ion channel. Positive allosteric modulators can greatly increase the  $P_{open}$  of  $\alpha 7$  nAChR in multiple ways. They may change the energy barriers into and out of the open channel state

such that immediately after a jump in agonist concentration there is a transient increase in  $P_{open}$  (Type I PAM). They may also alter the absolute energy differences between conducting and desensitized states, producing more current under equilibrium conditions. It has also been proposed that Type II PAMs, such as PNU-120596, convert one or more desensitized states into open channel states. Our data suggest that for  $\alpha 7$ , PNU-120596 promotes the stability of a state analogous to the  $O'$  of heteromeric receptors and that this is coupled with a decrease in the stability of the  $D_s$  state and a conversion of  $D_s$  into a subconductance. The  $O'$  events occur as bursts of prolonged openings, persisting sometimes for several seconds, with intraburst closures to the  $D_s$  subconductance. As in heteromeric nAChR, prolonged activation of  $\alpha 7$  is associated with the likelihood of conversion to an alternative desensitized state ( $D_i$ ) that is stable in PAM-modified receptors as long as agonist is present and reversible once agonist is removed. Responses to simultaneous co-applications of varying concentrations of agonist and PAM indicate that the stability of the  $D_i$  state in  $\alpha 7$  is promoted by high levels of binding both agonist and PNU-120596 and that there is mutually positive cooperativity for the binding of ACh and PNU-120596. The current evoked by co-applications is biphasic when intermediate concentrations of agonist and PAM are used, with a rapid transient phase associated with the initial perturbation from the resting condition, and a slow phase as an equilibrium between the multiple conformational states is approached. The equilibrium that is approached depends on both the concentration of agonist and PAM, such that pseudo steady-state activation is optimal when concentrations of both agents are low to intermediate. When agonist concentrations are relatively high, applications of competitive antagonists (MLA or DH $\beta$ E) can reduce the negative effect of high agonist occupancy and promote increased current. Our data therefore indicate how activation of  $\alpha 7$  is achieved in a dynamic window of agonist and PAM concentrations that can determine both transient and steady-state  $P_{open}$ . Our understanding of these mechanisms will allow us to better meet the challenge of optimizing PAMs for future therapeutic development.

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**Differential allosteric modulation of high- and low-sensitivity forms of the  $\alpha 4\beta 2$  nAChR: Evidence for distinct modulator binding sites**

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Multiple lines of evidence suggest  $\alpha 4\beta 2$  nicotinic receptors (nAChRs) to be integral components in the neurocircuitry of cognition, suggesting this receptor to be a potential drug target for treatment of cognitive dysfunction. The  $\alpha 4\beta 2$  nAChR is known to exist in two distinct isoforms, caused by differential subunit stoichiometries, displaying high (HS) and low (LS) sensitivity towards acetylcholine, respectively. There is mounting evidence that both are expressed in the mammalian brain but the functional significance of each isoform is obscure. Allosteric modulation of  $\alpha 4\beta 2$  nAChRs presents an attractive approach towards targeting this receptor and the compound NS9283 was previously reported by our group to act as an efficacious and selective positive allosteric modulator (PAM) at the low-sensitivity  $\alpha 4\beta 2$  nAChR. This compound was demonstrated to produce cognitive augmentation in vivo across a broad range of behavioral pharmacology paradigms, highlighting the importance of LS- $\alpha 4\beta 2$  nAChRs for cognitive function.