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Studies on the Hippocampal Formation: From Basic Development to: Studies on Schizophrenia

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1. Introduction

The development of the hippocampus and the regulation of its innervation by growth factors has been a particular interest of Karolinska Institute investigators (Ayer-LeLievre *et al.*, 1998). The intraocular transplant preparation allowed isolation of portions of the hippocampus for detailed developmental study (Goldowitz *et al.*, 1982). A particularly advantageous feature of the preparation was its facility for the study of adrenergic and cholinergic innervation, first from the host animal's peripheral autonomic nervous system and, as the technique matured, from co-grafted brainstem and midbrain neurons (Olson *et al.*, 1980; Goldowitz *et al.*, 1984a; 1984b). Professor Lars Olson and his long-standing colleagues Professor Åke Seiger and Dr. Barry Hoffer have always been eager to have the Institute's investigation become translated into clinical research that might improve patients' treatment. The intraocular transplantation of human tissue included samples derived from the fetuses of woman who had schizophrenia, to study abnormalities in the growth and development of their hippocampus (Freedman *et al.*, 1994). Basic studies of the hippocampus conducted at the Institute during this period have over the past decade led to the initiation of new treatment strategies for schizophrenia (Freedman *et al.*, 1993; 2008). In this chapter we review the progress of using the in oculo method to understand some key features of hippocampal development and the application to the clinical setting.

2. The Developmental Potential of the Isolated Dentate Gyrus and Neighboring Hippocampus

A time-honored approach to the understanding the intrinsic (genetic) and extrinsic (environmental, epigenetic) mechanisms that underlie development of the CNS is to isolate the tissue of interest from its neighbors and place it into a controlled environment. At the Karolinska Inst., Dr. Lars Olson had revived a seldom-used technique, the transplantation of tissue to the anterior chamber of the eye, to bring a fresh approach to this type of experimental

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questions. This method had much to recommend itself over other approaches such as putting tissue under the kidney capsule or into cell culture, where either tissue was not readily visible and accessible for further manipulation or the conditions were not under physiologic conditions, respectively. The plusses of the technique were myriad including the nurturing environment of the free epithelial surface of the iris, the availability of three sources of innervation, the ability to visually monitor transplants, and finally the accessibility to manipulate the environs of the transplanted tissue. In a step-wise fashion, we examined some of the basic principles that served in the development of the area dentate of the hippocampal formation with particular attention to the afferentation of this structure.

First, we examined the developmental potential of the dentate and adjacent hippocampus (termed area dentata) isolated from its extrinsic inputs. The histotypic development of these transplants was quite impressive, forming from an initially, loosely organized neuroepithelium of the embryonic day 18 rat dentate anlagen to a structure with a well-differentiated dorsal and ventral limb (Figure 1). Perhaps more surprising was the normal appearance of the granule cell dendritic tree in most cases examined. These findings, along with the presence of normal mossy fiber projections and synapses at the light and electron microscopic level, spoke to the transplant containing all of the relevant cues important for the development of several key aspects of the dentate. Of note, in all transplants we had also included cells that gave rise to a partial regio inferior. Whether these cells are important for the relatively normal development of the dentate in oculo, presumably via the associational connections to the dentate granule cells, is something that could be critically tested by transplanting more discretely dissected regions of the dentate. Some years later we had done a lineage analysis of the dentate in the mouse (Martin et al, 2002), and it would be interesting to know if the clonal organization of the dentate was similar in oculo as in vivo. Another interesting point to examine how the transplanted dentate affects the adult neurogenic population in the sub-granular zone that has been an area of intense investigation in recent times. The in oculo system would offer a setting to explore how various experimental conditions might alter adult neurogenesis.

One of the advantages of the anterior eye chamber as a model system is that multiple transplants can be placed on the iris surface and the interaction between tissues can be examined. In this manner, we explored how the prior presence of a transplanted region that normally innervated the dentate in vivo, the locus coeruleus, septum, or entorhinal cortex, might alter dentate development and provide the dentate with afferent fibers. It was being appreciated in the Olson lab, from work by a student Hakan Björklund, that the cerebral cortex was very responsive to the presence of a juxtaposed locus coeruleus (Björklund et al., 1983). In distinction to the cortex, the growth trajectory and cytoarchitecture of the dentate graft were not significantly altered by mimicking the in vivo situation, even though each co-transplant supplied nerve fibers to the dentate (Figure 2). The catecholaminergic fibers of the locus coeruleus supplied the most impressive innervation of the dentate. Given a joint survival period of a few months and even a limited number of noradrenergic neurons, the locus richly blanketed the dentate with nerve fibers (Figure 3). This was not the case with the two major inputs to the dentate, the septum and the entorhinal cortex. The entorhinal innervation was histotypic (to the outer part of the dentate granule cell receptive field) but extremely limited to just the region where the transplants touched. On the other hand, input from cholinergic neurons of the septum was robust but limited to specific regions of the dentate transplant (Figure 4). This suggested a competitive interaction within the dentate neuropil. One of the major intrinsic fiber systems is the mossy fibers that are the afferents of the dentate granule cells and demonstrable with the Timm's stain for heavy metals. The examination of neighboring sections with acetylcholinesterase histochemistry and Timm's stain revealed an exclusionary relationship between these fiber systems (Figure 4).

This interesting relationship between dentate and septum motivated the examination of how extrinsic inputs to the transplanted dentate might modulate the extrinsic sources of dentate innervation, in particular the hyperinnervation of dentate by the locus coeruleus. To test this, triple transplants were made in the eye between locus coeruleus and dentate and either the entorhinal cortex or septum. The schematic in Figure 5 summarizes the result; while entorhinal cortex had no effect on locus innervation, the presence of a neighboring or intervening septal transplant markedly dampened the noradrenergic innervation of the dentate (Figure 6).

The in oculo system has provided unique glimpses into the regulation of nerve fiber ingrowth into the dentate, although the molecular mechanisms that serve these phenomena are yet to be discovered. More recent work in the Granholm lab has begun to molecular determinants of hippocampal development using the in oculo approach (Willis et al., 2005; Nagy et al., 1998). It would be interesting to explore if the competitive interaction between afferent systems in the transplanted hippocampal formation might underlie the “input dysfunction” that is discussed as a basis for the schizophrenic condition discussed by Freedman in the second part of this chapter. The impressive evidence for the role of the $\alpha 7$ -nicotinic receptor in schizophrenia is intriguing relative to the in oculo findings that the septum’s cholinergic input is a determinant in the afferentation of the hippocampal formation. The in oculo paradigm would appear to have applicability to current investigations into the cellular and molecular determinants of hippocampal development and function.

3.1. Hippocampal Inhibitory Dysfunction in Schizophrenia

Early observations of how people with schizophrenia react to their environment noted a peculiarity in the ability of persons with schizophrenia to appear unaware of the environment and yet overly responsive to it. Eugen Bleuler, who first used the term schizophrenia, also first developed the concept of an attentional dysfunction in schizophrenia (1911). The most dramatic example of the phenomenon of seeming to ignore the environment is catatonia, a rarely seen syndrome in schizophrenia today. The patient gradually stops responding to stimuli and then eventually stops moving altogether. Patients with catatonia have hyperactive electroencephalographic activity, consistent with the minds being quite active, rather than asleep or anesthetized. They respond to barbiturates with a paradoxical “awakening”, in which they resume normal movement. This response suggests that they have actively withdrawn from the world around them, which they sometimes later report was to try to block out the noise around them. When the barbiturate partially inhibits their brain’s responsiveness, they lose their withdrawal and temporarily resume normal interaction. They often report that they were fully aware, indeed acutely hyper-aware, of their surroundings during the catatonia and repeat in detail the conversations that their care-givers had about them.

During World War II, Britain called on experimental psychologists to help improve the ability of plane spotters to identify aircraft quickly and at great distance, when features might be difficult to discern. Donald Broadbent (1958) learned that there were two quite separate aspects to the task. The first was a detection problem, to be able to see the plane when it was just a dot in the clouds. The second was a classification problem, to be able to check identifying features to determine what country the aircraft that made the dot represented. People concentrating on detection were poor at classification and, vice versa, those who concentrated on classification were poor at detecting aircraft early. Broadbent hypothesized that these were two separate features of perception and that people should be trained to do each independently.

Broadbent saw the two different types of tasks as distinct steps in the earliest stages of perception. He reasoned that the part of the brain that makes decisions, such as which plane is which, has limited capacity and that this part of the brain needed mechanisms to prevent itself from being overwhelmed with stimuli. If the brain were always trying to process every stimulus fully, its decision-making capacity would be quickly overwhelmed and greatly slowed. One

problem is how to decide prior to full processing which stimuli are important enough to be processed. Broadbent proposed that the primary filter needed to have a very simple principle. It did not need to eliminate all extraneous stimuli, but it needed to eliminate a substantial fraction of them, with little processing requirement. He proposed that if stimuli could be processed briefly to determine if they were being repeated, then the filter could simply eliminate those that were repetitive. Repeated information would generally have little significance to most goals of the organism, and hence the brain could safely ignore repeated stimuli.

Peter Venables realized that Broadbent's model could be applied to understanding the situation of patients with schizophrenia. They were being flooded by stimuli because of the absence of a fully functional filtering mechanism. He termed their condition an input dysfunction (1964). More commonly, we use the term sensory gating deficit. The control of stimuli reaching the brain's processing centers was lost, as Bleuler had earlier proposed. Patients might then be acutely psychotic and respond to all stimuli, including their internal thoughts, or, more chronically they might withdraw from stimuli, either voluntarily by being isolative or involuntarily through catatonia, to escape this flood of information.

3.2 Sensory Gating Dysfunction and the Hippocampus

We developed an electrophysiological demonstration of the hypothesized sensory gating deficit by presenting two auditory click stimuli to patients and recording their cerebral averaged evoked response using standard EEG technology. The normal response, shown by Subject A in Figure 7, is to have a larger evoked response to the first stimulus than to the second. The amplitude of the P50 wave, a positive wave occurring approximately 50 msec after the stimulus, was used to quantify the response. A lower ratio of the amplitude of the second to the first response indicates that there was significant inhibitory sensory gating. Normal subjects generally have ratios ≤ 0.40 ; most persons with schizophrenia exceed this value (Adler *et al.*, 1982; Freedman *et al.*, 1997).

This deficit in sensory gating schizophrenia has been replicated by Judd *et al.*, 1992; Cadenhead *et al.*, 2000; Thoma *et al.*, 2003; Yee *et al.*, 1998; Hong *et al.*, 2004; Hall *et al.*, 2007; Price *et al.*, 2005; and Louchard-del la Chapelle *et al.*, 2005. A meta-analysis of 45 studies was highly significant (Patterson *et al.*, 2008). Studies that have used the beta EEG filtering parameters that we specified have generally reproduced our findings (Brochhaus-Dumke *et al.*, 2008), whereas those that use other frequencies have not (Hong *et al.*, 2008). One of the advantages of the paradigm is that it is entirely passive and therefore it is amenable to modeling in animals, including those under anesthesia (Bickford-Wimer *et al.*, 1990). The deficit has been modeled in animals by other groups as well (Boutros *et al.*, 1998; Garcia-Rill *et al.*, 2007; Javitt *et al.*, 2000; Radek *et al.*, 2006; Maxwell *et al.*, 2006). The rodent model showed that one of the sites of responses that decremented rapidly to repeated stimuli was the hippocampus. There have been demonstrations of hippocampal hyperactivity in schizophrenia using other imaging paradigms (Malaspina *et al.*, 1999).

3.3. Neurobiology of Hippocampal Alpha 7-Nicotinic Acetylcholine Receptors

Decreased hippocampal response to repeated stimuli in rodents depends upon the input from the midbrain, because lesion of the fimbria-fornix causes the hippocampus to become disinhibited. We studied the effects of a series of cholinergic antagonist on rats with intact innervation to establish whether the cholinergic input from the medial septal nucleus was involved and to identify the receptor type. The experiments identified low affinity nicotinic receptors, antagonized by α -bungarotoxin, as the key receptors necessary for the inhibition of hippocampal evoked response to repeated auditory stimuli (Luntz-Leybman *et al.*, 1992). Alpha-bungarotoxin labels interneurons and these neurons can be co-labeled with antibodies for GABA in the rat hippocampus (Figure 8). The neurons are found in all hippocampal areas

and various representatives can be co-labeled with cholecystokinin, somatostatin, and neuropeptide Y (Freedman *et al.*, 1993). They are thus a subset of hippocampal interneurons in most hippocampal regions. Their prominence in CA3 stratum oriens and the dentate hilus corresponds with the increased cholinergic innervation of this region, but they are also prominent in CA1. In addition to the labeling of cell bodies and processes, there is diffuse labeling in CA2 and CA3 in particular, which may be reflective of labeling on presynaptic terminals. Postsynaptic responses of interneurons to acetylcholine are blocked by receptor antagonists like α -bungarotoxin (Alkondon *et al.*, 1993; Frazier *et al.*, 1998). Thus, the α -bungarotoxin-sensitive receptor emerged as a candidate mechanism for the loss of sensory gating function in schizophrenia. The gene for this receptor was subsequently cloned as *Chrna7* and it was identified as the $\alpha 7$ -nicotinic acetylcholine receptor (Sequela *et al.*, 1993).

Alpha 7-nicotinic receptors form a five-membered homomeric ring that upon activation by agonists admits cations into the cell, including both calcium and sodium ions. The receptors are co-localized with nitric oxide synthetase, and nitric oxide appears to be one of the second messengers activated by these receptors (Figure 9; Adams *et al.*, 1997). Blockade of the receptors increases nerve growth factor expression in the hippocampus, one of several pieces of evidence that the receptors have a role in hippocampal function beyond simple information processing (Figure 10; Freedman *et al.*, 1993).

Chrna7 is polymorphic and has different levels of expression in various inbred mouse strains. DBA/2 mice have diminished levels of $\alpha 7$ -nicotinic receptors, compared to C3H mice. DBA/2 mice also have diminished inhibitory sensory gating in the hippocampus (Figure 11; Stevens *et al.*, 1996). Using polymorphic markers in *Chrna7*, we compared introgression of the C3H and DBA/2 variants into C3H parental strains. As expected, there was no change in expression of $\alpha 7$ -nicotinic receptors or sensory gating function with the introgression of C3H alleles into C3H parental strains. However, the introgression of DBA/2 variants caused loss of sensory gating function and loss of expression of $\alpha 7$ -nicotinic receptors. The receptors were not only diminished, but their distribution in the hippocampus was also changed. C3H mice have their most prominent expression in CA2 and CA3, whereas DBA/2 mice have their most prominent expression in CA1. This pattern was reproduced by introgression of the DBA/2 allele into the C3H parental background (Figure 11; Adams *et al.*, 2001).

Schizophrenic hippocampal $\alpha 7$ -nicotinic receptor-expressing neurons are fewer and have decreased elaboration of neuronal processes. The other markers associated with $\alpha 7$ -nicotinic receptors in rodents are also abnormal in schizophrenia postmortem brain. There is decreased expression of cholecystokinin and somatostatin (Ferrier *et al.*, 1983). Interneurons that contain neuropeptide Y are dystrophic, with diminished extension of processes (Iritani *et al.*, 2000). Interneurons that contain nitric oxide synthetase are diminished in the hippocampus and other cortical structures and appear not to be migrating from the cortical subplate (Akbarian *et al.*, 1993). Other more general markers of interneuron integrity, GABA reuptake sites and synapsin expression, are also diminished (Simpson *et al.*, 1989; Browning *et al.*, 1993; Vawter *et al.*, 2002).

Diminished postmortem expression was also found in the frontal cortex and in the nucleus reticularis thalamis (Court *et al.*, 1999; Guan *et al.*, 1998; Marutle *et al.*, 2001). The nucleus reticularis thalamis does not express prominent $\alpha 7$ -nicotinic receptors in rodents, but it does in humans and other primates, perhaps consistent with the greater role of this nucleus for inhibitory gating of sensory input to the primate neocortex (Breese *et al.*, 1997).

3.4. *CHRNA7* and the Transmission of Schizophrenia

Like many features of schizophrenia, sensory gating dysfunction is co-transmitted in families with the illness (Siegel *et al.*, 1984). Therefore, a genetic linkage study of its heritability was

undertaken in families with multiple cases of schizophrenia. Sites of linkage were found on several chromosomes, including chromosome 15q14. Investigators at the Salk Institute subsequently localized the human *CHRNA7* at 15q14. A dinucleotide repeat sequence was isolated from a yeast artificial chromosome containing the *CHRNA7* sequence, and the linkage was repeated, with a two-point LOD score for sensory gating dysfunction of 5.2, providing strong evidence for genetic linkage at this site (Figure 7; Freedman *et al.*, 1997). The LOD score for schizophrenia itself was also positive, but less significant, because there are fewer cases of schizophrenia in the families. Many relatives have the sensory gating dysfunction but not schizophrenia. The sensory gating dysfunction is thus an example of an endophenotype, a phenotype more closely linked to the gene than the illness itself that is necessary but not sufficient to cause the illness. Because the genetic linkage was performed across the entire genome, there was no presupposition that any particular gene was involved. The convergence at chromosome 15q14 of linkage the gating dysfunction with the location of *CHRNA7*, the gene for a candidate mechanism for gating dysfunction, strengthened the evidence of the involvement of the $\alpha 7$ -nicotinic receptor.

Seven subsequent independent studies have shown that the 15q13 *CHRNA7* locus is also linked or associated with schizophrenia (Kaufmann *et al.*, 1998; Gejman *et al.*, 2001; Liu *et al.*, 2001; Riley *et al.*, 2000; Stassen *et al.*, 2002; Tsuang *et al.*, 2001; Xu *et al.*, 2001). Ethnic groups include Europeans, European-Americans, Bantus, African-Americans, Han Chinese, and Azoreans. The range of phenotypes with which *CHRNA7* is associated also strengthens the finding. Association between *CHRNA7* and P50 sensory gating has been replicated (Raux *et al.*, 2003). The NIMH Consortium on the Genetics of Schizophrenia (COGS) found association between *CHRNA7* and prepulse inhibition (Greenwood *et al.*, 2008), and association has also been found to episodic memory dysfunction in schizophrenia (Dempster *et al.*, 2006). Nevertheless, like all genetic findings in schizophrenia, these findings have not been universally replicated. Association study of 14 candidate genes by the NIMH Molecular Genetics Collaboration using Genome Wide Association (GWAS) standards found none with genome-wide significance. However, the intron 4 *CHRNA7* marker that showed positive signals—rs10438342 G allele, relative risk 1.10, $P = 0.043$ (Sanders *et al.*, 2008)—is similarly positive in another large GWAS study, relative risk 1.09, $P = 0.017$, Stefansson *et al.*, 2008). In the Stefansson *et al.* study and a similar by Stone *et al.* (2008), there were rare individuals with schizophrenia who had deletions of a small region of chromosome 15 that contains *CHRNA7*. Many of these deletions were *de novo*, appearing in the affected individual but not in either parent. These cases also had no family history of schizophrenia. In most individuals with schizophrenia, however, the coding region of the gene is intact and polymorphisms, if any, have been found in the promoter region of the gene (Leonard *et al.*, 2002). Thus, consistent with the postmortem expression, there appears to be diminished expression of a normal receptor.

3.5. Alpha 7 Nicotinic Acetylcholine Receptors as a New Therapeutic Target in Schizophrenia

A straightforward therapeutic strategy to overcome the inhibitory gating deficit is to stimulate $\alpha 7$ -nicotinic receptors. Nicotine transiently activates $\alpha 7$ -nicotinic receptors and transiently enhances P50 sensory gating in schizophrenia (Adler *et al.*, 1993). However, the effect rapidly dissipates before it produces any meaningful clinical effect. A more suitable drug would be an agonist less likely to cause tachyphylaxis, the loss of response to repeated doses. Tachyphylaxis is a classical characteristic of nicotinic receptors and reflects receptor desensitization among other neuronal responses. DMXB-A (3-2,4 dimethoxybenzylidene anabaseine) is a selective $\alpha 7$ -nicotinic receptor agonist (de Fiebre *et al.*, 1995). It has 40% bioavailability in plasma after oral administration and no obvious severe side effects. DMXB-A, administered intragastrically, increased the inhibition in the DBA/2 mouse model of the sensory gating deficit in schizophrenia with less tachyphylaxis than nicotine (Stevens *et al.*, 1998). We completed

stability studies and preclinical toxicology in two species to obtain an FDA Investigational New Drug exemption for its clinical testing and performed a Phase 1 study that showed that it increased P50 inhibition in schizophrenia (Olinicy *et al.*, 2006).

There was significant improvement in the Phase 1 study in the neuropsychological performance of the patients, particularly their ability to pay attention during repetitive tasks, which is one of the purposes of sensory gating—to exclude distraction and thereby protect concentration. In a Phase 2 trial with one month's administration, DMXB-A also improved attention, as well as the negative symptoms alogia and anhedonia, which are often resistant to dopamine receptor antagonist antipsychotic drugs (Freedman *et al.*, 2008).

3.6. An Approach to Developmental Problems in the Hippocampus in Schizophrenia

CHRNA7 has roles beyond adult brain dysfunction in schizophrenia. Expression of $\alpha 7$ -nicotinic acetylcholine receptors begins in neuroblasts and is maximal in both developing rodents and humans, when levels are over four times adult values (Figure 12; Adams *et al.*, 2002; Court *et al.*, 1997). Alpha7-nicotinic acetylcholine receptors are expressed in CA1 near birth at particularly high levels (Adams *et al.*, 2003). Their activation facilitates maturation of glutamate and GABA synapses (Aramakis *et al.*, 2000; Liu *et al.*, 2006). DBA/2 mice not only have diminished $\alpha 7$ -nicotinic acetylcholine receptors, but they also have diminished levels of kainate and AMPA-type glutamate receptors, compared to NMDA-type receptors, a pattern also found in schizophrenia (Zilles *et al.*, 2000; Porter *et al.*, 1997). Thus, the developmental role of $\alpha 7$ -nicotinic acetylcholine receptors suggests an influence on the ultimate functioning of the brain, and its dysfunction in schizophrenia, that may be more important than its role in sensory gating in adult life. Cholinergic innervation develops near birth and therefore during much of brain development, acetylcholine is not available to activate these receptors. However, free choline in high concentrations, as are found in amniotic fluid, can activate $\alpha 7$ -nicotinic acetylcholine receptors (Ilcol *et al.*, 2002; Alkondon *et al.*, 1997; Frazier *et al.*, 1998). Choline has already been shown to be essential for normal hippocampal development (Meck *et al.*, 1989; Albright *et al.*, 1999).

Just as we have stimulated $\alpha 7$ -nicotinic acetylcholine receptors in schizophrenic adults with DMXB-A to improve brain function, it is possible that stimulation of $\alpha 7$ -nicotinic acetylcholine receptors, during gestation may overcome the adverse effects of receptor deficiency on neuronal development. Unfortunately, the endogenous ligand choline is often deficient in pregnant women because of inadequate nutrition, genetic abnormalities in the synthetic enzyme phenethanolamine methyl transferase, or sequestration of choline in the maternal liver because of stress (Gossell-Williams *et al.*, 2005; Liu *et al.*, 2007). We therefore attempted dietary choline supplementation. Figure 13 shows the effects in the DBA/2 mouse model.

Supplementation of mouse dams during pregnancy and nursing resulted in offspring who had improved hippocampal sensory gating in adulthood, compared to those who received normal mouse diets. Neither group received supplementation after weaning (Stevens *et al.*, 2008). Based on these data, the Food and Drug Administration has granted us an Investigational New Drug exemption to assess choline supplementation in a double-blind, placebo controlled trial in humans. Choline, administered as phosphatidylcholine, has already shown positive effects on cognition in the offspring of supplemented women (Zeisel, 2006). The purpose of the test is to determine if it will also ameliorate the sensory gating deficit and other endophenotypes associated with schizophrenia, to decrease the eventual risk of developing the illness.

4. Summary

Hippocampal development and function are a focus for many neurobiologists concerned about the brain dysfunction in human illness. Basic studies at the Karolinska Institute have shown the innervation of the hippocampus by cholinergic, adrenergic, and glutamatergic afferents is

an exquisitely controlled phenomenon. Early intervention in the fetal period to improve brain development and prevent future mental illness is a possible translational outcome of this research.

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Figure 1.

a). Hippocampal formation from a 30-mm crown-to-rump length (CRL) embryo stained with cresyl violet. Boxed-in area approximates the region dissected to be used as the area dentata transplant.. b). An in oculo area dentata transplant from a 30-mm CRL embryo after 7 months in oculo. $\times 12$. c). Cresyl violet stained frozen section of an area dentata transplant from a 28–30 mm CRL embryo.. d). Cresyl-violet stained paraffin section of the area dentata in situ from a 15-day-old rat. . Scale bar in A and B=500 μ m, C and D=250 μ m. From Goldowitz et al., 1982.

Figure 2.

The weekly growth (in mm²) of area dentata transplants from 30–35 mm CRL embryos. Areae dentatae were transplanted to anterior eye chambers that had sham operated (Sham + AD), or had received a septal transplant (Septum + AD), or an entorhinal cortical transplant (ERC + AD). The increase in area dentata transplant size was somewhat less in the double transplant situation than in the single area dentata group. The inset compares the size of the area dentata transplants during the first 5 days after transplantation in a group of single area dentata transplants (solid line) and in a group of double area dentata/entorhinal cortex transplants (broken lines). From Goldowitz et al., 1984a.

Figure 3.

Falck-Hillarp histofluorescence of locus coeruleus/area dentata double transplants. a–c). Ten weeks joint survival. Fluorescent locus coeruleus neurons are shown in a and c with enlarged nuclei. There were approximately 91 noradrenergic neurons in this locus coeruleus transplant. The border between locus coeruleus and area dentata transplants is highlighted in a by arrows. Noradrenergic fibers are sparse in the locus coeruleus transplant and more prevalent in the area dentata transplant. For the most part, these noradrenergic fibers in the area dentata have prominent varicosities with small intervaricose segments (see b). d,e). Four-month joint survival. The locus coeruleus transplant contained approximately 200 noradrenergic neurons. In (d), the crack in the tissue marks the locus coeruleus/area dentata boundary. The noradrenergic innervation of area dentata is very dense. At higher magnification, in (e), the dense noradrenergic innervation of the granule cell region of the area dentata transplant is shown. f). Six months joint survival. This locus coeruleus transplant contained approximately 390 noradrenergic neurons. Noradrenergic hyperinnervation was characteristic of area dentata transplants, with an adjoining locus coeruleus transplant containing about 100 or more noradrenergic neurons with a joint survival of 6 months. Scale bar for a,d,f =50 μ m; b,c,e=25 μ m. From Goldowitz et al., 1984a.

Figure 4.

Double, sequential transplants of septum and area dentata. a). In oculo appearance of the small septal transplant (left) joined with the larger area dentata transplant (right). Scale bar=500 μ m. b). Septal/area dentata transplants processed for AChE-histochemistry 7 months after joint survival. The septal transplant is on the left. Arrows point to the AChE-rich region occupied by cholinergic cells. The adjacent area dentata transplant has both a zone rich in AChE-positive fibers (arrowheads) and a zone poor in AChE-positive fibers. c,d). Septal/area dentata transplants taken for AChE and Timm's histochemistry 8 months after joint survival. Dense AChE-positive material is present in the septal transplant in (c), and AChE-positive fibers are prevalent in two regions of the area dentate transplant (arrowheads). These two regions in an adjacent section stained for Timm's method (d) lack the typical mossy fiber staining that is found elsewhere in the area dentata transplant (arrowheads, Timm's positive mossy-fiber-like staining). e,f). Similar comparison as in (c) and (d) from another double transplant taken for AChE and Timm's histochemistry after 8 months joint survival. The area dentata region most proximal to the AChE-positive rich septal transplant (arrows) in (e) receives a modest amount of AChE-positive fibers (arrowheads), while the distal region receives very few AChE-positive fibers. It is this AChE-poor zone in the area dentata transplant that has extensive mossy fiber-like staining with the Timm's procedure in (f). Area dentata proximal to the septal transplant has very little mossy fiber-like staining although there are many pyramidal cell targets. Scale bar for b-f=50 μ m. From Goldowitz et al., 1984a.

Figure 5.

Schematic representation of the four types of intraocular triple transplantation combinations are shown in b–e (Goldowitz et al., 1984b) compared to the double transplant shown earlier (Goldowitz et al., 1984a). AD = area dentata, ERC = entorhinal cortex, S = septum, LC = locus coeruleus. The density of LC-derived noradrenergic innervation of AD is schematically depicted by the density of cross-hatching (i.e., the denser the hatching the denser the noradrenergic innervation of the area dentata transplant). Note that the noradrenergic innervation in other transplants than AD is not shown. The order of grafting in (a) is AD, then LC, and in (b–e) ERC or S, then AD, then LC. From Goldowitz et al., 1984b.

Figure 6.

Triple transplant with septal nuclei (S) intervening between area dentata (AD) and locus coeruleus (LC) transplants. a) The granule cell layer (arrowheads) and scattered pyramidal and polymorphic cells in the area dentata transplant are evident in this section stained with cresyl violet. The crack in the section passes through the septal transplant. At either end of the transplants is iris tissue. b) The fluorescent noradrenergic fibers are shown in this photomontage taken from a section adjacent to (a). In the center of the septal neuropil are the “clubs” or “swellings” (arrowheads) of histofluorescent axons that are characteristic of this triple transplant configuration, but are unusual in the other triple transplant configurations that were examined. The area dentata also contained such swellings (arrows). An unusual feature of this transplant configuration was the greater density of histofluorescent axons in the locus coeruleus compared to the area dentata. The histofluorescent axons at the edge of the septal transplant appeared to be destined for the area dentata neuropil. Other noradrenergic fibers reached the area dentata via the iris. c) This photomicrograph shows some of the approximately 288 fluorescent neurons present in the locus coeruleus transplant. The septal bridge is relatively fiber-free and the moderately innervated area dentate is shown to the left. d) The density of fluorescent noradrenergic fibers in the granule cell region of area dentata is shown at higher magnification in this photomicrograph. Calibration bars: a) 0.5 mm; b–d) 100 μ m. From Goldowitz et al., 1984b.

Figure 7. The role of $\alpha 7$ nAChRs and *CHRNA7* in auditory sensory gating and schizophrenia

A pedigree with two schizophrenic siblings (black symbols). Sibling A has a P50 auditory evoked response (positive polarity down) to the first of paired stimuli and normal inhibition of response to the second stimulus. Sibling B, who has schizophrenia, lacks this inhibitory sensory gating function and has a large response to the second sound, compared to Sibling A. Sibling C, who is unaffected, shares Sibling B's electrophysiological trait. Abnormal sensory gating (A) segregates with the 2 allele of D15S1360, which is in intron 2 of *CHRNA7*. Normal gating (N) segregates with the 1 allele. From Freedman *et al.*, 1997.

The P50 was selected and its amplitude measured by a computer algorithm that identified the largest positive wave between 40 and 70 ms following the initial stimulus. The wave following the second stimulus was selected to be within ± 10 ms of the latency of the first wave. This criterion was derived from hippocampal single neuron recordings in laboratory animals, where the timing of neuronal discharge to each stimulus could be rigorously determined. From Freedman *et al.*, 1996). Hence, the earlier wave in the response to the second stimulus in Sibling A is not a P50 response to this stimulus.

From Freedman *et al.*, 1997

Figure 8.

Co-localization of ^{125}I - α -bungarotoxin labeling of $\alpha 7$ -nicotinic acetylcholine receptors (right panels) and GABA-immunocytochemical reactivity as detected by fluorescence histochemistry (left panels) on rat hippocampal interneurons. For all panels but b, the fluorescence histochemistry was performed on perfused and fixed brain sections, followed by ^{125}I - α -bungarotoxin binding. For b, the ^{125}I - α -bungarotoxin was injected intraventricularly, and then the brain was perfused, fixed, and sectioned. Panels a and b show large neurons (arrowheads) that contain GABA and also bind ^{125}I - α -bungarotoxin, located in the stratum oriens of CA3. Similar CA3 neurons are shown in stratum oriens and stratum lucidum in d. c shows a series of smaller neurons on the inner layer of the distal portion of the dorsal blade of the dentate gyrus. e shows several small neurons in CA1, in striatum radiatum, and in stratum lacunosum moleculare. Scale bar: 50 μm for a–d, 100 μm for e. From Freedman *et al.*, 1993.

Figure 9.

Co-localization of $\alpha 7$ -nicotinic acetylcholine receptors and nitric oxide synthetase in rat hippocampal interneurons. Photomicrographs of interneurons in stratum oriens of area CA1 which were first found to be immunoreactive for nitric oxide synthetase (indicated with white arrowheads in A, B, C) and then subsequently found to also bind α -bungarotoxin (indicated with black arrowheads in the alternate A, B and C frames). The small black or white arrows indicate blood vessels visible in associated pairs of photomicrographs (e.g. A and A, B and B, C and C). Calibration bar=48 μ m. From Adams *et al.*, 1997.

Figure 10.

Blockade of $\alpha 7$ -nicotinic acetylcholine receptors activates nerve growth factor gene transcription. In situ hybridization using a probe for Nerve Growth Factor mRNA in rat hippocampus, 3 hr after intraventricular administration of α -bungarotoxin (1 pg in 10 μ L; panel a) or an equal volume of Ringer's solution (b). The animal treated with α -bungarotoxin shows prominent labeling in the dentate. The animal treated with Ringer's shows no labeling in the dentate, with scattered cells labeled in the hilus. Scale bar for A and B=100 μ m. From Freedman *et al.*, 1993.

Figure 11.

DBA/2 mice have abnormal inhibitory sensory gating and expression of hippocampal $\alpha 7$ -nicotinic acetylcholine receptors, linked to the *Chrna7* locus. Left panel. C3H mice have hippocampal evoked responses to repeated auditory stimuli that resemble those of normal humans, whereas DBA-2 mice have responses similar to persons with schizophrenia. Right panel. Photomicrographs of ^{125}I - α -bungarotoxin labeling of transverse sections of hippocampus. Panel (A) illustrates the pattern of ^{125}I - α -bungarotoxin labeling characteristic of parental C3H mice, showing that C3H mice have prominent labeling at the CA3/CA2 border (arrowheads). The photomicrograph in (B) is from a backcrossed strain which has a C3H *Chrna7* marker introgressed into a C3H genetic background; the characteristic C3H α -bungarotoxin binding phenotype is preserved. At higher power (C, D), the α -bungarotoxin-positive neurons embedded in the dense, diffuse binding can be more easily discerned (arrows). DBA/2 mice (E) lack this diffuse labeling, but instead display numerous α -bungarotoxin-positive neurons in CA1 lacunosum-moleculare (arrows). Introgression of the DBA/2 *Chrna7* marker into a C3H background to produce a second backcrossed strain results in a pattern of α -bungarotoxin binding characteristic of the DBA/2 phenotype (F). Panels G and H illustrate the area CA1 lacunosum-moleculare α -bungarotoxin-positive neurons (arrows) at a higher power. These mice also have abnormal inhibitory sensory gating like the DBA/2 parental strain. Scale bars (A, B, E, F)=125 μm ; (C, D, G, H)=50 μm . Modified from Adams *et al.*, 2001.

Figure 12.

Alpha7-nicotinic acetylcholine receptors are maximally expressed in the perinatal period. Graph shows the levels of ^{125}I - α -bungarotoxin binding in the hippocampus of the developing rat from just prior to birth to adulthood (postnatal day 60). From Adams *et al.*, 2002.

Figure 13.

Effects of perinatal choline supplementation on the development of sensory gating. DBA/2 mice have diminished expression of $\alpha 7$ -nicotinic cholinergic receptors, due to *Chrna7* polymorphisms, and also have hippocampal sensory gating deficits that resemble the P50 gating deficits in schizophrenia. Choline was supplemented at twice the normal dietary level in the maternal diet from conception through weaning. Thereafter, both groups of mice received diets with normal choline levels. Each point is the ratio of amplitude of the hippocampal auditory evoked responses to paired stimuli, amplitude of the second response divided by the first. In adulthood, the offspring had decreased amplitude ratios, indicative of improved sensory gating. Data reported in Stevens *et al.*, 2008.