is also a negative allosteric modulator of $GABA_A$ α5 receptors with more drug-like attributes than 2-228. The proposed molecule will selectively activate α7 nAChRs over other neuronal nAChRs (e.g. α4β2, α3β4). In addition, the proposed molecule selectively inhibits activity mediated by $GABA_A$ α5 receptors relative to other $GABA_A$ subunit containing receptors (e.g. α1, α2, α3). 522-054 is an analog of 2-228 that has a similar dual allosteric profile as 2-228 but with improved absorption. 522-054 is active in the radial arm maze (RAM) and 5-choice serial reaction (5-CSR) model at doses that suggests synergy between α7 nAChRs and $GABA_A$ α5 for cognition may be possible to design into one molecule.

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2.15

Effects of 4R,6R-cembratriene diol on human α7 nicotinic acetylcholine receptor

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Neuronal nicotinic acetylcholine receptors (nAChR) have been targeted for developing drug treatments in a wide variety of illnesses and conditions that affect humans. The α7 nAChR was reported to have a role in the Alzheimer's disease, Parkinson's disease, schizophrenia, Tourette's syndrome, and anxiety disorders. The 4R, 6R-cembratriene diol (4R) is a cyclic diterpenoid that displays neuroprotective properties by a mechanism involving the α7 nAChR. The present work was undertaken to study the effects of 4R on human α7 nAChR expressed in SHSY5Y cells (obtained from Novartis Pharma AG). Using α[^{125}I]bungarotoxin binding assay, we determined that the level of expression was 1.5 pmoles receptor per mg protein. Whole-cell patch-clamp recordings indicated that nicotinic agonist (300 μM ACh, 80 μM nicotine, 1.6 mM choline and 1 μM epibatidine) evoked inward currents with amplitudes of 50-1500pA. Currents induced by 300 μM ACh and 1 μM epibatidine were totally inhibited by 10 nM methyllycaconitine (MLA), a selective α7 antagonist. 4R displayed a complex pattern of effects on the current evoked by 300 μM ACh. 4R inhibited the response to ACh at low concentration (30 nM 4R; 50% inhibition) and at high concentration (30 μM 4R; 95% inhibition), but not intermediate concentrations (1-10 μM 4R, no inhibition). 10 nM MLA totally inhibited the current remaining in the presence of 30 nM 4R, but only partially the current observed in the presence of 10 μM 4R. These results are consistent with the interpretation that 4R acts both as an inhibitor and as a positive modulator of human α7 nAChR.

Conflict of interest: Vesna Eterovic and Richard Hann have patents related to the use of Cembranoids; SHSY5Y cells expressing alpha7 nAChRs were obtained from Novartis Pharma AG.

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2.16

Characterization of type I and type II positive allosteric modulators of α7 nicotinic acetylcholine receptors

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Positive allosteric modulators (PAMs) of nicotinic acetylcholine receptors (nAChRs) have attracted considerable interest. They are useful experimental tools to study the pharmacological and biophysical properties of nAChRs. In addition, it has been suggested that they may have potential therapeutic use in the treatment of cognitive deficits associated with disorders such as schizophrenia and Alzheimer's disease. An extensive series of compounds have been identified that act as selective positive allosteric modulators of α7 nAChRs. All potentiate peak agonist-evoked responses but differences have been reported in their effect on receptor desensitization. These compounds have been designated as being either "type I" or "type II" potentiators, on the basis of their differing effects on receptor desensitization [1,2]. Type I compounds (e.g. LY-2087101 and NS-1738) have little or no effect on the rate of desensitization, whereas type II compounds such as PNU-120596 dramatically reduce rates of receptor desensitization. We have previously obtained evidence to indicate that positive allosteric modulators of α7 nAChRs may bind to an intrasubunit transmembrane site [3]. We have now extended those studies with the aim of determining whether all α7-selective positive allosteric modulators share a common binding site. In part, these studies have been prompted by recent evidence suggesting that potentiation by some allosteric modulators (e.g. NS-1738) may be influenced by additional nAChR domains [4] (findings that have been reproduced in our own lab). In order to examine this question, we have performed studies with a series of chimeric and mutated nAChRs expressed in *Xenopus* oocytes. A variety of experimental approaches have been used, including those designed to investigate competitive ligand binding, as well as studies examining receptor modification by cysteine-reactive reagents. Data obtained from these studies supports the hypothesis that both type I modulators (such as LY-2087101 and NS-1738) and type II modulators (such as PNU-120596) interact competitively on α7 nAChRs at a common allosteric site.

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Section 3. Nicotine addiction and smoking

3.1

Chronic nicotine exposure differentially alters gene expression in VTA from adolescent and adult rats

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Smokers who begin in adolescence are at a higher risk of developing dependence than those who begin as adults. We have previously demonstrated that adolescent male Sprague Dawley rats have a distinct pattern of expression of the three major neuronal nicotinic acetylcholine receptors (nAChR) subtypes and differential response to chronic nicotine treatment in multiple brain regions compared to their adult counterparts [1]. A different pattern of CNS nicotinic receptor expression may play a role in the initiation of smoking among adolescents. Furthermore, the distinct pattern of responses of nAChR subtypes to nicotine during adolescence may contribute to the higher daily consumption and decreased probability of cessation observed in smokers who initiate tobacco use during adolescence. We used a similar chronic nicotine exposure model to examine the effects of chronic nicotine treatment on whole genome expression in the ventral tegmental area (VTA). Adolescent and

adult male SD rats received 6 mg/kg/day via osmotic mini pump for 14 days and were sacrificed immediately after nicotine treatment or after an additional 30 days without treatment. Four brain punches of the VTA were taken from each animal and mRNA was hybridized to Affymetrix Rat Genome 230 2.0 arrays. Two-way ANOVA of age and treatment was performed with 10% FDR using Partek Genomics Suite. We identified three classes of differentially expressed genes including transient, persistent, and late phase genes. There were a total of 267 transient phase genes (80 adol specific, 176 adult specific, 11 shared), 106 persistent phase genes (63 adol specific, 34 adult specific, 9 shared), and 1011 late phase genes (546 adol specific, 103 adult specific and 362 shared). Ontological analysis revealed a number of overrepresented classes of genes regulating nervous system development and function specific to adolescent nicotine exposure. These include 43 genes regulating neurite development, growth and morphology. Other genes of interest specific to adolescent nicotine exposure were 6 genes regulating circadian rhythms, and 23 genes involved in schizophrenia. Furthermore, these genes form an extensive interaction network, whereas those genes specific to the adult form no network. Lastly, network analysis revealed significant regulation of the synaptic long term potentiation canonical pathway in the adolescent treatment group. This suggests chronic nicotine causes large scale changes in plasticity in the adolescent brain not seen in the adults. Further examination of these genes may help reveal the underlying causes of the observed increased vulnerability of adolescent smokers.

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3.2

Nicotine persistently activates prefrontal layer VI pyramidal neurons through α5 subunit-containing α4β2* nicotinic acetylcholine receptors

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We have recently shown that corticothalamic neurons in layer VI of prefrontal cortex are excited by nicotinic receptor stimulation during development [1]. These neurons are the major source of corticothalamic feedback projections and play a key role in attention. Yet, it is not well understood how layer VI neurons are affected by acetylcholine and nicotine in adulthood. Human imaging work has shown that nicotine from one cigarette saturates cortical nicotinic receptors for several hours [2]. This finding has been widely interpreted to suggest that smoking results in the inactivation of cortical nicotinic receptors through desensitization. However, it is unclear that nicotine would equally desensitize all subtypes of α4β2* nicotinic receptors in the cortex. In particular, the properties of nicotinic receptors may be altered by the presence of the α5 accessory subunit (encoded by the CHRNA5 gene). Here, we investigate the effects of nicotinic stimulation on layer VI pyramidal neurons in adult mice. Since both acetylcholine (1 μM to 1 mM) and nicotine (300 nM) can elicit significant inward currents in layer VI neurons of wildtype mice, we tested the contribution of the nicotinic receptor α5 subunit by examining these responses in mice in which this receptor subunit has been genetically deleted [3]. Layer VI neurons in these α5−/− mice showed a maximal inward current with acetylcholine which was approximately one third of that observed in α5+/+ mice. In both genotypes, the cholinergic currents were recorded in the presence of 200 nM atropine, were reversibly inhibited by the α4β2*-selective antagonist DHβE (3 μM), and were resistant to 2 μM TTX, suggesting that they are mediated directly by receptors on the recorded cells. Similar to our findings with acetylcholine, 300 nM nicotine elicited a persistent inward current in α5−/− mice which was approximately one third of that in α5+/+ mice. Interestingly, this application of nicotine had a significantly greater ability to desensitize layer VI neurons to subsequent application of acetylcholine in α5−/− mice compared with α5+/+ mice. Results from this study suggest that the presence of nicotinic receptor α5 subunits in layer VI neurons is necessary for their normal response to acetylcholine, contributes significantly to their persistent activation by nicotine and protects against the desensitizing effects of nicotine. Prolonged binding of nicotine to prefrontal layer VI nicotinic receptors following cigarette smoking likely has sustained effects on attention gating through corticothalamic pathways because of their expression of the nicotinic receptor α5 subunit.

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3.3

Distinct pharmacological profiles for nicotinic AChR-evoked noradrenaline release in rat frontal cortex and hippocampus

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Nicotinic acetylcholine receptors (nAChRs) are widely distributed in the mammalian brain and modulate many neurotransmitter systems. Noradrenaline (NA) is important for spatial learning in hippocampus (HC) and alertness/attention in frontal cortex (FC). The modulation of NA release by nAChRs has been extensively studied in the HC, showing that NA release in this area is predominantly governed by α3β4* and α7 nAChRs [1]. Here, we compare the effects of nicotinic agonists on [^{3}H]NA release from FC prisms, using a 96 well filtration assay, and report distinct differences in the regulation of [^{3}H]NA release compared with HC. In FC, nicotine and the β2* nAChR-selective agonist 5-I-A-85380 elicit [^{3}H]NA release (EC_{50} = 0.78 μM and 5.8 nM respectively) and these responses are blocked by β2* nAChR antagonist DHβE. In contrast, in the HC these agonists are less potent (EC_{50} > 10 μM nicotine and >0.1 μM 5IA) but more efficacious. These responses are insensitive to DHβE, in agreement with previous findings [2]. Furthermore, [^{3}H]NA release from the FC is insensitive to the α7 nAChR agonist choline, which is effective in releasing [^{3}H]NA from HC prisms, via an indirect action. Responses to cytisine also differed between these regions. Thus in contrast to the HC, β2* nAChRs in the FC are implicated in modulating NA release, but α7 nAChRs are not involved. This distinction may reflect the two populations of noradrenergic neurons that have