LOCALISING NICOTINE RECEPTOR SUBUNITS IN THE HUMAN FOETAL BRAIN USING DAB IMMUNOHISTOCHEMISTRY

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Introduction

Nicotine along with many other compounds is found in cigarette smoke. The drug binds to nicotine receptors which are ion channels and can be found in the brain.

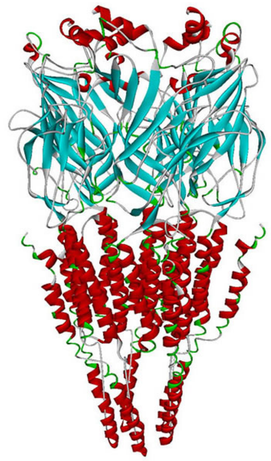

Figure 1 - Molecular model of the alpha 7 receptor courtesy of Dr. Jerrel Yakel at the National Institute of Environmental Health Sciences (2008)

There are many subtypes of this receptor, which are made up of different components and cause slightly different effects to our physiology and mental state.

One of the receptor subtypes is of particular interest to this research group. Known as **alpha 7**, the increased prevalence of this receptor in the brain is thought to be associated with smoking during pregnancy.

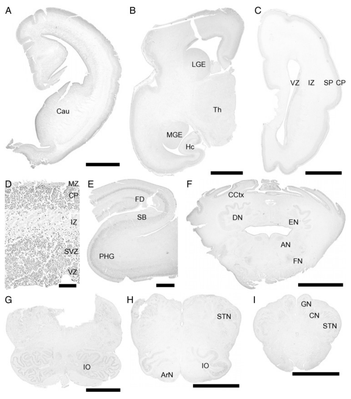

Figure 2 - Labelled brain regions AN, abducens nucleus; ArN, arcuate nucleus; Cau, caudate; CCtx, cerebellar cortex; CN, cuneate nucleus; CP, cortical plate; DN, dentate nucleus; EN, emboliform nucleus; FD, fascia dentata; FN, facial nucleus; GN, gracile nucleus; Hc, hippocampal formation; IO, inferior olivary nucleus; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; MZ, marginal zone; PHG, parahippocampal gyrus; SB, subiculum; SP, subplate; STN, spinal trigeminal nucleus; SVZ, subventricular zone; Th, thalamus; VZ, ventricular zone
Priddle and Crow, 2013. Cerebral Cortex.

In an effort to identify where in the brain this receptor can be found and quantify its presence during different periods of foetal development, staining of foetal brain slices using antibodies specific to the alpha 7 receptor were used alongside known receptors in the brain with staining regions.

It is important to have some understanding of the structure of the brain and the key areas to subsequently determine where receptors migrate to during development.

Methodology

Immunohistochemistry (IHC) is a series of techniques that makes use of antibodies that are designed to bind specifically to a choice protein.

Antibodies to alpha 7 were purchased commercially and a protocol designed as follows. Due to the novel nature of the experiment, concentration of the antibody was unknown. From surrounding literature, dilutions of 1:200 and 1:500 were used.

1. Dewax sections in xylene for 10min twice.
2. Wash sections in 2 changes of 100% ethanol for 2min each time
3. Stand in 3% hydrogen peroxide for 10min
4. Stand in boiling Na-Citrate buffer (pH 6) for 20min
5. Stand in PBS for 10min
6. Incubate sections with 2.5% horse serum for 20min
7. Apply primary antibody with 3% horse serum blocking agent and incubate in a cold room overnight
8. Wash in PBS for 5min three times
9. Incubate sections with ImmPRESS reagent for 30min
10. Wash in PBS for 5min three times
11. Incubate sections with DAB until good development occurs or 10min max
12. Wash in distilled water for 5min
13. Dehydrate sections in 70%, 95% and two 100% ethanol baths for 2min each
14. Incubate slides in Histoclear for 2mins twice
15. Apply coverslips with Histomount

Figure 3 - DAB Immunochemistry Protocol when using slides fixed with paraffin.

Staining is achieved using DAB, a commercial system that oxidises to a characteristic brown/orange colour when bound.

Sections from 8, 12 and 16 post conceptual weeks were used. Careful handling of the sections and hygienic conditions were important to ensure integrity and validity of the binding.

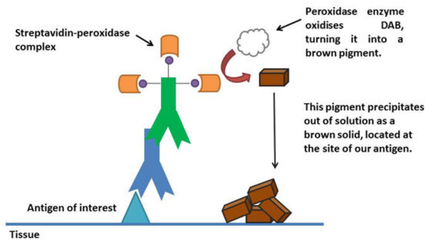

Figure 4 - Principle of DAB Immunochemistry where oxidation of DAB results in a dark brown stain that can be visualised under microscope.
Magub, 2016, Getting the Stain You Want

Developed slides were then visualised under light microscope and photos taken.

Results

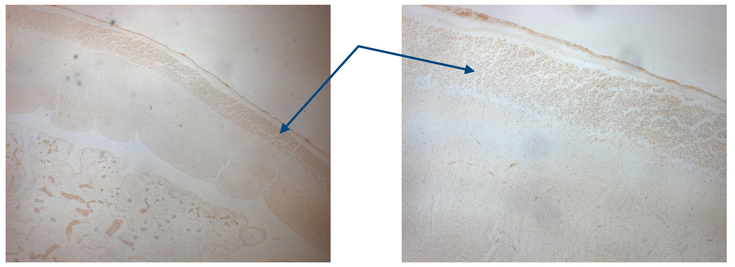

Figure 5 - DAB staining of alpha 7 antibodies bound to alpha 7 receptors marked with arrows on the sub cortical zone migrating outward from the ventricles.

The images depict some staining from the alpha 7 antibody used at 1:200. The 1:500 dilution was not thought to give any visible staining distinguishable from background.

To ensure correct protocol, known stains of alpha 4 were used adjacent to alpha 7 experiments with staining shown in figure 5.

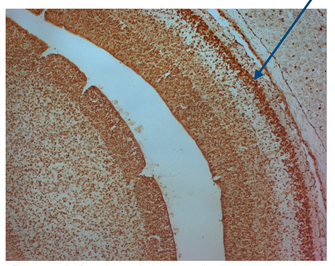

Figure 6 - DAB staining of alpha 4 antibodies bound to alpha 4 receptors marked with arrows on the sub cortical zone migrating outward from the ventricles.

Discussion

Based on the preliminary trials, it suggests that there may be some presence of alpha 7 receptors in as early as 12 weeks post conception.

Further experiments are required to quantify the levels of receptor in the developing foetal brain and some data is available to support this research.

When coupled with phenotypic information, this research may lead to a greater understanding of the implications of smoking during pregnancy.

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