## 2.4

### Distinct neural pathways mediate alpha7 nicotinic acetylcholine receptor-dependent activation of the forebrain

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$\alpha_7$ nicotinic acetylcholine receptor (nAChR) agonists are promising candidates for the treatment of cognitive deficits in schizophrenia and Alzheimer's disease, and have shown pro-cognitive effects in clinical trials. However, the neural systems involved in these effects remain elusive. We have previously shown that the selective $\alpha_7$ nAChR agonist SSR180711 activates the medial prefrontal cortex (mPFC) and shell of the nucleus accumbens (ACC-shell) in rats. Here we use retrograde tracing from the mPFC with Cholera Toxin B and immunoreactivity of the immediate-early gene c-Fos, a marker of neuronal activation, to show that cortically projecting neurons in the Horizontal limb of the Diagonal Band of Broca (HDB) of the basal forebrain are activated by acute administration of SSR180711 (10 mg/kg). We further show that in the HDB, SSR180711 potently and selectively activates cholinergic neurons, and that depletion of these cholinergic neurons with 192 IgG-Saporin abolishes the SSR180711-induced activation of the mPFC, but not the ACCshell. Contrarily, depletion of dopaminergic neurons in the ventral tegmental area (VTA) with 6-OHDA abolishes the SSR180711-induced activation of the ACCshell, but not the mPFC or HDB. These results suggest that two different neural systems are activated by SSR180711, involving HDB to mPFC and VTA to ACCshell projections, respectively. The basal forebrain and mPFC are important for attentional function, and may subserve the pro-cognitive effects of $\alpha_7$ nAChR agonists, whereas activation of the ACCshell has been implicated in beneficial effects on the positive symptoms of schizophrenia. Thus, these systems likely mediate different behavioral effects of $\alpha_7$ nAChR activation.

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## 2.5

### Selectivity of ABT-089 for α4β2* and α6β2* nicotinic acetylcholine receptors in brain

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Numerous pharmaceutical efforts have targeted neuronal nicotinic receptors (nAChRs) for amelioration of cognitive deficits. While α4β2 and α7 are the more prominent nAChR in brain, other heteromeric nAChR can have important impact on agonist pharmacology. ABT-089 is a pioneer nAChR agonist found to enhance cognitive function with an exceptionally low incidence of adverse effects. To further investigate the mechanism of action of ABT-089, we evaluated its function in mouse brain preparations in which we have characterized the subunit composition of native nAChR. Among α4β2*-nAChR, ABT-089 had partial agonist activity (7–23% of nicotine) and high selectivity for α4α5β2 nAChR as evidenced by loss of activity in thalamus of $\alpha5^{-/-}$ mice. ABT-089 stimulated [$^3$H]-dopamine release (57%) exceeded the activity at α4β2* nAChR, that could be explained by the activity at α6β2* nAChR. The concentration–response relationship for ABT-089 stimulation of α6β2* nAChR was biphasic. $EC_{50}$ and efficacy values for ABT-089, respectively, were 28 μM and 98% at the less sensitive α6β2* nAChR and 0.11 μM and 36% at the more sensitive subtype (the most sensitive target for ABT-089 identified to date). ABT-089 had essentially no agonist or antagonist activity at concentrations ≤300 μM at α3β4-nAChR measured by [$^3$H]-acetylcholine release from interpeduncular nucleus. Thus, ABT-089 is a β2* nAChR ligand with demonstrable agonist activity at α4β2* and α6β2* receptors. As one form of α6β2* nAChR is sensitive to sub-μM concentrations, we propose that that this receptor in particular may contribute to the enhanced cognitive performance following low doses of ABT-089.

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## 2.6

### Role of Sp1 and AP-2 in the transcriptional regulation of the CHRNA7 gene and the link to schizophrenia

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The alpha 7 neuronal nicotinic acetylcholine receptor subunit gene (*CHRNA7*) has been implicated as a candidate gene for schizophrenia by both genetic linkage at 15q14 and biochemical data. The promoter region of the *CHRNA7* gene is GC-rich, has multiple transcription start sites, and contains a TATA-like sequence. The promoter also contains multiple single nucleotide polymorphisms linked to schizophrenia, some of which significantly affect *CHRNA7* transcription and have been found to differentially bind transcription factors. Expression of the transcription factor Sp1 (Specificity protein 1) has been shown to be disrupted in schizophrenia. We have found that *CHRNA7* is regulated, in part, by Sp1, as well as by another factor known to bind GC-rich sequences, AP-2 (Activating Protein 2). EMSAs were performed using neuroblastoma cell extract, showing Sp-1 and AP-2 binding sites within the promoter region. Mutations of these sites were made and tested with a luciferase reporter assay using factor-free environments. Chromatin immunoprecipitation (ChIP) was also performed using antibodies to these factors for *in vivo* data. These studies suggest that transcription of *CHRNA7*, like many genes with GC-rich promoters, is regulated by binding of Sp1 and AP-2, and that the level of these factors directly affects CHRNA7 expression, supporting a role for ubiquitous transcription factors in the pathophysiology of schizophrenia.

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