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Identifying Novel Cognitive Therapy Targets: Expression of nAChR mRNA in Rat Hippocampal Interneurons

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ABSTRACT

We quantified mRNA subunit expression in the CA1 rat hippocampus. The α3 and β2 nAChR subunits are the highest expressed mRNA subunits while the α4 is the least. Current nAChR targeted cognitive therapies focus on the α4β2 and α7 nAChRs. These results provide a new avenue in cognitive therapies by targeting α3 containing nAChRs.

Abbreviations: nicotinic acetylcholine receptor (nAChR)

METHODS

To obtain the interneurons, coronal brain slices (either 300 or 350 μm thick) were made from 8 to 35 day old Wistar rats using a Vibratome 1000-Plus. The slices were cut in ice-cold oxygenated (95% O2, 5% CO2) artificial cerebrospinal fluid (ACSF) and placed in room-temperature oxygenated ACSF for at least 30 minutes prior to placing in microscope recording chamber.

Individual hippocampal interneurons from the CA1 *stratum oriens* and *stratum radiatum* were visually identified using an upright microscope with infrared light, and aspirated into a standard whole-cell patch-clamp pipette containing 5 μL Intracellular Fluid.

The primers and probes were designed using either Vector NTI version 7.0 or Primer Express version 2.0 (ABI Prism) software. A cDNA library representing each interneuron was made by running a reverse transcription reaction using BIORAD iScript cDNA Synthesis Kit with a final volume of 10 μl.

A multiplex PCR reaction was run (15 cycles) for each aspirated interneuron using all neuronal nAChR primers and the primer for 18S rRNA with a final volume of 75 μl. The α6 subunit was not examined because initial experiments showed no detection of this subunit in any hippocampal interneurons examined. The multiplex reaction was run using Platinum® Taq DNA Polymerase and PCR nucleotides (10mM). A second round of PCR was run (60 cycles) for each specific target using an ABI 7000 Sequence Detection System utilizing BIORAD iTaq Supermix with ROX. Cycle threshold values for each target were compared to the reference gene 18S for analysis (more in Real-Time Analysis).

Standard curves (efficiency tests) for each cDNA target were developed by running 60-cycle real-time quantitative PCR assays on positive controls (rat whole-brain homogenate) for six known concentrations (100, 33.3, 10, 3.33, 1, 0.333 ng cDNA/μL). Upstream (primer +) and downstream (primer –) primer concentrations were adjusted to optimize amplification. The efficiency of the amplification reaction is calculated using the slope of the log(concentration) vs. CT plot. Reaction efficiencies were run in triplicate and the amplification efficiencies were compared using an ANOVA to determine if there were significant differences between any of the primer/probe sets.

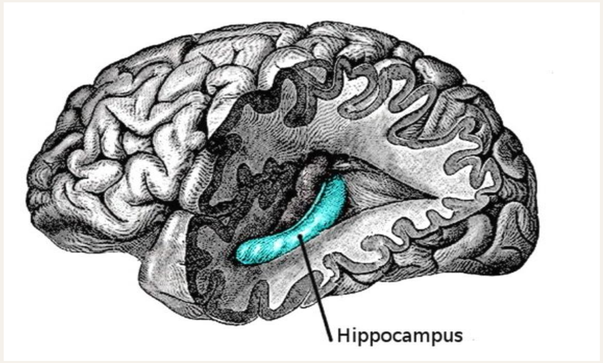

Figure 1. The hippocampus (highlighted) is part of the limbic system of the brain. It plays a vital role in long-term memory. The hippocampus is implicated in cognitive diseases like Alzheimer's disease.

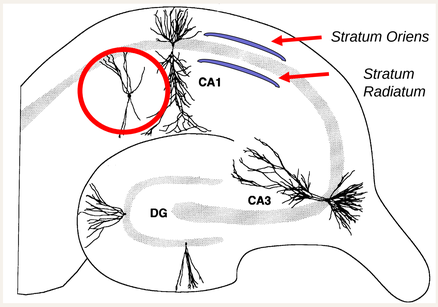

Figure 2. Depiction of the rat hippocampus. The interneurons (circled) are few in number, but play a necessary role in synchronous firing.

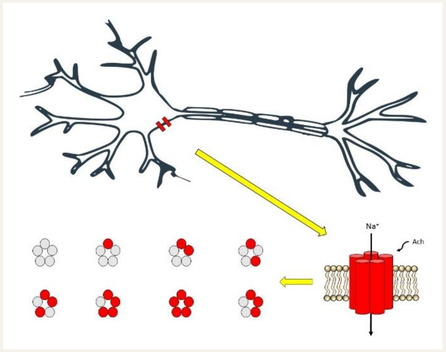

Figure 3. nAChRs are transmembrane proteins that act as ion channels. Their activation on the soma or dendrites of a neuron can result in EPSPs (excitatory currents). nAChRs can form eight different possible combinations of α (gray) and β (red) subunits. There are many more possible combinations that can be used to form a functional nAChR considering the number of α and β subunits. nAChRs play a necessary role in learning and memory.

A

Proportion of Cells containing each subunit	
Subunit	Proportion
α3	0.54
β2	0.54
α5	0.43
α7	0.42
β4	0.42
β3	0.40
α2	0.37
α4	0.27

B

Subunit Co-expression of nAChR mRNA	
Percentage	Subunit Co-expression
52%	α3 and β2
49%	α3 and α5
44%	α7 and β2
42%	α5 and β2
41%	α3 and β4
41%	β2 and β4
39%	α3 and β3
38%	β2 and β3

Figure 4. A. Proportion of interneurons expressing each unique nAChR mRNA. α3 is the most often expressed mRNA while the α4 is the least often expressed mRNA. B. The α3 and β2 subunits co-express at the highest rate.

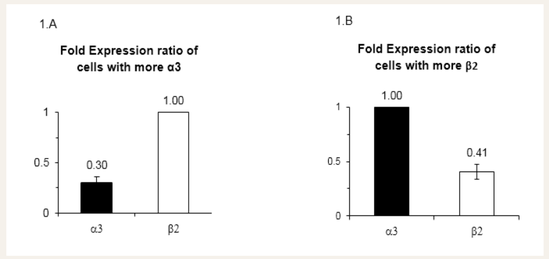

Figure 5. The population of α3β2 can be separated into two subgroups: those that have 3x more β2 subunits and those that have 3x more α3 subunits. This suggests two subtypes of nAChRs may serve as better targets than current therapies.

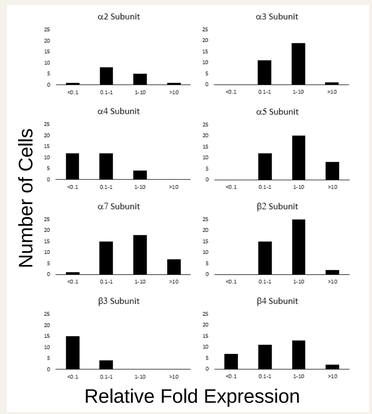

Figure 6. Relative expression of each subunit as compared to the median fold expression of all cells. The α4 subunit has a surprisingly low expression level as compared to the rest of the brain.

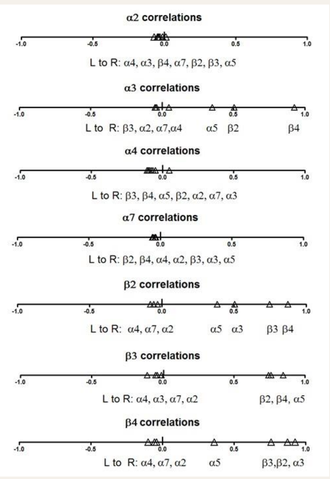

Figure 7. The two-subunit Pearson correlations reveal that the β subunits likely have fixed ratios regardless of region (*stratum oriens* and *stratum radiatum*) as evidenced by very high Pearson correlation coefficients. Likewise, the α5 and the α3 subunits have high Pearson correlation coefficients.

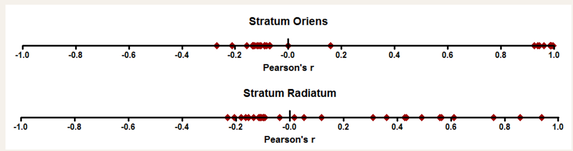

Figure 8. The *stratum oriens* and *stratum radiatum* have distinct and significant differences in their Pearson correlation coefficients of two-subunits.

CONCLUSIONS

- CA1 hippocampal interneurons may be functionally diverse considering the diversity of nAChR mRNA expression
- nAChR mRNA expression may provide clues about protein expression
  - It does not necessarily represent a 1:1 expression ratio
- The α3 subunit is highly expressed in the given regions while the α4 has the lowest expression
  - α3 directed cognitive therapies may prove more effective than current α4 directed therapies
- The *stratum oriens* and *stratum radiatum* have very different arrays of Pearson correlation coefficients for two-subunits
  - The data suggests the *stratum oriens* is likely stricter in its expression of nAChR mRNA ratios