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COMMENTARY

α4β2 Nicotinic acetylcholine receptors, willing if able

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Li and Steinbach apply nonstationary noise analysis to the whole-cell current responses of low sensitivity α4β2 nAChR stably-expressed in HEK cells. These receptors represent one of the most important nAChR subtypes in brain, and also one of the most difficult to study in native tissues. They found the activating properties of the full agonists ACh and nicotine to be similar with regard to P_{open} and single channel conductance, whereas the weak α4β2 partial agonist cytisine caused channels to open with low probability but increased single channel conductance. When optimally stimulated by either of the full agonists, approximately 80% of the available receptors opened at the peak of the response. However, comparisons of whole-cell current to estimates of total cell surface receptors, indicated that only about 7% of the total receptor population can be activated. These observations provide important and intriguing new pieces of the brain nicotine receptor puzzle.

Abbreviations
nAChR, nicotinic acetylcholine receptors; NMJ, neuromuscular junction; P_{open}, probability of a single channel being open

Much of our basic understanding of synaptic transmission has come from studies of the neuromuscular junction (NMJ). Bernard Katz and his colleagues initiated the era of molecular neuroscience with the demonstration of quantal transmitter release at the NMJ. The NMJ was the first synapse made visible under the electron microscope, and with *in situ* autoradiographic studies of α-bungarotoxin binding, it was possible to localize and even quantify the nicotinic acetylcholine receptors (nAChR) of the NMJ (Salpeter, 1987). Our first images of single molecular mediators of synaptic function were of nicotinic receptors; electromicrographic images of the receptor molecules came from the Torpedo electric organ (Brisson and Unwin, 1985), and dynamic functional images of nAChR from the first single-channel recordings (Sakmann, 1978). For many of us, the NMJ remains a model from which we base our hypotheses for synapses. We find ultrastructural specializations in the brain that place high densities of postsynaptic receptors in close proximity to sites of quantal vesicular release. We have the expectation that each vesicle released will provide a brief high concentration of transmitter, opening postsynaptic receptors with very high probability, and that both rapid removal of transmitter and intrinsic desensitization will provide punctate point-to-point neurotransmission.

Indeed, the model of the NMJ fits several aspects of the synaptic transmission mediated by AMPA-type glutamate receptors in the brain, but ironically, the greatest failure of the neuromuscular model has been in trying to apply it to the function of nAChR in brain. Most of the high affinity nicotine receptors of the brain appear to function presynaptically (Wonnacott, 1997), and even when nicotinic

receptors can be confirmed to be present on neuronal cell bodies, they are not associated with proximal presynaptic acetylcholine release sites. In the brain most of the release sites for acetylcholine are diffusely situated on axonal varicosities and provide a sort of volume transmission to both nicotinic and muscarinic acetylcholine receptors (Descarries *et al.*, 1997).

While the nicotinic receptors of the NMJ have always been the synaptic receptors most accessible to study, the nicotinic receptors in the brain remain the most obscured. However, in recent years, methods for heterologous expression of the neuronal nicotinic receptor genes have brought these receptors into the light and made them available for extensive physiological and pharmacological characterizations. In this issue of the *British Journal of Pharmacology*, Li and Steinbach (2010) applied non-stationary noise analysis to macroscopic agonist-evoked responses of neuronal α4β2 nicotinic acetylcholine receptors, stably expressed in a mammalian cell line.

The method of non-stationary noise analysis is based on variances in the current generated by a population of receptors and the relationship determined by the total number of receptors, their single-channel current amplitude, and the probability that any single channel is open. The authors showed that when a full agonist is used at an optimal concentration, there is a transient phase of synchronous channel activation when the probability of any single 'activatable' channel being open is as high as 80%. In this regard at least, these brain-type receptors behave like their counterparts at the NMJ.

The channel blocking activity of nicotine notwithstanding, activation properties of the full agonists were similar. Somewhat surprisingly, the weak α4β2 partial agonist, cytisine, caused channels to open with low probability but larger single channel conductance than the ACh or nicotine-evoked currents. The new smoking cessation drug, varenicline, is a cytisine derivative and so it may have a similar mechanism.

Receptors containing α4β2 subunits have been strongly associated with both the cognitive and addictive effects of nicotine (Gotti *et al.*, 2007). There are two primary forms of pentameric α4β2 receptors, based on the subunit stoichiometry, and both forms are likely to exist in native brain tissue (Grady *et al.*, 2010). The Li and Steinbach study pertains to the low sensitivity (LS) α4(3)β2(2) form, which is most readily expressed in mammalian cell lines and requires relatively high concentrations of nicotine or ACh for optimal activation. The authors saw maximal stimulation of the α4β2 receptors with ACh concentrations in the range of 300 μM to 1 mM and used solution application times of tens of milliseconds. We cannot really say for sure how much or for how long ACh concentrations fluctuate at the α4β2 receptors in brain; however, with diffuse release of acetylcholine, and active acetylcholine esterases present in the brain, it is unlikely that receptors in native brain tissue receive such signals. Likewise, maximal stimulation by nicotine required concentrations of 30–100 μM, far higher than would be expected in the brain of a smoker. Therefore, although capable of synchronous high P_{open}, responses, the authors acknowledge that native LS α4β2 receptors are unlikely to function in this manner *in vivo*.

Another important finding of the Li and Steinbach study is that there is a large, 15-fold, disparity between the apparent number of 'activatable' receptors on a single cell determined by functional studies and the number of putative surface receptors determined by radioligand binding experiments. This implies that although an optimized stimulus may have an 80% probability of opening an 'activatable' receptor, that same stimulus is inert for 93% of the receptors. Combining these two factors, any single surface receptor has less than a 6% chance of being opened, even by a stimulus well outside the expected physiological range. Therefore, the study by Li and Steinbach (2010) is equally as exciting for the questions that it brings to light as for the important new data it provides.

Insights into the nature of disparity between the surface populations of functional and nonfunctional receptors could be extremely important for enlarging our understanding of nAChR function in the brain. Is the same disparity found in native tissues? Does it also exist for the high sensitivity form of α4β2? Are the populations of functional and nonfunctional receptors interconvertible and, if so, on what time scale? Is the distribution of receptors into the functional and nonfunctional pools sensitive to other neurotransmitter systems, signal transduction pathways, or chronic exposure to nicotine?

Sometimes, finding a new piece to a puzzle reveals that the puzzle extends in new unexpected directions.

References

Brisson A, Unwin PNT (1985). Quaternary structure of the acetylcholine receptor. Nature 315: 474–477.

Descarries L, Gisiger V, Steriade M (1997). Diffuse transmission by acetylcholine in the CNS. Prog Neurobiol 53: 603–625.

Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M (2007). Heterogeneity and complexity of native brain nicotinic receptors. Biochem Pharmacol 74: 1102–1111.

Grady SR, Salminen O, McIntosh JM, Marks MJ, Collins AC (2010). Mouse striatal dopamine nerve terminals express alpha4alpha5beta2 and two stoichiometric forms of alpha4beta2*-nicotinic acetylcholine receptors. J Mol Neurosci 40: 91–95.

Li P, Steinbach JH (2010). The neuronal nicotinic α4β2 receptor has a high maximal probability of being open. Br J Pharmacol 160: 1906–1915.

Sakmann B (1978). Acetylcholine-induced ionic channels in rat skeletal muscle. FASEB J 37: 2654–2659.

Salpeter MM (1987). *The Vertebrate Neuromuscular Junction*. Alan R. Liss: New York.

Wonnacott S (1997). Presynaptic nicotinic ACh receptors. TINS 20: 92–98.