An infection model of the neurocompromised brain in psychiatric disorders.

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Summary: *At least one in three humans has been infected by and is harboring the protozoan parasite Toxoplasma in their brain tissue. Toxoplasma has refined its parasitic strategy during evolution to intricately balance its auxotrophic needs and the massive remodeling of the infected host cell, yet remain under the immunological radar and not kill the abundant human hosts. It is virulent only when the human host is immunocompromised. A hypothesis is forwarded here that Toxoplasma is pathological also when the host is neurocompromised, meaning that the brain is genetically predisposed for neurodevelopmental and neurodegenerative diseases. This widespread parasite is a prime candidate as a significant environmental factor to the onset of schizophrenia, idiopathic epilepsy and Alzheimer's Disease.*

In his essay "Bugs in the Brain" (Sapolsky, 2003), the neuroendocrinologist and primatologist Robert Sapolsky marvels at a pond containing swimming protozoans and reflects on the protozoan parasite *Toxoplasma* for its ability to manipulate the mammalian brain. The "behavioral manipulation" hypothesis in parasitology states that a parasite can alter its host behavior specifically to increase its own transmission efficiency (Webster, 2007). *Toxoplasma* is arguably the most advanced and successful parasitic protozoan, capable of infecting all nucleated cells and is widespread in most animals: mammals, birds and fishes (Flegr, 2007). However, it can only undergo sexual reproduction in the intestine of cats, hence when infected with this parasite, rodents reverse their aversion to cat's odor to a suicidal attraction and facilitate their own predation. In doing so, *Toxoplasma* also compromises the circuitry of the learned fear and anxiety response in the infected rodent brain (Vyas and Sapolsky, unpublished data).

Toxoplasmosis is a continuous human condition, since infection can occur at any stage of your lifetime, from that of a fetus in the womb, through your childhood, adulthood, and into your aging years (Figure 1, 5A). At least one out of three humans on earth has been infected with this "refined" protozoan and is most likely carrying *Toxoplasma* cysts and bradyzoites, embedded in their muscle or brain tissues. Unlike the more notorious protozoan parasites – *Plasmodium*, *Trypanosoma* and *Leishmania* - which wreak endemic havoc, lowering human life expectancies and stifling economic development, in the underdeveloped regions of the world, *Toxoplasma* has evolved to live with minimal collateral damage and under the immunological radar for the duration of the lifespan of the human host. It is pathological under only two human conditions of vulnerability; being immunocompromised and/or being "neurocompromised". The first concept is well established and the second is being introduced.

Toxoplasma is so successful as a parasite that in spite of its common distribution, it was not until the early years of the AIDS epidemic that it was brought to the attention of the biomedical community. Destruction of CD4+ T cells by HIV reactivates the cryptic bradyzoites/cysts and its active proliferation is the most common cause of intracranial mass lesions in AIDS patients (Skiest, 2002). Direct infection of the fetus in the first and second trimester is either lethal or results in significant defects in brain development, such as enlargement of the third and lateral ventricles, hydrocephaly, microcephaly and intracranial calcifications, frequently of the basal ganglia, choroid plexus, and meninges (Rorman et al., 2006). With brain lesion and gross birth defects as the smoking guns, it is indisputable that

Toxoplasma is pathological in immunocompromised hosts. This realization launched several decades of research of our understanding of *Toxoplasma* in immunology, cellular and molecular biology. With the exception of on-going studies in microglial activation, the neurobiology of *Toxoplasma* infection remains relatively unexplored, resisted mainly by the parasitology community, insisting that the latent infection is subclinical and inconsequential. The latent parasites are viewed as out of sight - albeit in the brain - and out of mind. Is it ?

A growing body of serological studies are implicating that chronic toxoplasmosis is associated with complex brain illnesses such as cryptogenic/idiopathic epilepsy (Stommel et al., 2001; Yazar et al., 2003) and schizophrenia, in which a team of psychiatrists, led by E. Fuller Torrey and Robert Yolken at the Stanley Medical Research Institute, have championed the *Toxoplasma* and viral infection model of schizophrenia (Torrey & Yolken, 2003; Mortensen et al., 2007) and bipolar disorder. Peripheral to this central hypothesis, additional serological studies are also suggestive that *Toxoplasma* infection is also associated with risk taking behavior, lower IQs, antisocial behaviors in men, promiscuity in women and slower reaction times, leading to an increase in car accidents (Flegr, 2007). This emerging field, unfortunately, has generated much media buzz but lacks supporting evidence in neurodevelopment, neurophysiology and neuropharmacology. Hence, there is not a comprehensive mechanistic and circuitry model. One pharmacological report in 1985 of dopamine being elevated in the infected mouse brain and a second of dopamine antagonists reversing the rodent fear behavior have served as the staple for association with complex behaviors and mental illness syndromes (reviewed in Webster, 2007).

Schizophrenia is a severe, debilitating mental disorder characterized by profound disturbances of cognition, emotion and social functioning. The lifetime morbid risk is surprisingly uniform at slightly less than 1% across different populations and different cultures. The most troubling aspect of the infection hypothesis for schizophrenia is that it is one-dimensional, focusing solely on the epidemiological data of only one environmental factor and completely ignoring the genetic components of psychosis, which can account for at least 50% concordance among monozygotic twins. The converse is also true of the schizophrenia genetic studies, which commonly examine cause and effect of gene and locus variants without taking also into consideration any of the environmental factors. Linkage studies have suggested that multiple candidate chromosomal regions may harbor genes associated with schizophrenia and schizoaffective spectrum, although with variable and inconsistent results.. Numerous high interest candidate genes (dysbindin, DISC1, neuregulin, G72, COMT, PROD, RGS4, GRM3, etc..) have been analyzed from these chromosomal regions, but they all have only a small effect or a weak association with schizophrenia (Harrison & Weinberger, 2005).

There are no published studies suggesting a connection between *Toxoplasma* and the onset of Alzheimer's Disease (AD), but a sharp increase in *Toxoplasma* infection at age 50-60 (Fig. 5A) strikingly coincides with the onset of Alzheimer's Disease. Alzheimer inflicts an estimated 4.5 million Americans and and 24 million people worldwide with predictions that these figures will rise rapidly to 14.3 million Americans and 81 million worldwide. As the age expectancy in developed nations rises, accumulating effects of parasitic burden compounded with the effects of aging can possibly lower this biological threshold of pathology, especially in those with predisposition for familial or sporadic Alzheimer's Disease. Alzheimer's disease (AD) is an age-related neurodegenerative disorder that is characterized by a progressive loss in memory and deterioration of the higher cognitive functions. The brain of an individual with AD exhibits extracellular senile plaques of aggregated amyloid-beta-peptide (Abeta), intracellular neurofibrillary tangles (NFTs) that consist of hyperphosphorylated tau protein (P-tau) and a profound loss of basal forebrain cholinergic neurons that innervate the hippocampus and the neocortex. *Toxoplasma* infection of ApoE-deficient mice shows a lower plasma level of cholesterol and an increase in atherosclerotic lesions (Portugal et al., 2004), but its effects on the brain tissue and the possible amyloid deposits have not been examined.

Hypothesis 1. *I propose that chronic infection with common Toxoplasma can generate neuropathology only in the "neurocompromised" brain, meaning the population with genetic predisposition to brain illness such as schizophrenia, epilepsy, bipolar affective disorder and Alzheimer's disease. This in theory should not exclude nongenetic factors as cause of susceptible brains, such as physical injuries, strokes, hypoxia and other acute brain infections. Since Toxoplasma has evolved to survive in harmony within the human brain, disruptive factors offsetting this equilibrium between the host and parasite can be detrimental if pushed beyond a biological threshold, resulting in neuropathology.*

This hypothesis should not interpreted in terms of a simple variable, e.g. if a developing brain from a human or mouse with a DISC1 translocation when infected with *Toxoplasma* will generate schizophrenia symptoms of synaptic disconnectivity between the frontal cortex, temporal cortex and the anterior thalamus. The neurocompromised brain can be defined from either models of 'common disease – common alleles' or 'common disease – rare allele' (McClellan et al., 2007). The former indicates that the disease results from the additive impact of multiple common small effects, genetic variants that exceed a biological threshold. The later model explains that the common and complex disease, such as schizophrenia and Alzheimer's disease, results from mutations that are highly penetrant and rare, perhaps even specific to patients or families. These two models can be mutually inclusive (McClellan et al., 2007). For an example, a frameshift mutation of DISC1 that expresses a truncated protein has been found to be cosegregated in schizophrenic siblings over four generations of a Scottish kindred. This is a rare large effect allele because Green and coworkers (2006) have not found this mutation in any of 655 schizophrenia cases but have found the translocation in two of 694 non-psychiatrically screened controls. Expanded association studies of DISC1 have identified other common haplotypes of DISC1 that were associated with schizophrenia and other mental illness in European and North American populations, but not in Japanese or Scottish populations. DISC1 transcript is downregulated (0.74 fold) in fibroblast culture containing *Toxoplasma* infected cells (Fouts and Boothroyd, unpublished data). *Toxoplasma* infection can potentially have a significant influence in at least 1:3 humans in these association studies, perhaps contributing to an additive effect on some DISC1 haplotypes, particularly in combination with subsets of additional susceptible genes.

To establish candidate genes that constitute the neurocompromised brain for psychosis and dementia, I have first taken a global approach of assimilating a master list of host genes (~650) (Table 1) that have been reported to be altered by *Toxoplasma* infection in gene chip studies of diverse cell types (Figure 2-3), including an unpublished list of altered gene expression from the complete mouse brain that has been chronically infected with *Toxoplasma* for over a year (R. McLeod, personal communication). Second, this list of *Toxoplasma* regulated genes (TRG) is compared to databases of susceptible candidate genes for schizophrenia/ schizoaffective disorder and Alzheimer's Disease that are accessible at NCBI, Alzheimer Research Forum (AlzGene Database) and Schizophrenia Research Forum (SZGene Database) (Figure 5D-E, 15). Third, TRGs are further cataloged into seven different functional compartments that are manipulable by the parasite, including (1) host secreted factors and receptors, (2) host metabolism, (3) host extracellular matrix and adhesion, (4) host cytoplasmic signaling cascades, (5) host cytoskeleton, (6) host nucleus, and (7) host cytokine, antigen processing and presentation (Table 2). Fourth, to determine whether these genes are expressed in the brain and their regional localization, in situ hybridization data of the adult mouse brain (P56, male) have been collected from the Allen Brain Atlas. Most of these ~650 TRGs are expressed in the adult mouse brain. Figure 5D-E summarize the candidate genes that define the neurocompromised brain and are the potential trigger points by *Toxoplasma* in developing psychosis or neurodegeneration.

Several footnotes should be highlighted. The TRG list is incomplete, consolidating only on results from the microarray studies and has not included reports in the published literature.

The TRG list is nonspecific, including studies using tachyzoite, bradyzoite, and all three strains of *Toxoplasma*. There is, however, not a significant difference between host gene response to a tachyzoite or a bradyzoite infection (Fouts et al., 2006). In situ hybridization data of the adult mouse brain have been collected and analyzed, but data from the embryonic and early postnatal stages in mouse that are available in other databases have not been included: GENSAT at Rockefeller University (green, Figure 5E), GenePaint at Max-Planck Institute in Hannover, and eMAP at the University of Edinburg. The list of SZ susceptible genes is also incomplete since the SZGene database is still under construction by Dr. Lars Bertram.

To understand the parasitic consequences at the cellular level, a simplified map of signaling pathways that are important in neurodevelopment and neurotransmission is constructed (Figure 4). TIGs are mapped to most of these pathways, but *Toxoplasma* appears to have preference in regulating the host organism at the levels of nuclear transcription and cell surface signaling, hence it is able to control its own host cell as well as neighboring brain cells (Fig. 4B-C). ApoE, LDL, LRP, Notch and Wnt signaling pathways are heavily clustered with TIGs. These pathways are particularly important to the pathology of Alzheimer's (10B) and in a lesser degree also to schizophrenia. Albeit the TRG list is incomplete and requiring direct experimental confirmation (see proposed research), it is quite astonishing to see the potential capability of *Toxoplasma* to manipulate the human brain from molecular, to cellular, to the organ level. Even more impressive is that its latent infection does not generate more catastrophic consequences to the mammalian brain, illustrating a refined control by the protozoan invader.

No matter how refined it is, *Toxoplasma* is a protozoan and hence an auxotroph, lacking the metabolic machineries to synthesize purines, selected amino acids (e.g. tryptophan) and sterols. *Toxoplasma* is an obligate intracellular parasite, being capable of vegetative growth and proliferation only inside a host cell, where it can salvage and divert nutrients from the host cytoplasm (Figure 6-8). Even if the parasites carry within their genome the required metabolic enzymes, they still take advantage of the readily available pool of precursors from the host cytoplasm, e.g. by importing folates. Table 3 identifies the TRGs that are essential to the metabolism of neurotransmitters. Metabolic parasitism is a significant risk to the host when the parasites are harbored in the brain, because metabolism of neurotransmitters is critical to neuro-neurotransmission and glia-neurotransmission. These serve as fundamental basics to circuitry connectivities that drive neurocognition.

Models are constructed to demonstrate the broader consequences of having intracellular parasites acting as a metabolic drain in brain cells (Figure 6-8). Whether at a slow or rapid rate, a parasitic draining in cellular pools of arachidonic acid (Figure 6B), tyrosine and tryptophan (Figure 6C), methionine and cysteine (Figure 6C), glutamate and glutamine (Figure 7B) and purines (Figure 6E) can significantly alter neurotransmission, neurodevelopment and host behaviors. The draining of host cholesterol (Coppens et al., 2006) is particularly significant within the intricate balance of sterol signaling involving the ApoE/LRP/LDL, Notch, Wnt and presenilin signaling pathways (Figure 8A); these processes are pivotal to embryonic and adult neurogenesis and the taupathology leading to plaque formation in neurodegeneration (Grilli et al., 2003).

Hypothesis 2. *Consistent with the neurodevelopment theory for schizophrenia, I propose that Toxoplasma infection, at either the late embryonic or postnatal stage of the schizophrenic neurocompromised brain, increases the risk of developing schizophrenia and its onset during the adolescence.*

Even with the vast amount of literature and the many hypotheses for the etiology of schizophrenia, the sizeable list of *Toxoplasma* regulable genes can provide correlations for most of these hypotheses. Only a few are discussed here.

Congenital toxoplasmosis, the direct infection of the fetus in the first or second trimester, produces the more extreme cortical development defects, such as microcephaly, hydrocephaly, abnormal development of the ventricles and intracranial calcification (Rorman et al., 2006). These are symptoms of probable defects in the migration, positioning and connectivity of neuronal progenitors. Two interesting examples are the down regulation by *Toxoplasma* of PAFAH1B1/LIS1 and DAB1. Both, when mutated, are the causes of different class I lissencephalopathies, brain developmental diseases which are characterized by decreased complexity of cortical folds, or gyri (Feng & Walsh, 2001; Olson & Walsh, 2002). PAFAH1B1 is involved in the regulation of microtubules by interactions with dynein and nudel (Figure 4). When PAFAH1B1 is mutated, differentiated neurons are produced, but many are mislocalized (Figure 7B-C), typically in a broad ectopic lamina internal to the normal cortical layers. In the second case, DAB1 is a key cytoplasmic adaptor that docks to the NPXY cytoplasmic motifs of receptors (APOER, VLDLR, Notch), in which the DAB1 knock out mouse has a similar phenotype as the *reeler* mouse; these mice develop a reduced and inverted neocortex with cerebellar hypoplasia. Reelin is a large glycoprotein secreted by Cajal–Retzius cell during embryonic cortical development in the cortex, hippocampus and dentate gyrus. Reelin and DAB1 are required for the splitting of the preplate and the proper positioning of the neuronal cortical layers in the cortical plate.

Morphometric imaging studies of schizophrenic brains commonly report three structural abnormalities: enlargement of the lateral and third ventricles and the reduction in gray matter and the hippocampus. Lateral and third ventricle enlargement has also been reported in congenital toxoplasmosis, albeit brain structural studies of congenital and adult toxoplasmosis are sparse and rudimentary. Ventricular enlargement has been associated with polymorphism of the IL-1RN and IL1B gene (Papiol et al. 2005), in which IL1B is a TIG and a candidate susceptibility gene for both SZ and AD.

In a study of early onset schizophrenics that progress throughout adolescence, dynamic tracking over a period of 5 years reveals waves in gray matter loss that travels anteriorly from the parietal region into the temporal lobes, supplementary motor cortices, and frontal eye fields. This progression correlates functionally with the expected sequential deficits after the first-episode of psychosis in language and associative thinking, neuromotor, sensory and visual search (Thompson et al. 2004). Variants of RGS4 impact the functional connectivity during working memory tasks and can gradually induce the reduction of white and gray matter in specific activated regions (Prasad et al., 2005). RGS4 is a G-protein coupled modulator for metabotrophic glutamate, serotonin, dopamine and neuregulin receptors. It is a candidate for TRG, SZ and AD.

Hippocampal atrophy is also a pathology of multiple psychiatric diseases, including schizophrenia, epilepsy and Alzheimer (Figure 11). Hippocampal volume reduction has also been associated with the BDNF Val66Met allele (Bueller et al., 2006) in humans susceptible to chronic depression. FGFR1 and FGF2 are critical to the development and size of the hippocampus (Fagan et al., 1997). Volume reduction of this critical node in most brain circuitries is also associated with the deletion of the 22q11.1 chromosomal fragment, a

candidate region for increasing the risk of schizophrenia (Debbane et al., 2006). COMT, PRODH, NOGOR and CECR1 are located in this region, in which the first three are being rigorously examined as schizophrenia susceptible genes. BDNF, FGFR1, COMT and CERC1 are *Toxoplasma* regulable genes.

Hypothesis 3. *I propose that chronic infection of Toxoplasma in early and late adulthood affects adult neurogenesis in the dentate gyrus, and induces hippocampal atrophy*.

Toxoplasma infection can disrupt embryonic neurogenesis and cortical development, suggesting that adult neurogenesis can be affected by similar mechanisms. Neurogenesis in the adult brain is restricted to the subventricular zone in the olfactory region and the dentate gyrus in the hippocampus (Figure 11). The hippocampus is of high interests in examining the damages by *Toxoplasma* chronic infection due to the parasitic association with crytogenic epilepsy, schizophrenia and the current proposed link to Alzheimer's diseases.

With a proposed viral infectious etiology, temporal lobe is the most common form of seizure in which epileptogenesis orginates from the hippocampus, amygdala and parahippocampal gyrus. The dentate gyrus regulates transmission between the entorhinal cortex and the hippocampus, gating hippocampal excitability (Figure 11). Both hilar cell loss and mossy fiber sprouting in the dentate gyrus of the hippocampus have been associated with the development of epilepsy in experimental seizure models and in patients with temporal lobe epilepsy (Statler, 2006). Generalized traumatic brain injury during childhood can develop post-traumatic epileptogenesis that is centralized to the dentate gyrus, in which recurrent seizure will increase neurogenesis. *Toxoplasma* infection can possibly induce epilepsy and alter neurogenesis by a similar mechanism. Relevant to the etiology and physiology of epilepsy (Ben-Ari, 2006; Gurnett & Hedera, 2007), a number of ion channels are regulable by *Toxoplasma* and expressed in the hippocampus (Table 2, Catalog 1)

The hippocampus has long been implicated in the pathology of schizophrenia, based on multiple lines of evidence of neuroanatomical, neurodevelopmental, neurophysiological and genetic abnormalities. Disconnectivity of the hippocampal and prefrontal cortex circuitry is central to the cognitive dysfunction in psychosis. By neonatal lesioning of the ventral hippocampus, rodent and nonhuman primate models exhibiting neurobiological and behavioural features of schizophrenia have been generated; these mice are shown to have disconnectivity in development and plasticity of prefrontal cortical and hippocampal circuitry by (Lipska 2004). The prominent model of psychosis is a deficit in executive functions, a cluster of cognitive brain functions involved in attention, planning, sequencing, decision making, initiating, and inhibiting behaviors which are associated with the prefrontal cortex (Goldman-Rakic, 1994). The alternative view is that long-term memory, specifically the encoding and contextual binding memory processes associated with the hippocampus and entorhinal cortex, is an equally, if not more salient, feature of the impaired cognitive profile of schizophrenia (Boyer et al., 2007). Either way, neurogenesis from the dentate gyrus possibly plays a crucial role in the earlier wiring and the continued development of network plasticity.

Interactions between the dorsal hippocampus and the lateral nucleus of the amygdala control the development of cognitive representations of contexts during Pavlovian fear conditioning (Bouton et al. 2006). Hippocampal damage in an infected mouse is a possible mechanism through which *Toxoplasma* can blunt the learned fear and anxiety response to cats.

Sex hormones are implicated in notable developmental differences between the hippocampal structure and function between males and females, including size, symmetry, the number of granule cell neurons in the dentate gyrus, and dendritic branching in the CA3 pyramidal cells (MacLusky et al. 2005). Androgens are important in controlling the number of dendritic

spines synapses and the responses to stress on CA1 pyramidal neurons. Onset of schizophrenia is earlier in boys than girls by at least 2 years and females are much more susceptible to Alzheimer's disease. It is implied, but not directly measured, that the testostorone level is higher in *Toxoplasma* infected humans, supported by a lower second- to fourth-digit length ratio, greater body height in men, longer duration of pregnancy, and higher sex ratio of more male births (Flegr, 2007). The androgen receptor (AR) is upregulated by *Toxoplasma* and it is expressed in the dentate gyrus of mice.

The hippocampus and entorhinal cortex are the first regions of the brain to suffer damage in the Alzheimer's diseased brain, marked by pyramidal cell loss and disruption of the perforant path connections, hence the insult is the cause of memory problems and disorientation. Accumulation of A-beta amyloid has been found to markedly impair hippocampal long-term potentiation (LTP), a widely studied cellular model of synaptic plasticity that is thought to underlie learning and memory. Natural aging also suppresses hippocampal neurogenesis, which is required for some types of hippocampal-dependent learning. The multiple lines of abnormalities, proposed to be generated by chronic and episodic reactivated toxoplasmosis in the brain, have provided compelling rationales to examine the parasitic effects on hippocampal neurogenesis and the Alzheimer's neurocompromised brain.

Proposed research (Year 1-5)

The hypothesis of *Toxoplasma* effects on the neurocompromised brain has been painted with a broad and imprecise stroke, based on microarray analyses. Although microarray studies are prone to high false positive and false negative rates, the list of TRGs provides a global perspective and a springboard in constructing cause and effect models to be tested. I propose to examine, foremost in the adult mouse brain, the parasitic effects on neurogenesis and neurodevelopment of the dentate gyrus (**Proposal 1**) and then to back track to the neonate and early postnatal developmental periods (**Proposal 2**). The adult stage is selected first because any evidence of *Toxoplasma* altering adult neurogenesis can be directly associated with all 3 diseases: Alzheimer's, epilepsy and schizophrenia. More importantly, restoring hippocampal neurogenesis is already feasible with the success of some drugs being targeted for mood disorders, with more still under development. Adult neurogenesis also provides the promise of providing a mechanism for immediate repair of the neurological damages existing in afflicted individuals.

For proposal 1, microarray analysis using statistically defined criteria for reliability/ significance (Blalock et al. 2005) will be used to analyze the dentate gyrus gene expression profile that will be altered in the adolescent and aged mouse; those that will be infected with type III strain *Toxoplasma* at embryonic day 10 and P0. The gene list will be scored for susceptible genes to define the possible interface between *Toxoplasma* effects on neurogenesis in the schizophrenic, epileptic and Alzheimer neurocompromised brains. Candidate genes will be confirmed by RNA blots and in situ hybridization of brain slices, using primers already defined by the Allen Brain Atlas. Protein levels will be analyzed by biochemical blotting using available antibodies. Biochemical and microscopical confirmation will also include the *in vitro* infection of organotypic hippocampal brain slices obtained from uninfected mice; slices will be infected with cysts/bradyzoites that are obtained from infected mouse brain. Candidate genes will be screened against the GENSAT mouse resource at Rockefeller University to identify available transgenic GFP reporter mouse strains. A higher resolution morphological and biochemical analysis of gene specific changes due to parasitic infection will be mapped.

For proposal 2, a similar strategy is followed to examine parasite-induced gene profile at the ventricular zones of the developing embryo at later gestation and immediately after birth. The GFP reporter mice will allow the gene-specific mapping of abnormalities in cortical development, specifically for any abnormalities in the construction of the hippocampal fomation.

If time and resources will be permitted, electrophysiological recording of infected hippocampal brain slices, and/or in situ physiological recording of the prefrontal cortex of infected animals will provide additional confirmation, particularly when coupled with cognitive tasks, such as working and episodic memory. Applications of these routine neurobiological techniques and resources will be groundbreaking for the parasitology field, especially for understanding the pathology and cures for neurotrophic protozoan parasites causing toxoplasmosis, cerebral malaria and African sleeping sickness.

Long Range Goals (Year 6-15)

With a list of candidate genes that when exposed to latent infection of Toxoplasma can increase the risks of developing schizophrenia and Alzheimer from the mouse model, we can confirm the links to human neuropathologies. For example, polymorphism of a specific subset of candidate genes can be investigated in the population of schizophrenics with seropositivity for Toxoplasma. A similar strategy can be expanded to examine familial and sporadic Alzheimer's patients.

The Toxoplasma infection hypothesis is particularly attractive to the prevention of psychosis and dementia for several practical reasons. A sound public health strategy can be effective to prevent progression of pathology in susceptible individuals, as by teaching them to avoid eating unwashed fruit and undercooked meat. A vaccine for humans and household cats are being developed against the unique surface proteins (SAGs) and secretory proteins (ROPs, MICs, GRAs) of the parasite. Chemotherapeutic approaches are being targeted against the unique auxotrophic properties of the protozoan, particularly compounds that cross the blood-brain barrier to kill the resistant brain bradyzoites.

I anticipate that the human and mouse virulence loci for Toxoplasma will be mapped by the laboratories of David Sibley (Washington U.) and John Boothroyd (Stanford University) within the next 5 years. The generation of avirulent strains will provide a powerful eukaryotic and neurotrophic vector in providing gene therapy to neurological diseases, particularly for those specialists with a working knowledge of parasites and who already have developed mouse models and techniques in studying neurodevelopment and neurogenesis. My goal is to be prepared and poised to develop such a translational research program, hopefully at Yale University.

What Dr. Robert Sapolsky did not see in that pond of protozoans is a sea of potential for drug discovery. To survive in that pond, protozoans have evolved effective mechanisms to kill off constant competitors for auxotrophic resources, either by secreting toxins and metabolites, or by contact killing which is likely to be induced by extractable surface macromolecules. For example, certain species of the centric diatom Thalassiosira can instantaneously kill dinoflagellates within close microscopic proximity. Dinoflagellates are evolutionary relatives to the apicomplexan parasites sharing similar metabolic, cellular and molecular templates which would likely be susceptible to these compounds. Currently, there are culture collections of over 6,000 freshwater and marine microalgae and protozoans that are being maintained in the U.S., waiting to be screened for antiparasitic and neurotrophic drugs. As a promise to the late Dr. David B. Czarnecki, who has curated over 1,000 of these cultures for the last 20 years, I hope to provide a leadership role to the phycological community in persuing this vision and to develop a strategic plan for harvesting this goldmine of potential drugs for parasitic and neurological diseases.

Figure Legends

Figure 1. *Toxoplasma* is widespread, being able to proliferate in all warm-blooded mammals and their resistant cysts and bradyzoites can be transmitted to humans of all ages by ingestion of contaminated meat, soil or water. Transplacental transmission of the fetus in earlier stages of pregnancy can lead to neonatal death, whereas infection between the 10th and 24th week of gestation can produce neurodevelopmental defects such as hydrocephaly, microcephaly, cerebral calcification and mental retardation. In addition to its neurotrophism, the retina is particularly attractive to *Toxoplasma* infection and can lead to inflammation and varying degrees of blindness (see Rorman et al., 2006).

Figure 2. (A) Postnatal and adult infections generally trigger an acute infection when ingested cysts, within the intestine, switch to an actively proliferating parasite and can infect any nucleated cells, triggering cell-mediated and adaptive CD4+ immune response (Buzoni-Gatel & Werts, 2006). Profiling of host gene response using gene microarray has been studied in a number of human or mouse cell types (Table 1). (B) Tachyzoite invasion into fibroblasts in vitro and its rapid replication within a parasite vacuole over a 30-hours period, duplicating every ~8 hours. Without the inhibition such as cell-mediated immunity, parasites will completely lyse the host cell within 48-72 hours and egress to find new host cells. Diagrams of intestinal and maternal barriers are from Barragan and Sibley (2003).

Figure 3. Parasite can hijack a monocyte, or a dendritic cell, and use it as a "Trojan Horse" to evade the immune surveillance to travel to the brain cerebral vascular network (B), where it transmigrates across the blood-brain barrier into the brain parenchyma (A, C), where *Toxoplasma* egresses, reinfects and reverts to the bradyzoite stage in glia and neuron cells, due to the bradyzoite conversion factors. The absence of these factors, such as T cell depletion in immunocompromised patients, can trigger the reactivation of the tachyzoite stage and the inflammation response generating brain lesions (Suzuki, 2002; Wilson & Hunter, 2004). It is possible, but not proven that *Toxoplasma*-carrying monocyte can enter the brain via the cerebral ventricle, in which the 3rd and lateral ventricles are accessible directly to the neurogenesis zones.

Table 1. Summary of host gene responses to *Toxoplasma gondii* infection from microarray gene profiles, termed *Toxoplasma* regulable genes (TRG). The astrocyte study is not from a direct infection with the parasite, but it is relevant because infected astrocytes secrete IFN-γ which is required to maintain the parasite in the bradyzoite stage. Similarly, tri-pyrrole is a MAPKK inhibitor that can alter the fibroblast cellular conditions that facilitates the parasite conversion to bradyzoites. The infected mouse brain study unfortunately includes the whole mouse brain that does not provide structure-specific information; it also includes the cerebral vascular system and peripheral immune and vascular cells, which explain the dominance (>90%) of immune-related genes.

Figure 4. A synopsis of current models of information flow from the cell surface linking cytoplasmic signaling pathways to gene expression, with special emphasis on brain cells (expanded from Arendt 2003). Green indicates TRGs and red arrows indicate an inhibitory effect.

Figure 5. (A) A comparison of the developmental windows of vulnerabilities leading to neuropathologies and an age surveillance of *Toxoplasma* seroprevalence in Northern Greece for 20 years (Diza et al., 2005). The peak of human population that is infected with *Toxoplasma* in the elderly strikingly coincides with the onset of Alzheimer's disease. (B) The stages of brain development. Diseases with late-puberty onset such as schizophrenia are proposed to relate to the adaptive developmental phases: myelination, synaptic production and pruning. (C) *Toxoplasma* can affect host gene response inter- as well as extracellularly; the latter is either by secreting factors or direct cell-to-cell communication. At a more organismal level,

the infection can alter the immune and endocrine homeostasis, which will have consequences on peripherally uninfected cells. (D) *Toxoplasma* regulated genes are also as candidate susceptible genes for schizophrenia/schizoaffective disorders (blue), Alzheimer's disease (red), and both (purple). (E) Correlation of reported effects by *Toxoplasma* infection to the expression of SZ and AD "neurocompromised" candidate genes, in the dentate gyrus and olfactory regions. Expression in the DG and OLF is qualitatively extracted from the Allen Brain Atlas – see Figure 15. Transgenic mice expressing specific gene tagged with GFP are accessible in the GENSAT Mouse Resource (green) to be used for tracking the effects of *Toxoplasma* infection at the cellular and system levels.

Abbreviation: **ASCL1**[8] (MASH1/Achaete-scute complex homolog-like 1), **BMP1**[4] (bone morphogenic protein 1), **C3**[10] (Complement component C3), **CECR1**[4] (Cat eye syndrome candidate 1), **CHI3L1**[8] (chitinase 3-like 1), **COL4A1**[4] (Collagen IV alpha 1), **COL4A2**[4] (Collagen IV alpha 2), **COMT** [5] (Catechol-O-methyltransferase), **DCT**[2] (dopachrome tautomerase), **DISC1**[5] (Disrupted in schizophrenia 1), **DPM1**[10] (Dolichol-phosphate mannosyltransferase), **DUSP6**[2] (Dual specificity phosphatase 6), **FBLN2**[4] (Fibulin 2), **FGF14**[5] (fibroblast growth factor 14), **GCH1**[2,3] (GTP cyclohydrolase 1), **GRM4**[7] (glutamate receptor, metabotropic 4), **IGF1**[4, 8] (insulin-like growth factor 1/somatomedin C), **MPZL1** (myelin protein zero-like 1), **MT-ND1**[10] (NADH dehydrogenase subunit 1), **MVK**[4] (Mevalonate kinase), **NTSR2**[8] (Neurotensin receptor 2), **PEBP1**[8] (Phosphatidylethanolamine binding protein), **PHGDHL1**[4] (Phosphoglycerate dehydrogenase like 1), **PIP5K2A**[10] (PI 5-kinase type II alpha), **S100B**[8] (S100 protein, beta polypeptide, neural), **SERTAD4**[4] (SERTA domain containing 4), **AR**[4] (Androgen receptor), **BDNF**[4] (brain derived neurotrophic factor), **CCL2**[1,2,9,1] (Chemokine (C-C motif) ligand 2), **IL1B**[2] (interleukin 1, beta), **PTGS2**[2] (prostaglandin-endoperoxide synthase 2), **RGS4**[3] (Regulator of G-protein signalling 4), **SLC6A4**[7] (Serotonin (5HT) Transporter), **SOD2**[2] (superoxide dismutase 2 mitochondrial), **CASP3**[1] (Caspase 3), **CCL5**[4,6,8] (Chemokine (C-C motif) ligand 5), **CH25H**[4] (25-hydroxylase), CXCL1[2,4] (Chemokine (C-X-C motif) ligand 1), **CXCL10**[8] (Chemokine (C-X-C motif) ligand 10), **FADD**[10] (Fas-associating protein), **FAS**[4] (TNF receptor superfamily, member 6), **FGF1**[4] (fibroblast growth factor 1), **GAPDH**[10] (Glyceraldehyde-3-phosphate dehydrogenase), **GBP2**[6,8] (Guanylate nucleotide binding protein 2), **GNA11**[3] (G protein alpha 11/Gq), **HMGCR**[4] (HMG-CoA reductase), **ICAM1**[2,9] (intercellular adhesion molecule 1/CD54), **IL4**[9] (Interleukin 4), IL6[1,2,9] (Interleukin 6), **LCK**[10] (Proto-oncogene tyrosine-protein kinase), **LRP1**[4] (low density lipoprotein-related protein 1//ApoER), LRP8[4] (low density lipoprotein-related protein 8/ApoER2), **MAPT**[8] (Microtubule-associated protein tau), **MMP3**[2,8] (matrix metallopeptidase 3), **MYST4**[10] (histone acetyltransferase 4), **NFKBIA**[2,10] (NFKB inhibitor, alpha), **NME1**[10] (diphosphate kinase 1, isoform 2), **NOS3**[10] (Nitric oxide synthase 3), **PCK2**[4] (Phosphoenolpyruvate carboxykinase 2), PLAU[2] (plasminogen activator urokinase), POLB[10] (DNA polymerase beta), **PRG1**[3,10] (proteoglycan 1, secretory granule), **PSAP**[4] (Prosaposin), **PSMB8**[6,8,10] (Proteasome subunit, beta type 8), **PSMB9**[6,8] (Proteasome subunit, beta type 9), **PSMB10**[8] (Proteasome subunit, beta type 10), **RPS15**[10] (Ribosomal protein S15), **SEPT3**[8] (Septin 3), **SERPINA3N**[6,8] (Serine/cysteine peptidase inhibitor A3N), **SOAT1**[4] (Sterol O-acyltrasnferase 1), **TAP1**[8] (Transporter 1 ATP-binding cassette sub-family B), **TAP2**[6] (Transporter 2 ATP-binding cassette sub-family B), **TRAF2**[4] **(**TNF receptor-associated factor 2**)**

Table 2. Cataloging of SZ/SZPD and AD susceptible genes that are regulable by *Toxoplasma* infection. Catalogs (not shown) provide localization data of expressed mRNA in the mouse hippocampus and olfactory region, extracted from the Allen Brain Atlas. Not all of the ~650 TRGs have been cataloged. This list cannot differentiate between the effects on infected versus noninfected cells. * In situ localization data is not available in the ABA database.

Table 3. Potential interference of neurotransmitter metabolism by *Toxoplasma*. Neurotransmitter information is obtained from NCBI. Abbreviation: **ACADL**[10] (Acyl-

coenzyme A dehydrogenase), **ACAT1**[4] (Acetyl-Coenzyme A acetyltransferase 1), **ALDH3B2**[4] (Aldehyde dehydrogenase 3B2), **ASNS**[4] (Asparagine synthetase), **ASS1**[4] (Argininosuccinate synthetase 1), **CA5A**[7] (carbonic anhydrase VA, mitochondrial), **COMT**[5] (Catechol-O-methyltransferase), **CSF2**[4] (colony stimulating factor 2), **DCT**[2] (dopachrome tautomerase), **DGKZ**[7] (Diacylglycerol kinase zeta), **DPYD**[10] (Dihydropyrimidine dehydrogenase NADP), **GARS**[4] (Glycyl-tRNA synthetase), **GLS2**[8] (Glutaminase 2), **GNA11**[3] (G protein alpha 11), **GRM4**[7] (glutamate receptor, metabotropic 4), **GRP**[4] (Gastrin-releasing peptide), **GSTM2**[10] (Glutathione S-transferase M2), **HADHA**[10] (Hydroxyacyl-Coenzyme A), **HMGCS1**[4] (HMG-CoA synthase 1), **HSD17B12**[4] (Hydroxysteroid (17-beta) dehydrogenase 12), **IDH**[10] (Isocitrate dehydrogenase 1), **IDH2**[10] (Isocitrate dehydrogenase 2), **IGF1**[4,8] (Insulin-like growth factor 1), **IL4**[9] (Interleukine 4), **KYNU**[4] (Kynureninase), **MAP2K1**[1,10] (Mitogen-activated protein kinase kinase 1), **MAP2K6**[4] (Mitogen-activated protein kinase kinase 6), **MDH1**[10] (Malate dehydrogenase cytosolic), **MYST4**[10] (MYST histone acetyltransferase), **NME1**[10] (Non-metastatic cells 1 nucleoside-diphosphate kinase), **NME2**[10] (Non-metastatic cells 2 nucleoside-diphosphate kinase), **NME6**[10] (Non-metastatic cells 6 nucleoside-diphosphate kinase), **NTSR2**[8] (Neurotensin receptor 2), **PAFAH1B1/LIS1**[4] (platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa), **PBEF1**[4,2] (pre-B-cell colony enhancing factor 1), **PGK1**[10] (Phosphoglycerate kinase 1), **PIK3CG**[10] (Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform), **PIP5K2A**[10] (Phosphatidylinositol-4-phosphate 5-kinase type II alpha), **PLA2G7**[7] (Phospholipase A2, group VII), **POLR1D**[10] (DNA-directed RNA polymerase I), **POLR2D**[10] (DNA-directed RNA polymerase II), **PPAP2B**[4] (Phosphatidic acid phosphatase type 2B), **PRKCB1**[10] (Protein kinase C beta 1), **PSAT1**[4] (Phosphoserine aminotransferase 1), **SOAT1**[4] (Sterol O-acyltrasnferase 1), **TAP2**[6] (Transporter 2 ATP-binding cassette sub-family B), **TJP**[4] (Tight junction protein 1), **TKT**[4] (Transketolase)

Figure 6. (A) A synopsis of *Toxoplasma* control of the host cells in an acute tachyzoite phase. Upon entry into the host cell, *Toxoplasma* evades the lysosomal degradation pathway and establishes am autonomous parasite vacuole. As the vacuole matures, host mitochondria and endoplasmic reticulum adhere to the parasite vacuole membrane, which is surrounded by host intermediate filaments, e.g. vimentin, GFAP. Kinases and phosphatases secreted from the parasite enter the host nucleus. Vesicles containing cholesterol are diverted from the host endosomal and lysosomal pathways; host microtubules mediate the delivery of vesicles and their contents into the parasite vacuole. Pores in the vacuole membrane allow the movement of biomolecules (lipids, ions, ATP, purines, pyrimidines, folates, lipids, amino acids, arachidonic acids) into the vacuole where they can be transported into the parasite cytoplasm. Theoretical modeling of the disruptive effects on neurotransmission, neurodevelopment and pathologies that can result from parasite depletion of host (B) arachidonic and retinoic acids, (C) folate, cysteine, methionine, (D) tyrosine and tryptophan and (E) purine and adenosine.

Figure 7. Correlation of parasite disruption of host glutamergic (B), dopaminergic and serotonergic (see Figure 6D) pathways to abnormal neurocircuitries in schizophrenia (C-D). Parasites acting as a metabolic sink that drain the precursor pools of glutamine/glutamate and tyrosine/tryptophan can disrupt the intricate balance of glutamergic, GABAergic and dopaminergic interneurons that connect (A) the circuitries for working and episodic/ contextual memories. In schizophrenic brains, disconnectivity in circuitries yielding a net increase in cortical glutamate are well documented and reviewed in Stone (2006) and Lewis and Gonzalez-Burgos (2005). Redrawn from (A) Meyer-Lindenberg and Weinberger (2006), (C) Stone (2006) and (D) Lewis and Gonzalez-Burgos (2005).

Figure 8. Modeling of parasitic cholesterol depletion to neurodegeneration. (A) Model of cholesterol, LDL and lipoprotein depletion by *Toxoplasma* (pink) associated with ApoE/cholesterol signaling pathways. (B) Implications of Notch, Wnt, ApoE/Reelin signaling pathways in neurodevelopment and the development of Alzheimer's diseases. Redrawn from

Grilli et al. (2003). (C) *Toxoplasma* regulable proteins (green) are involved in all three pathways connecting from surface binding to key cytoplasmic regulators, e.g. Dab1, Disc1, PAFAH1B/LIS1. *Toxoplasma* appears to regulate secreted factors that bind to G-coupled receptors.

Figure 9. Topography of embryonic development of the cortex. (A) Morphogenesis of the mouse forebrain starting from the closure of the neural tube at embryonic day 9 (E9), to the development of the two telencephalic vesicles (E13). Arrowheads are telecephalic vesicles and empty arrowheads are location of eyes. From Monuki and Walsh (2001). (B) Rostrolateral (left) and sagittal (right) view of the patterning centers of the mouse brain as determined by selected secreted morphogens mediating developmental signaling pathways. NR4A2/NURR1 and TH are involved in the development of DA neurons, originating from the floor plate, are potentially under *Toxoplasma* control. Redrawn from Monuki and Walsh (2000) and Vitalis (2005). (C-E) Mechanisms regulating interneuron migration to the cortex in an E12.5 mouse brain from the transverse (C, E) and sagital (D) views. Key TRPs include ASCL1, BDNF and PAVB. Redrawn from Sur and Rubenstein (2005), Wonders and Anderson (2005), Marin and Rubenstein (2003), Schuurmans & Guillemot (2002).

Figure 10. Embryonic neurogenesis and interneuron migration that establish the foundation of cortical wiring to the developing cortex. (A) Expression of proteins implicated in the induction of neurogenesis includes TRPs (red): SLC64A, NR4A2, ALDH2, TGF-α. (B) TRPs can potentially disrupt the normal migration of neurons along radial glia from the ventricular zone, as well as tangential migration from the thalamus. (C-D) More specifically in radial migration, *Toxoplasma* can potentially disrupt both locomotion and adhesion of migrating neurons along the radial glia to the pia. Redrawn or extracted directly from (B) Price et al. (2006), (B) Molnar et al. (2006), (D) Rakic (2006); see these reviews for more details.

Figure 11. Neurogenesis in the adult hippocampus and the consequences of hippocampus atrophy. (A-B) Two localized regions within the adult brain that still maintain neurogenesis are the subventricular zone, streaming neurons toward the olfactory bulb, and the dentate gyrus in the hippocampus. (C) Neurogenesis from the subgranular cell layer (green) sprouts dendrites that connect to CA3, then to the CA1 and subsequently to the entorhinal cortex. Circuits are formed also to the amygdala and prefrontal cortex that correlates to specific cognitive functions. (D) Growth and maturation of newborn granule cells in the dentate gyrus. GABA initially is stimulatory in immature neurons, but becomes inhibitory in mature neuron at the time that glutaminergic neurons are established from the entorhinal cortex. (E) Intrinsic programs and external factors that control neurogenesis in the dentate gyrus. Factors or pathways that are potentially affected by *Toxoplasma* infection are highlighted in pink. Redrawn from (A-B) Lennington et al. (2003), (E) Lledo et al. (2006), (D) Aimone et al. (2006), (E) McCaffery et al. (2006), Duman and Monteggia (2006).

Figure 12. Anatomy of the mouse adult brain as illustrated in the Allen Brain Atlas. Disruption in the connections (red) between the hippocampus formation (HPF) and prefrontal cortex results in the development of psychosis. Neurogenesis (pink) is active in the olfactory region and migrates from the dentate gyrus (DG) to the Amon's horn (CA).

Figure 13. In situ hybridization of TIGs associated with the WNT signaling pathways, including WNT2[4] (wingless 2), WNT4[4] (wingless 4), DKK1[4] (Dickkopf homolog 1), SPRY2 (Sprouty homolg 2), CCND3[10] (G1/S-specific Cyclin D3), PRKCB1[10] (Protein kinase C beta 1). Not included are TAX1BP3[7], JUN[4.2], MYC[2,10].

Figure 14. In situ hybridization of TIGs associated with cholesterol and Notch pathways in the dentate gyrus and olfactory regions. **Notch signaling pathway:** JAG1[4] (Jagged 1), NOV[4] (Nephroblastoma overexpressed gene). **Cholesterol signal pathway:** LRP1/ApoER[4], LRP8/ApoER2[4], MAPT[8], LDLR[4], APOC1[4], APOD[4], APBB2[3], B2M[6,9], HDLBP[4], PAFAH1B1/LIS1[4], CD36[6],

DAB1[5], DAB2[5], DAB2IP[5], PLTP[4], MSR1[8*], MSR2[8], STAB1[8]. **Cholesterol metabolism:** ACADL[10], ACAT1[4], SOAT1[4], ACOX2[4], ACSL1[4], CH25H[4], EBP[4], FDPS[4], GAPDH[10], HMGCR[4], HMGCS1[4], NSDHL[4], SC5D[4], SQLE[10]

Figure 15. In situ hybridization in the dentate gyrus of TIGs linked to susceptibility genes to the SZ and AD neurocompromised brains.

Abbreviation - BL: basal lamina, BZ: bradyzoite, CA: Ammon's Horn, Ch: Chandelier neuron, CP: cortical plate, CP: commissural plate, CX: cortex, DA: dopamine , DG: dentate gyrus, FA: fibrillary astrocyte, FL: Fibre Layer, FP: floor plate, GEN: ganglionic eminence, GLUT: glutamate, GP: glial progenitors, HT: hypothalamus, IZ: intermediate zone, LGE: lateral ganglionic eminence, LT: lamina terminalis, MGE: medial ganglionic eminence, ML: Molecular Layer, MN: migrating neuron, MZ: marginal zone, NP: neurogenic potentials, OC: optic chiasm, OLF: olfactory region, PA: protoplasmic astrocyte, PC: posterior commissure, PP: Preplate, PVM: parasitophorous membrane, Py: pyramidal neuron, RG: radial glia, RMZ: rostral migratory zone, SP: subplate, SVZ: subventricular zone, SZ: schizophrenia, SZPD: schizoaffective disorder, TRP: Toxoplasma regulated protein , TZ: tachyzoite, V3: 3rd ventricle, V4: 4th ventricle, VZ: ventricular zone, WM: white matter
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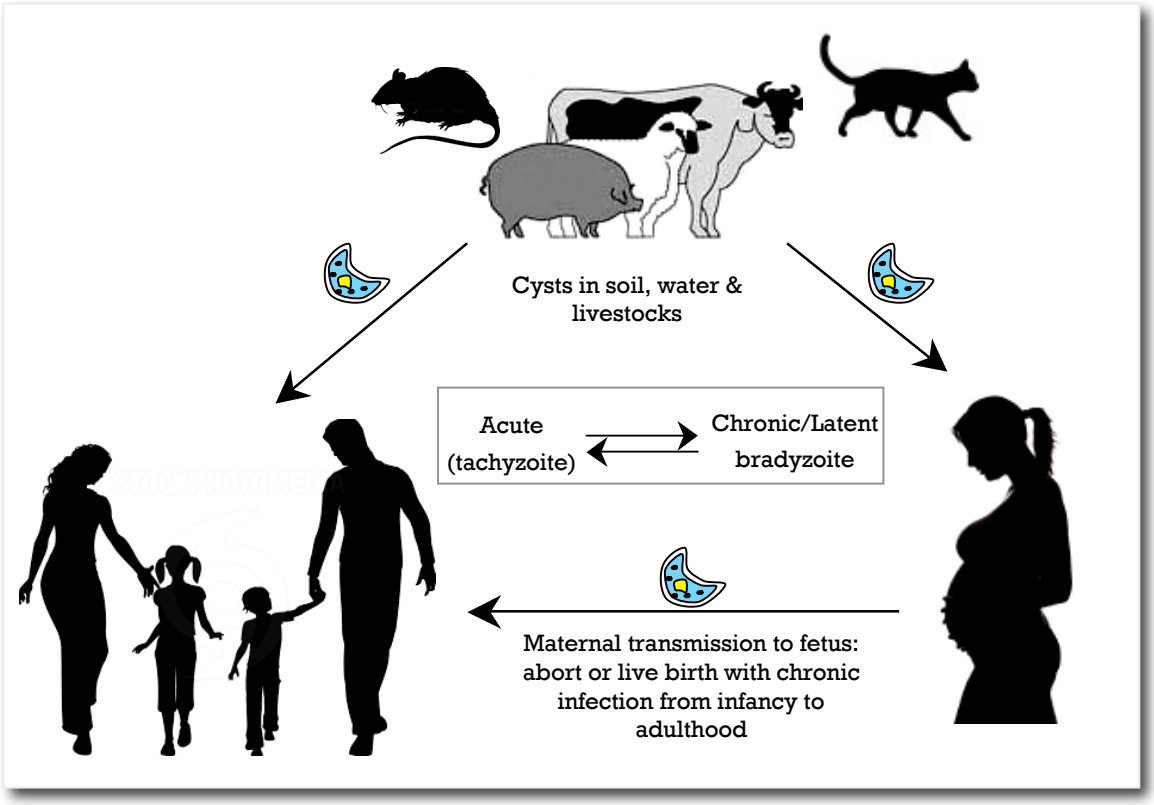


Figure 1

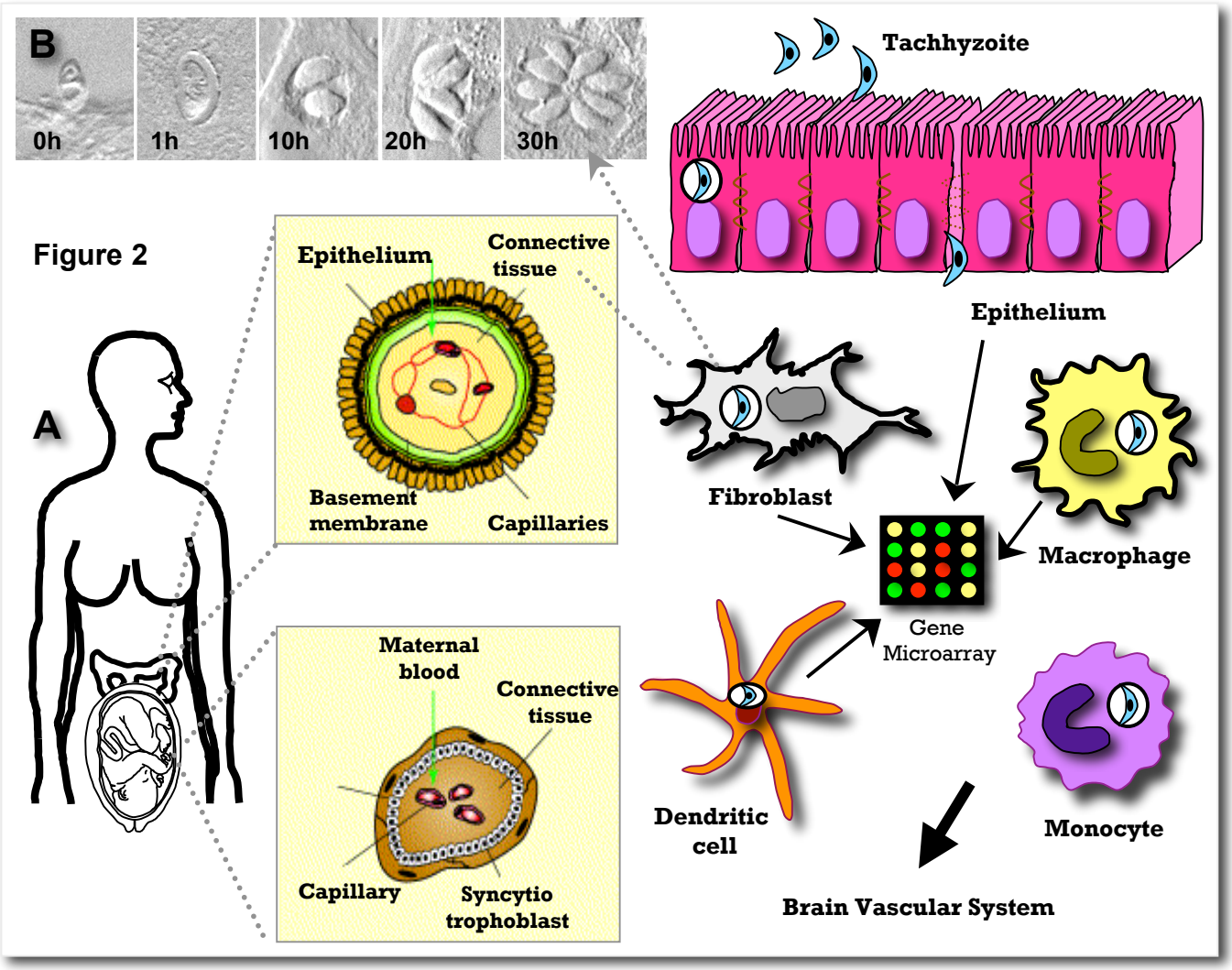


Figure 2

Figure 3

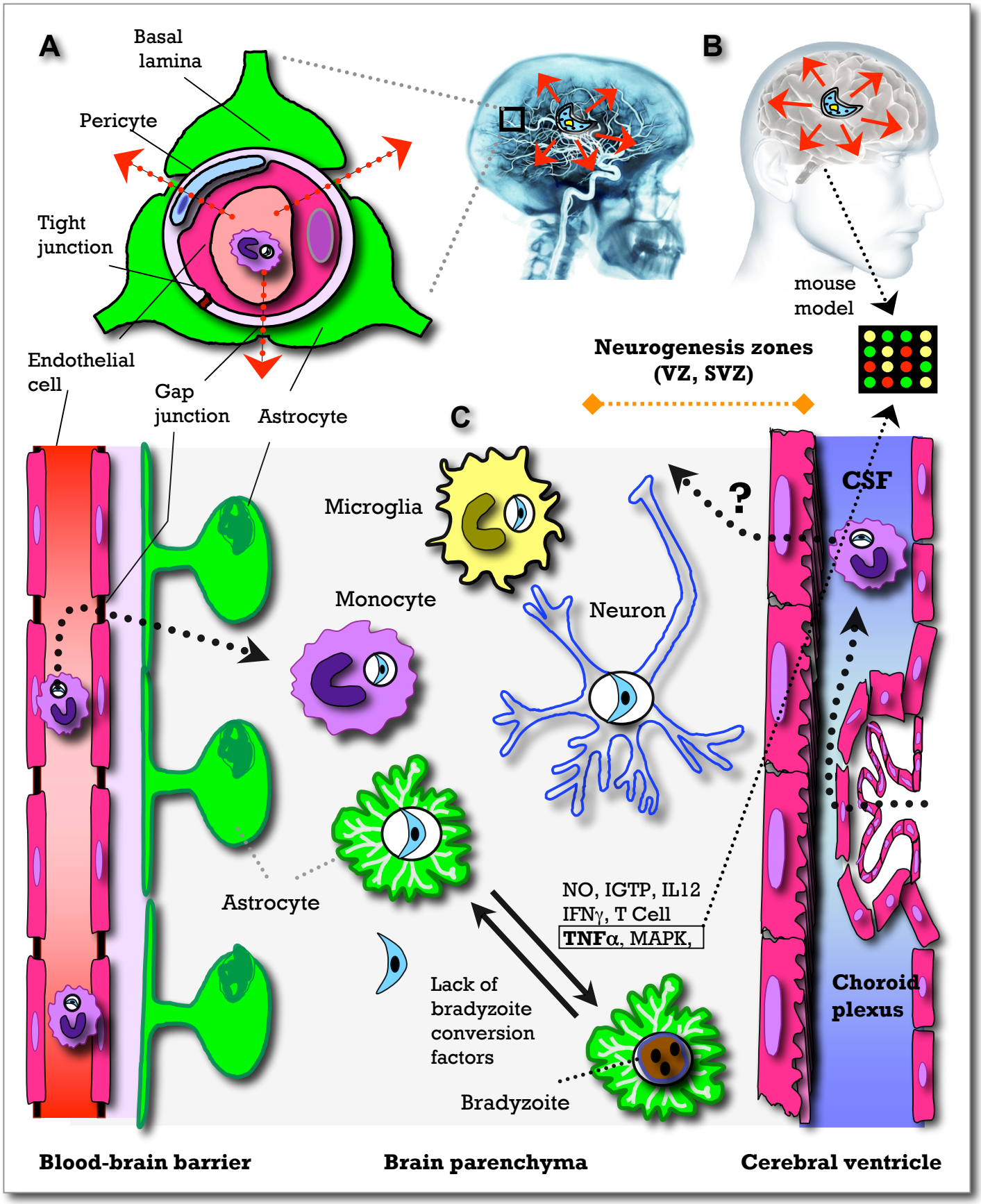
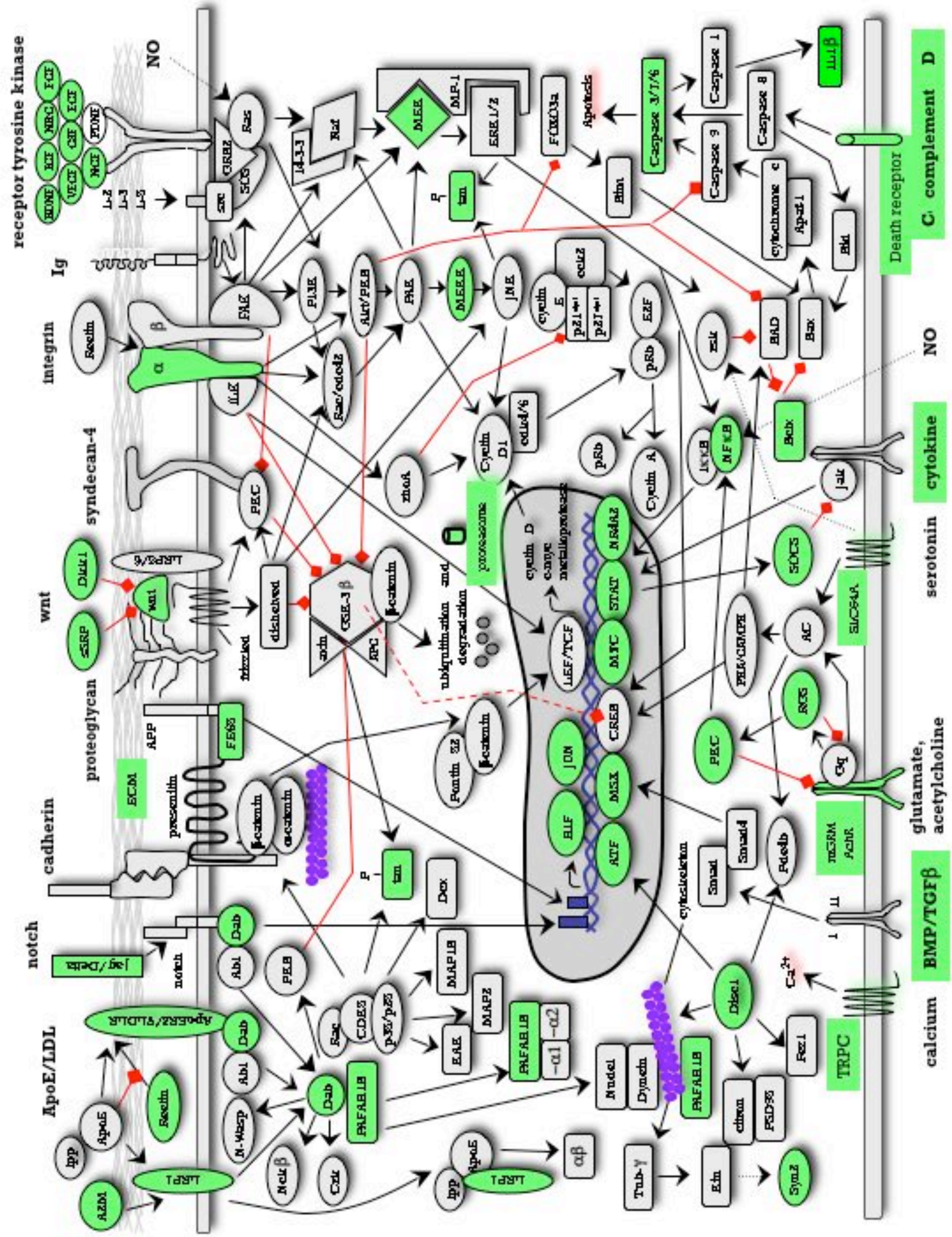

A
Basal lamina
Pericyte
Tight junction
Endothelial cell
Gap junction
Astrocyte
B
mouse model
Neurogenesis zones (VZ, SVZ)
C
Microglia
Monocyte
Neuron
?
CSF
Astrocyte
NO, IGTP, IL12
IFNγ, T Cell
TNFα, MAPK,
Lack of bradyzoite conversion factors
Bradyzoite
Choroid plexus
Blood-brain barrier
Brain parenchyma
Cerebral ventricle

Table 1. Summary of host gene response to *Toxoplasma gondii* infection from microarray gene profiles, termed Toxoplasma Regulatable Genes (TRG).

FIBROBLASTS [1-5]	EPITHELIUM [11]	DENDRITIC CELL [12]	MACROPHAGE [13]
ADAMTS5 [4], ADD3 [4], AKR1C2 [4], ALDH3B2 [4], ALOX5 [3], ALPI [4], AMH [4],ANGPTL2 [3], ANGPTL4 [4], AP1S1 [4], AP1S2 [3], APBB2 [3], APOC1 [4], APOD[4], APOL3 [4], AR [4], ASAM [4], ASNS [4], ASS1 [4], ATF3 [2], ATP8B1 [4], ATP9A [4], BCL2A1 [3], BCL2A1 [4], BDNF [4], BGN [4], BMP1 [4], BMP2 [4], BMP4 [4], BMP6 [3], BNIP3 [1], C16ORF45 [3], C1R [4], C1S [4], C5 [4], C8ORF1 [3], CASP3 [1], CCL1 [1,2,3], CCL11 [3, 4], CCL20 [3], CCL26 [3, 4], CCL5 [4], CCL7 [2], CCL8 [4], CD53 [2], CD55 [4], CD82 [3], CDC37L1 [3], CEBPG [4], CECR1 [4], CECR5 [4], CEP135 [3], CH25H [4], CHST2 [3], CISH [3], CLCF1 [3], CLSTN3 [3], COL1A1 [4], COL1A2 [4], COL3A1 [4], COL4A1 [4],COL4A2 [4], COL5A1 [4], COL5A2 [4], COL5A3 [4],COL6A1 [3, 4], COL6A2 [4], COL8A2 [4], COL14A1 [4], COL15A1 [4], COMMD10 [3], CPM [3], CSF2 [4], CSF3 [4], CST4 [3], CTF1 [4], CTSL [3], CTSO [4], CXCL1 [2, 4], CXCL2 [1, 2, 3, 4], CXCL3 [4], CXCL5 [1], CXCL6 [4], CXCL14 [4], DAB1 [5], DAB2 [5],DAB2IP [5], DCT [2], DDI1 [4], DDR1 [3]DDX58 [4], DIO2 [4], DISC1 [5], DKK1 [4], DNAJB9 [4], DUSP5 [2], DUSP6 [2], EBP [4], EGLN3 [3], EGR1 [2], EGR2 [2], ELOVL7 [3], ENC1 [4], EREG [2], ESM1 [2], ESM1 [4], ETV4 [4], FAS [4], FBLN1 [4], FBLN2 [4], FBN1 [4], FBN2 [4], FDPS, FGF1 [4], FGF9 [4], FGFR1 [5], FGF12 [4], FGF14 [5], FGFR1OP [5], FGFR1OP2 [5], FLT3 [4], FMNL1 [3], FN1 [4], FNTA [3], FOXA1 [3], FTL [4], GADD45A [1], GARS [4], GCH1 [2,3], GM2A [4], GNA11 [3], GOS2 [3], GPI [4], GRN [4], GRP [4], GRPEL1 [4], HBEGF [2], HDLBP [4], HIF1A [4], HMGCR [4], HMGCS1 [4], HOOK2 [3], HSD17B12 [4], HSPD1 [1], IARS2 [4], ICAM1 [2], IER3 [2, 3], IFI16 [4], IFITM2 [3], IGF1 [4], IGF2 [4], IL1B [2],IL23A [3], IL24 [4], IL33 [3], IL6 [1, 2], IL8 [1], INHBB [4], IRF1 [2], ITGA2 [2], ITGA8 [3], ITGAV [1], JAG1 [4], JUN [4], JUNB [2], KAL1 [4], KCNMB4 [3], KITLG [4], KRTHB6 [3], KYNU [4], LAMB3 [3], LBA1 [4], LCP1 [3], LDLR [4], LIF [2, 4], LIPL3 [4], LOC145009 [4], LOC339267 [3], LOC374443 [3], LOC387763 [3], LOC388610 [3], LOC926691 [3], LOX [3], LRP1 [4], LRP8 [4], LUM [3], LUM [4], MAFG [3], MAP2K1 [1], MAP2K6 [4], MAP3K1 [3], MARCKSL1 [1], MATN4 [4], MDFIC [3], MEOX1 [3], MFAP2 [4] , MFAP5 [4], MIR16 [4], MMP3 [2], MPZL1, MSX2 [2], MTHFD2 [4], MTHFS [3], MTSS1 [3], MVD [4], MVK [4], MYC [2], MYLIP [4], NFE2L3 [3], NFKB1 [2], NFKBIA [2], NGFB [4], NINJ1 [2], NOV [4], NR4A2 [2], NRG2 [2, 4], NSDHL [4], OSGIN1 [4], OSGIN2 [3], PAFAH1B1 [4], PBEF1 [2, 4], PCDH18 [3], PCK2 [4], PDGFA [4], PHGDHL1 [4], PHYH [4], PLAU [2], PLEKHA9 [3], PLTP [4] , PMAIP1 [2], PPAP2B [4], PRELP [4], PRG1 [3], PRKDC [1], PRR16 [4], PRSS23 [3], PSAP [4], PSAT1 [4], PTGIS [3, 4], PTGS2 [2], PTPN13 [3], RCSD1 [3], RELB [2], RGS4 [3], RIPK2 [3], SAT [3], SC5DL [4], SELE [3], SEPHS1 [3], SERPINB2 [2], SERTAD4 [4] , SESTD1 [4], SFRP1 [2], SH3PXD2A [4], SLC25A37 [3], SLC2A1 [4], SLC7A1 [4], SOAT1 [4], SOD1 [1], SOD2 [2], SPRY2 [2], ST3GAL5 [3], STAT1 [4], STAT3 [4], STC1 [2], STK4 [3], STS [4], SULF1 [3], TDO2 [3], TFF1 [4], TFF3 [2], TFPI2 [2, 4], TFRC [1], THBD [2], TJP1 [4], TKT [4],TNFAIP2 [2], TNFAIP3 [2], TNFAIP6 [1], TNNT1 [3], TNS3 [3], TOMM40L [4], TRAF1 [4], TRAF2 [4], TRPC6 [5], TSLP [3], TSPAN2 [4], USP25[4], VEGFB [4], WNT2 [4], WNT4 [4], XCL1 [4], XPO7 [3], ZNF436 [4], ZNF659 [3]	A2M, ACADL, ACO2, APR-3, ARPC2, ATP6, BECN1, C1R, C3, C4, C4BPA, CAP1, CCND3, CDC2L5, CDC45L, CFLAR, C-FOS, CIB1, CLK3, CNN3, COX3, CS, CTSL, CXCL12, CXCL9, CXCR4, CYTB, DAD1, DBI, DDIT3, DNASE2, DPM1, DPYD, DSTN, EBI3, EIF2B4, EIF4G2, ELK3, FADD, FARSLB, GAPDH, GGTA1, GLTP, GRN, GSTM, GSTM2, H1FX, HADHA, HLA-DMB, HNRPLL, HPSE, HSPA8, HSPCB, IDH1, IDH2, IFI30, IFIT1, IFIT3, IFIT4, IFITM3, IGHG, IL2RG, IL8RA, KARS, LCK, MAP2K2, MAX, MCP-1, MDH1, MR1, MTAP, MYC, MYST4, ND1, ND4, NFE2, NFKB2, NFKBIA, NME1, NME2, NME6, NR2F2, ORC4L, PCNA, PECI, PGAM2, PGK1, PIGO, PIK3CG, PIP5K2A, PKC, PKM1, PLAUR, POLB, POLR1D, POLR2D, PRG1, PRTN3, PSMB8, PSMD14, PSME1, PTPNS1, RABGEF1, RAP2A, RHOG, RIN2, RPS15, RSU1, S100A12, SARA2, SEPT7, SF3A3, SLA-DMA, SLA-DQA, SLA-DQB1, SLA-DRA, SLC15A4, SNX2, SQLE, STAT1, STAT6, STK17B, SUCLG1, TAF11, TEC, TOPORS, TTRAP, TYMS, UBE2D2, UBE2I, UBR1, UFD1L, UMPS, YME1L1	AGRN, RSAD2, UBE2S, IFI44L, HLAF, HLAJ, IF135, IFIT1, IFIT2, IFIT3, IFITM1, IFITM3, CXCR3, CXCL11, IRF4, IRF7, ISG15, ISG20, CCL8, CCL7, CXCL9, MN1, MX1, MX2, MYD88, OAS2, OASL, PLXNC1, PSMA2, PSMA3, SERPING1, STAT1, STAT4, TAPBP, TNFSF10	RSAD2, UBE2S, HLAF, IFIT1, IFIT2, IFIT3, IFITM1, CXCR3, CXCL11, IRF4, ISG15, ISG20, CCL8, CCL7, CXCL9 MN1, MX2, OAS2, OASL, PLXNC1, SERPING1, STAT1, STAT4
		VASCULAR ENDOTHELIUM [10]	**ASTROCYTE (+) IFN-γ [8]**
		CX3CL1, CXCL1, ICAM-1, IL8RA, CCL3, CCL5, CCL2, NFKB1, NOS3	ADAMTS12, ADAMTS15, ALDH1A1, ASCL1, ASPM, BAI2, BUB1, CALB1, CCL5, CCL9, CD74, CDH22, CFI, CHI3L1, CIDEA, CLDN2, CXCL4, CXCL9, CXCL10, CXCL11, DCN, E2F7, EBI2, EMB, FCGR2B, FXYD6, GAP43, GBP1, GBP2, GBP4, GLS2, GLYCAM1, H2-Aa, H2-AB1, H2-D1, H2-DMA, H2-DMB1, H2-DMB2, H2-EA, H2-EB1, H2-K1, H2-L1, H2-Q1, H2-Q7, H2-Q8, H2-T10, HGPD, HNT, IFI47, IFI203, IFI205, IFIT1, IFIT3, IGF1, IGSF4D, IGTP, IIGP1, IIGP2, IL18BP, IRF1, IRGM, ITGA6, ITIH3, LTC4S, LY6A, LY6C, MAPT, MKI67, MLF1, MMD2, MMP3, MPA2L, MRC1, MSR1, MSR2, MYB, NELL2, NSG2, NTSR2, OASL, PCDHB20, PEBP1, PLA1A, PLAC8, PPM1E, PSMB8, PSMB9, PSMB10, PVALB, RACGAP1, S100B, SCG2, SEPT3, SERPINA3G, SERPINA3N, SLAMF8, SLC14A1, SNX10, SOCS1, STAB1, STAT1, SYN2, TAP1, TGTP, TIMP4, UBD
		MULLER CELL (RETINAL GLIA) [9]	
		HLA-A201, B2M, CXCL2, CCL2, ICAM-1, IL8, IL4, IL-6, VCAM1	
		FIBROBLAST (BZ CONVERSION) [7]	
		BSCL2, C3orf34, CA5A, CD1A, CD69, CES3, CROCC, DGKZ, ARID2,DSC2, EEF1A2, EEF1D, EEF1D, EXOSC6, FRMPD1, FRS3, TIMM44, GAL3ST2, GJA7, GPM6B, GPR172B, GRM4, HEY2, IFNA21, IFNA24, IQSEC1, ITGB3BP, KCNE2, LAMA5, MRPS31, PLA2G7, PRDM12, RORB, RPL8, SLC6A4, TAX1BP3, TNNT3, TSPYL2, TTTY8, UPK3, ZNF179 [7]	
		ADULT MOUSE BRAIN [6]	
		B2M, BC057170, BCL2A1C, BST2, C1qa, C1qc, C1R, C1SB, C4a, C4b, C920025E04Rik, CCL5, CCL8, CD274, CD36, CYBB, D17H6S56E-3, DEFCR17, DRLD, EMR1, FYB, GBP2, GBP4, GBP5, GFAP, GIMAP4, IFI47, IFIT3, IGH-4, IGHG2C, IGHV1S44, IGHV1S46, IGH-VJ558, IGJ, IGL-V1, IGTP, IIGP1, IIGP2, LAG3 [6]LGALS3BP, MAFB, MGBP8, MPA2, PARP14, PSMB8, PSMB9, PSME1, SERPINA3N, SERPING1, SLPI, SOCS1, STAT1, TAP2 [6]	

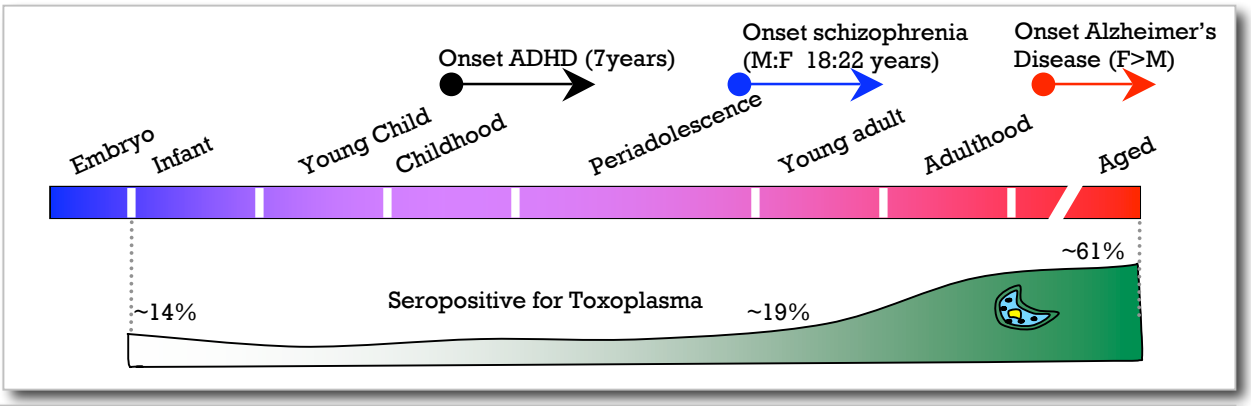
References. [1] Gail et al. 2001 (infected human HFF), [2] Bladder et al. 2001 (infected human HFF), [3] Saeij et al. 2006 (infected human HFF), [4] Fouts et al. 2006 (infected human HFF), [5] Fouts & Boothroyd unpubl. (infected human HFF) , [6] McLeod et al. unpublished (chronically infected murine brain), [7] Radke et al. 2006 (tri-pyrrole treated human HFF), [8] Halonen et al. 2006 (IFN-γ treated murine astrocyte), [9] Knight et al. 2006 (infected human retinal Müller cells – chemokine profiling), [10] Knight et al. 2005 (infected human vascular endothelial cells - chemokine profiling), [11] Okomo-Adhiambo et al. 2006 (infected porcine epithelium cell), [12] Chaussabel et al. 2003 (infected human dendrite), [13] Chaussabel et al. 2003 (infected human macrophage).

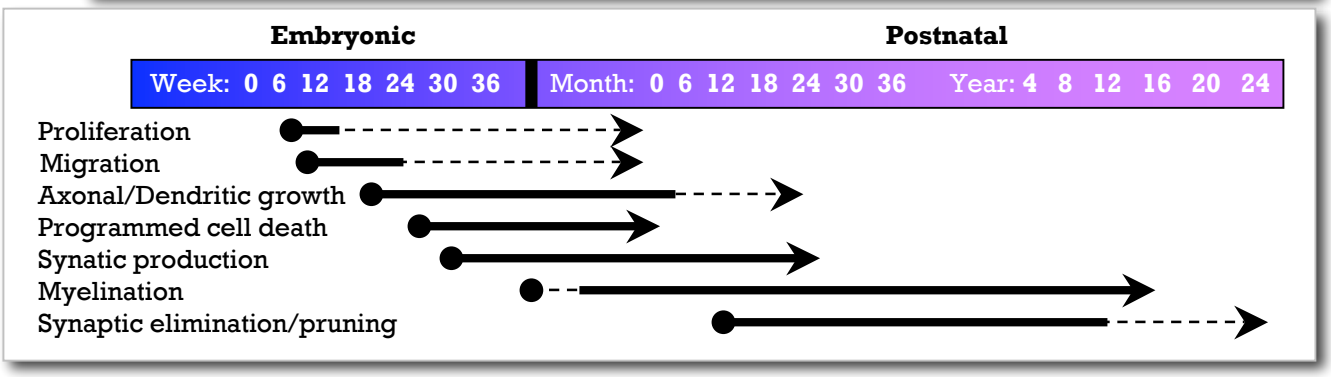
Figure 4

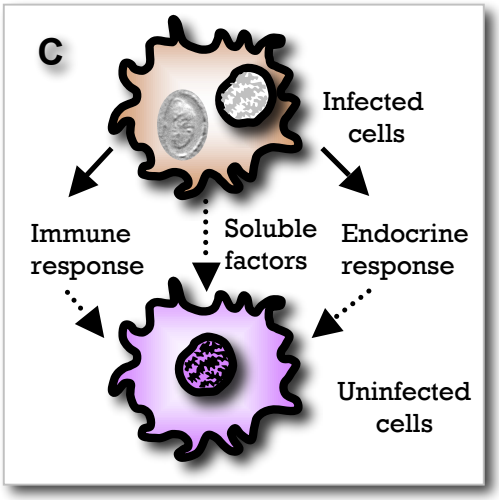


receptor tyrosine kinase
Ig
integrin
syndecan-4
wnt
proteoglycan
cadherin
notch
ApoE/LDL
NO
Death receptor
C: complement D
cytokine
serotonin
glutamate, acetylcholine
BMP/TGFβ
calcium
TRPC
Apoptosis
Caspase 3/7/6
Caspase 1
Caspase 8
Caspase 9
proteasome
ubiquitination and degradation
cytoskeleton

Figure 5







D

SZPD	SZPD/AD	AD
ASCL1, BMP1, C3, CECR1, CHI3L1, COL4A1, COL4A2, COMT, DCT, DISC1, DPM1, DUSP6, FBLN2, FGF14, GCH1, GRM4, IGF, MPZL, MT-ND1, MVK, NTSR2, PEBP1, PHGDHL1, PIP5K2A, S100B, SERTAD4, SLC25A37, STK4, SYN2, TFR, TNFAIP, TNFAIP3, TNFAIP6, TPH2	AR, BDNF, CCL2, IL1B, PTGS2, RGS4, SLC6A4 SOD2, IL8	CASP3, CCL5, CH25H, CXCL1, CXCL10, FADD, FAS, FGF1, GAPDH, GBP2, GNA11, HMGCR, ICAM1, IL4, IL6, LCK, LRP1, LRP8, MAPT, MMP3, MYST4, NFKBIA. NME1, PCK2, PLA, POLB, PRG, PSAP, PSMB10, PSMB8, PSMB9, RPS15, SEPT3, SERPINA3N, SOAT1, TAP1, TAP2, TRAF2

E

Express. (DG & OLF)	Toxo-induced Δ	SZ suscept. gene	SZ & AD gene	AD suscept. gene
Mid to high	↑	MVK [4], **GRM4** [7], PIP5K2A [10], SYN2 [8], TFRC [1], TNFAIP6 [1], DUSP6 [2]	CCL2 [1,2,9,10]	CASP3 [1], GAPDH [10], LRP8 [4], NME1 [10], POLB [10], RPS15 [10], TRAF2 [4], SOAT1 [4], HMGCR [4], NFKBIA [2,10],
	↓	**ASCL1** [8], COL4A2 [4], COMT [5], BMP1 [4], SERTAD4 [4]	PTGS2 [2], SOD2 [2], RGS4 [3], **BDNF** [4], AR [4]	FGF1 [4], LRP1 [4], MAPT [8], PSAP [4], **SEPT3** [8],
Neg. to Low	↑	PBEF1 [2,4], **GCH1** [2,3], C3 [10], MT-ND1 [10]	IL1B [2], IL8 [1,9]	FAS [4], GBP2 [6,8], LCK [10], PSMB9 [6,8], CCL5 [4,6,8], CH25H [4], CXCL1 [2,4], CXCL10 [8], ICAM1 [2,9], IL4 [9], IL6 [1,2,9], PLA1A [8], PRG1 [3,10], PSMB8 [6,8,10], PSMB10 [8], TAP1 [8], TAP2 [6], ICAM1 [2,9]
	↓	FBLN2 [4], SLC25A37 [3], FGF14 [5], TNFAIP2 [2], TNFAIP3, NTSR2 [8], CHI3L1 [8], DISC1 [5], **IGF1** [4,8], PHGDHL1 [4], CECR1 [4], COL4A1 [4], MPZL1 [3]	**SLC6A4** [7]	MYST4 [10], PCK2 [4], FADD [10], SERPINA3N [6,8], MMP3 [2,8], GNA11 [3]

Table 2. Cataloging of SZ/SZPD and AD susceptible genes that are regulatable by *Toxoplasma* infection. Catalogs (not shown) provide localization data of expressed mRNA in the mouse hippocampus and olfactory region, extracted from the Allen Brain Atlas.
*Data is not available in the ABA database.

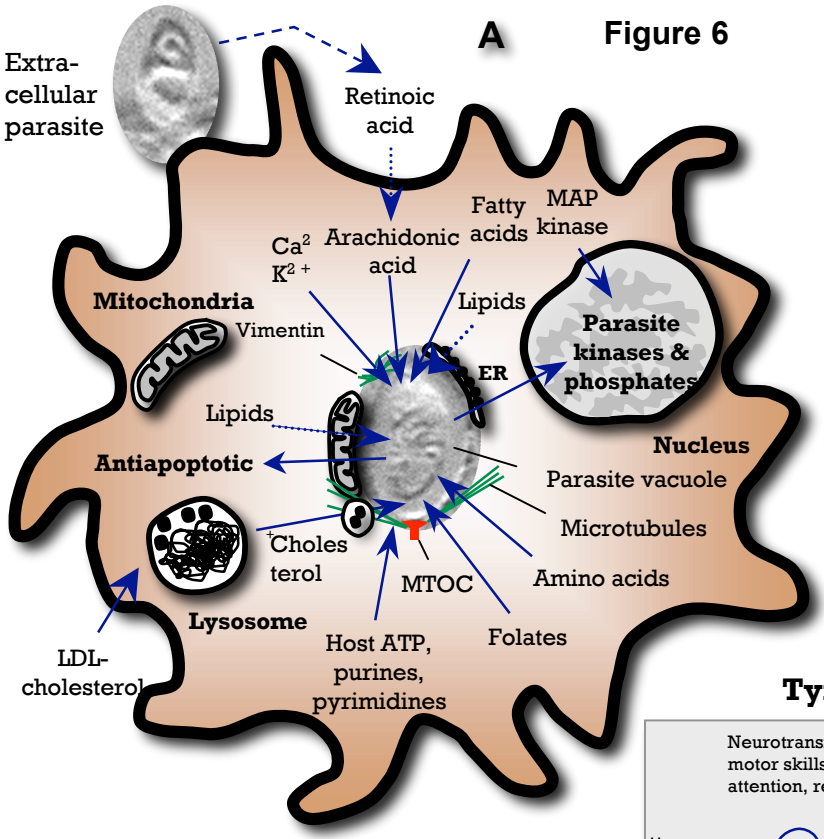
Catalog 1	**Regulation of Host Secreted Factor and Receptor**
	Growth & Inhibitory Factors: BDNF[4], BMP1*, BMP2[4], BMP4[4], BMP6[3], FGF1[4], FGF12[4], FGF14[5], NGFB[4], LIF[3,4], CSF2/GM-CSF[4], CSF/G-CSF[4], CTF1, IGF1[4,8], IGF2[4,8],GRP[4], KITLG[4], HBEGF[2], VEGFB[4], PLAU[2], JAG1[4], FN1[4], NOV[4] **Receptors:** FGFR1[5], NTSR2[8], TFR[1], FLT3[4], PLAUR[10], GRM4[7], AR[4] **Transporters & Channels:** ATP8B1[4], ATP9A[4], FXYD6[8], KCNE2[7], KCNMB4[3], SLC2A1[4], SLC6A4[7], SLC7A1[4], SLC14A1[8], SLC15A4[10*], SLC25A37[3].
Catalog 2	**Regulation of Host Metabolism**
	Cholesterol Signal Pathway: LRP1/ApoER[4], LRP8/ ApoER2[4], LDLR[4], APOC1[4], APOD[4], APBB2[3], B2M[6,9], HDLBP[4], MAPT[8], PAFAH1B1/LIS1[4], CD36[6], DAB1[5], DAB2[5], DAB2IP[5], PLTP[4], MSR1[8*], MSR2[8], STAB1[8]. **Cholesterol Metabolism:** ACADL[10], ACAT1[4], ACOX2[4], ACSL1[4], CH25H[4], EBP[4], FDPS[4], GAPDH[10], HMGCR[4], HMGCS1[4], NSDHL[4], SC5D[4], SOAT1[4], SQLE[10] **Purine & Pyrimidine Metabolism:** DPYD[10], MTAP[10], NME1[10], NME2[10], NME6[10], POLR1D[10*] POLR2D[10], TYMS[10], UMPS[10], CECR1[4*], CECR5[4], GCH1[2,3]. ***Glutamate:*** GLS[8] ***Tryptophan:*** HADHA[10], KYNU[4] ***Tyrosine:*** ALDH1A1[8], ALDH3B2[4], COMT[5], DCT[2], MYST4[10*]. ***Glycine, serine and threonine:*** GARS[4], HSD17B12[4], PSAT1[4] **Histidine:** ALDH3B2[4] **Lysine:** KARS[10], HADHA[10], ACAT1[4] **Arachidonic & Retinoic acid:** LTC4S[8], PTGIS[3,4], PTGS2[2], SEPT3[8], CCND3[10], TNFAIP2[2], ALOX5[3*]
Catalog 3	**Regulation of Host Extracellular Matrix & Adhesion**
	Collagen Fibrils: COL1A[4], COL1A2[4], COL3A1[4], COL4A1[4*], COL4A2[4], COL5A1[4], COL5A2[4], COL5A3[4], COL6A1[3,4], COL6A2[4], COL8A2[4], COL14A1[4], COL15A1[4], CD36[6], BGN[4], FN1[4], LUM[3,4]. **Integrin:** ITGA2[2], ITGA5[1], ITGA6[8], ITGA8[3], ITGB3BP[7], CIB1[10], CD53[2] **Laminin:** LAMA5[7], LAMB3[3] **ECMs:** CHI3L1[8], DCN[8], FBLN1[4], FBLN2[4], FBN1[4], FBN2[4], HPSE[10], LGALS3BP, MATN4[4], MFAP2[4], MFAP5[4], PRELP[4], TFPI2[2,4], KAL1[4] **Proteases**: ADAMTS5[4*], ADAMTS12[8], ADAMTS15[8], MMP3[2,8], PRTN3[10*], SERPINA3N[6,8], SERPINB2[2], SERPING1[6,11,12], SLPI[6], THBD[2], CTSL[3,10], CTSO[4], ITIH3[8]. **Cell Adhesion:** AGRN[11], CD274[6*], CDH22[8], CLDN2[8], DDR1[1], DSC2[7], EMR1[6], HNT[8], ICAM1[2,9*], LY6A[8*], MPZL1, NINJ1[2], PCDH18[3], PCDHB20[8], PLXNC1[11,12], SELE[3], TSPAN2[4], VCAM1[9]. **Cell Junction:** GJA7[7], TJP1[4]
Catalog 4	**Regulation of Host Cytoplasmic Signaling**
	Calcium-Phosphoinositol Pathway: TRPC6[5], STC1[2], CALB1[8], GAP43[8], PVALB[8], S100B[8], CLSTN3[3], DGKZ[7], LCK[10], PIK3CG[10], PIP5K2A[10], PRKCB1[10], PLA2G7[7], PPAP2B[4], PTPN13[3], RGS4[3], SCG2[8], NOS3[10], S100A12[10*], GNA11[3*] **MAPK Pathway:** FAS[4], TRAF2[4], MAP2K1[1,10], MAP2K6[4], MAP3K1[3], DDIT3[10], CASP3[1], NFKBIA[2,10], DUSP5[2], DUSP6[2], GADD45A[1], HSPA8[10], MAPT[8], STK4 **Apoptosis:** CFLAR[10], FADD[10], PMAIP1[2]. See other panels: CASP3[1],FAS[4], IL1B[2], MYD88[11], NFKB1[2], NFKB2[10], NFKBIA[2,10], NGFB[4], PIK3CG[10],TNFSF10[11], TRAF2[4] **Cell cycle:** BUB1[8], CDC45L[10], ORC4L[10], CCND3[10], GADD45A[1], PCNA[10], PRKDC[1],PSMA2[11], PSMA3[11], PSMB10[8], PSMB8[6,8,10], PSMB9[6,8], PSMD14[10], PSME1[6,10], TYMS[10] **Not included in analysis:** JAK-STAT & Toll Pathway

Catalog 5	**Regulation of Host Cytoskeleton**
	Intermediate Filament: GFAP[6] **Microtubule & Centrosome**: HOOK2[3], PAFAH1B1/LIS1[4], SPRY2[2], ASPM[8], POLB[10], CROCC[7], RACGAP1[8], MAPT[8] **Microfilament:** ABLIM1/2[3], ADD3[4], ARPC2[10], CAP1[10], CD82[3], CNN3[10], DISC1[5], DSTN[10], ENC1[4], FRMPD1[7], LCP1[3], MTSS1[3], MYLIP[4], STK17B[10], TNNT3[7]
Catalog 6	**Regulation of Host Nucleus**
	Transcriptional Factors & Regulators: ASCL1[8], ATF3[2], CEBPG[4], E2F7[8], EGR1/Krox24[2], EGR2/Krox20[2], ETV4[4], FOXA1[3], FYB[6], HEY2[7], HIF1A[4], JUN[4], MAFB[6], MAFG[3], MAX[10], MEOX1[3], MSX2[2], MYB[8], MYC[2,10], NFKB2[10], NR2F2[10], NR4A2[2], RELB[2], RORB[7], STAT1[4,6,8,10,11,12], STAT3[4], STAT4[11,12], STAT6[10], TAF11[10], ZNF179[7], NFE2[10], NFE2L3[3], ELK3[10*], NFKB1[2*], ZNF382[10*], ZNF436[4*], ZNF659[3*] **Chromatin Regulation & Remodeling:** ARID2[7], BNIP3[1], FOS[10], GADD45A[1], LOX[3], MEOX1[3], MKI67[8], MX2[11,12], MYST4[10], PARP14[6], PCNA[10], STK4[3], TOPORS[10], APBB2[3], CEBPG[4], FOXA1[3], HEY2[7], HIF1A[4], JUN[4], MAFG[3], MYC[2,10], STAT1[4,6,8,10,12,11], H1FX[10*] **DNA Repair:** LBA1[4], PRKDC[1], TTRAP[10], CEBPG[4], GADD45A[1], PCNA[10], POLB[10], POLR2D[10], TYMS[1] **Nuclear Pore:** XPO7[3] **See Purine & Pyrimidine Metabolism**
Catalog 7	**Regulation of Host Cytokine**
	Interleukin & Receptors: IL1B[2], IL4[9], IL6[1,2,9], IL8[1,9*], IL23A[3], IL24[4], , IL2RG[10*], IL8RA[10], IL18BP[8], CLCF1[3], TSLP[3]. Not available in ABA **Chemokine & Receptor:** CXCR3[11,12], CXCR4[10*]; **(C motif):** XCL1[4]; **(C-C motif)**: CCL1[3], CCL2[1,2,9,1], CCL5[4,6,8], CCL7[2,11,12], CCL8[4,6,11,12], CCL9[8], CCL11[3,4], CCL20[3], CCL26[3,4*]; **(C-X-C motif):** CXCL1[2,4], CXCL2[1,2,3,4,9], CXCL3[4*], CXCL4[8], CXCL5[1], CXCL6[4*], CXCL9[8,10,11,12], CXCL10[8], CXCL11[8,11,12], CXCL12[10], CXCL14; **(C-X-C moti)f:** CX3CL1. **Transforming growth factor beta:** AMH[4], INHBB[4], MYD88[11], BMP2[4], BMP4[4], BMP6[3], CXCL11[8,11,12], MYC **Interferon:** IFNA4[7], IFI16[4], IFI30[10], IFI47[6,8], IFI203[8], IFI205[8], IFIT1[8,10,11,12], IFIT2[11,12], IFIT3[6,8,10,11,12], IFITM1[11,12], IFITM3[10,11], IGTP[6,8], IRF4[11,12], IRF7[11], IRGM[8], ISG15[11,12], ISG20[11,12], IFNA21[7*], IFI44L[11*], IFIT4[10*], IFITM2[3*], IGTP1[6,8*], IGTP2[6,8*], IRF1[2,8*], GBP2[6,8*] **Tumor necrosis factor:** TNFSF10[11], TNFAIP2[2], TNFAIP3[2], TNFAIP6[1], FAS[4], TRAF1[4], TRAF2[4] **Cytokine -** CSF2[4], CSF3[4], CTF1[4], FLT3[4], KITLG[4], LIF[2,4], VEGFB[4]
	Regulation of Host Antigen Presentation & Processing
	Histocompatibility 2 class II antigens: H2-Aa[8], H2-AB1[8], H2-DMA[8], H2-DMB1[8], H2-DMB2[8], H2-EA[8], H2-EB1[8], H2-Q8[8], H2-T10[8], **Immunoglobulin G:** IGHG[10], IGJ[6], IGSF4D[8] **Agn Processing:** B2M[6,9], CD74[8], CTSL[3,10], HSP90AB1[10], HSPA8[10], IFI30[10], MR1[10], IFNA21[7], IFNA4[7], PSMB8[6,8], PSMB9[6,8], PSME1[6,10], RELB[2], TAP1[8], TAP2[6], TAPBP[1], H2-D1[8*], H2-K[8*], H2-L1[8*], H2-Q1[8*], H2-Q7[8*], IGH-4[6*], IGHG2C[6*], IGHV1S44[6*], IGHV1S46[6*], IGH-VJ558[6*], IGL-V1[6*], HLA-A201[9*], HLA-DMB[1*], HLAF[11,12*], HLAJ[11*], CD1A[7*]
	Supplement (miscellaneous)
	C3, PEBP1, SERTAD4, DPM1*, MT-ND1*,MVK*, PHGDHL1*, SYN2*, TPH2*, SOD2, PCK2, PRG1, PSAP*, RPS15*

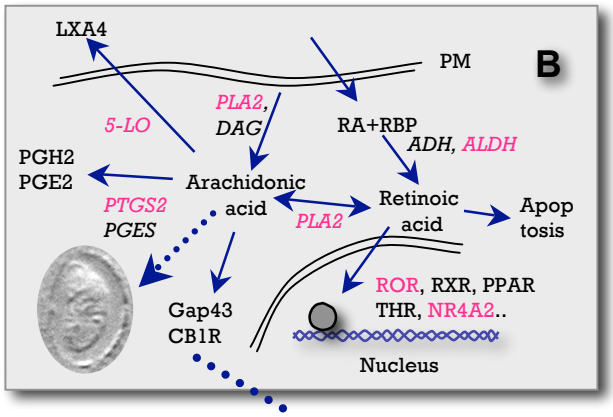
Table 3. Potential interference of neurotransmitter metabolism by *Toxoplasma*.

Toxoplasma-regulated gene	Acetylcholine	GABA	Dopamine	DOPA	Glycine	Aspartate	Glutamate	Epinephrine	Norepinephrine	Serotonin	Histamine	Thyroxine
ACADL[10]		√										
ACAT[4]		√					√					
ALDH3B2[4]			√	√		√		√	√			√
ASNS[4]					√	√	√					
ASS1[4]		√				√	√					
CA5A[7]					√	√	√					
COMT[5]			√	√				√	√			√
CSF2[4]											√	
DCT[2]				√				√	√			√
DGKZ[7]	√											
DPYD[10]		√				√						
GARS[4]					√	√	√					
GLS2[8]		√			√	√	√					
GNA11[3]			√				√		√	√		
GRM4[7]	√	√	√		√	√	√	√	√	√	√	√
GRP[4]	√	√	√		√	√	√	√	√	√	√	√
GSTM2[10]					√		√					
HADH[10]		√				√	√			√		
HADHA[10]		√				√	√					
HMGCS1[4]		√					√					
HSD17B12[4]	√	√			√	√	√					
IDH[10]					√		√					
IDH2[10]					√		√					
IGF1[4,8]							√					
IL4[9]											√	
KYNU[4]										√		
MAP2K1[1,10]			√				√		√	√	√	
MAP2K6[4]											√	
MDH1[10]						√						
MYST4[10]	√		√	√				√	√			√
NME1[10]					√							
NME2[10]					√							
NME6[10]					√							
NTSR2[8]	√	√	√		√	√	√	√	√	√	√	√
PAFAH1B1[4]	√											
PBEF1[4,2]						√						
PGK1[10]						√						
PIK3CG[10]											√	
PIP5K2A[10]	√											
PLA2G7[7]	√											
POLR1D[10]					√							
POLR2D[10]					√							
PPAP2B[4]	√											
PRKCB1[10]			√				√		√	√	√	
PSAT1[4]					√	√						
SOAT1[4]					√							
TAP2[6]							√					
TJP1[4]			√				√		√	√		
TKT[4]						√						

Figure 6



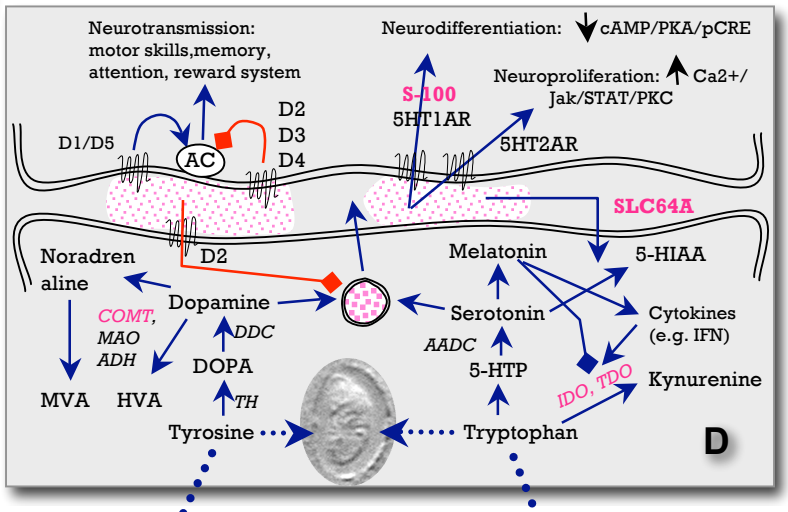
Arachidonic /retinoic acid depletion



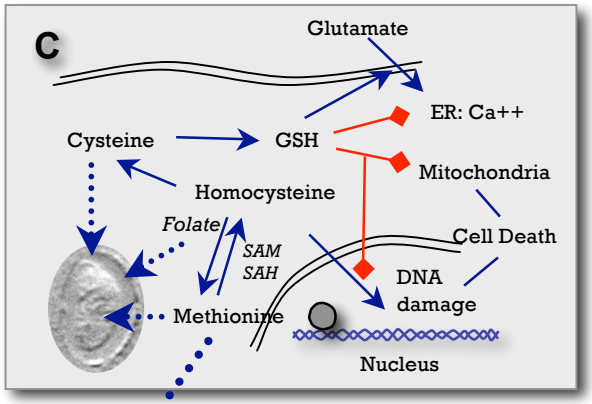
Neurodevelopment, neurotransmission, Endocannabinoid neuromodulation

Hypofunction retinol and arachidonic acid signaling in Schizophrenia, Alzheimer and Parkinson conditions.

Tyrosine & tryptophan depletion



Folate depletion



Folate deficiency/high homocysteine

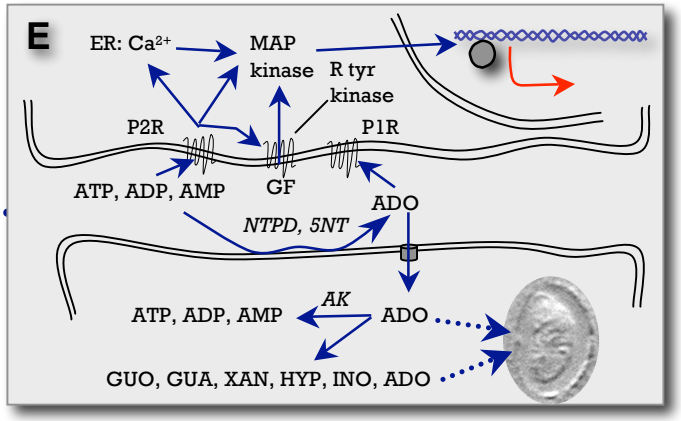
Cell cyle arrest in neural stem cell, impaired neuro- genesis and gliogenesis, synaptic dysgenesis, impaired myelination, neuronal and glial cell death, synaptic dysfunction

Development: Spina bifida, Meningocoele, Anencephaly, Autism
Adult: Depression, Schizophrenia
Aging: Alzheimer's Disease, Parkinson's Disease,

Alteration in multiple genes and pathways in psychosis converge to the hyperactivity of DRs, particularly DR2

Low level of circulating serotonin is associated with psychosis suicide, depression and HIV dementia, altered serotonergic circuitry in the PFC of Alzheimer's brains.

Purine/adenosine depletion



Nucleotide: stimulate cell proliferation, migration, differentiation, neurite extension, myelination, synapse formation, synaptic network, induction of cell death.

Figure 7

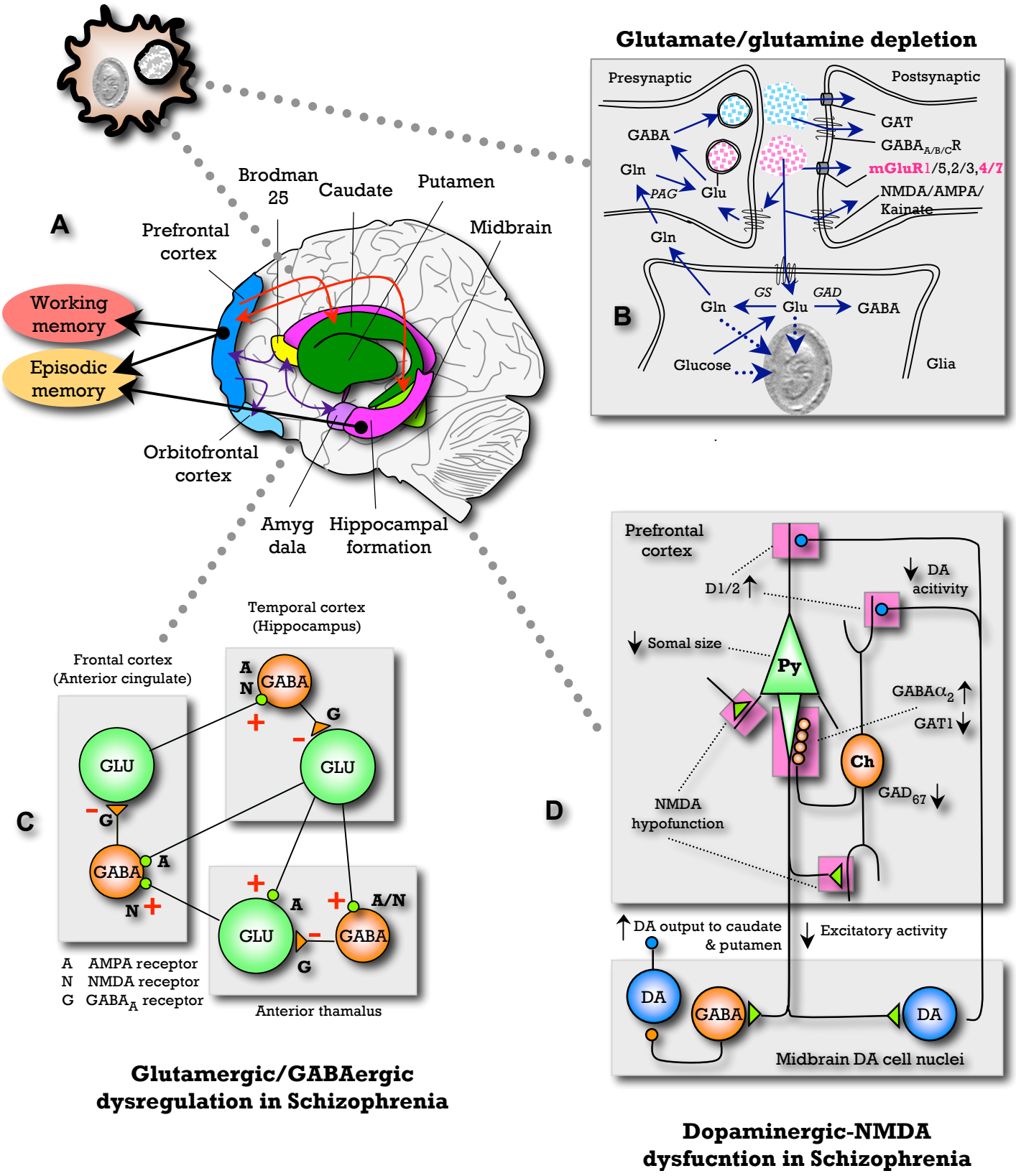
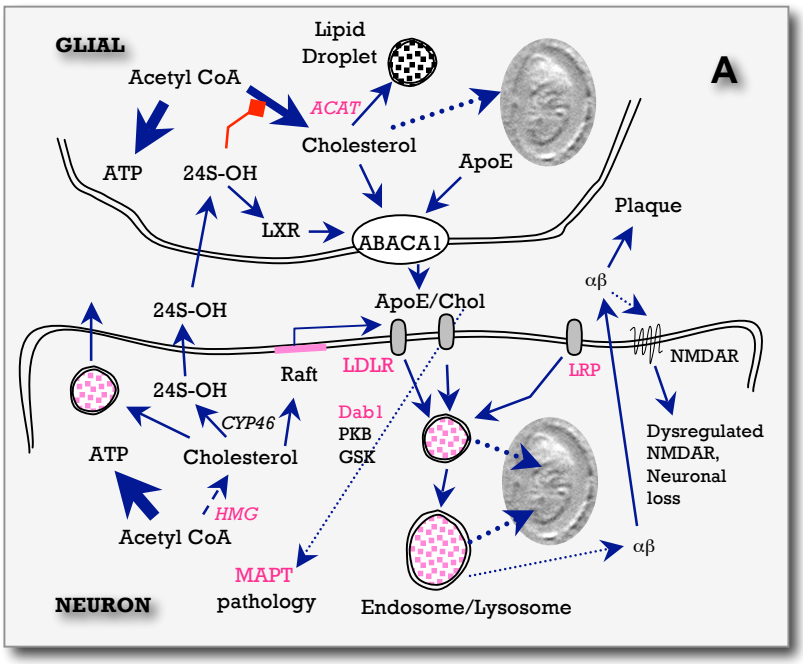
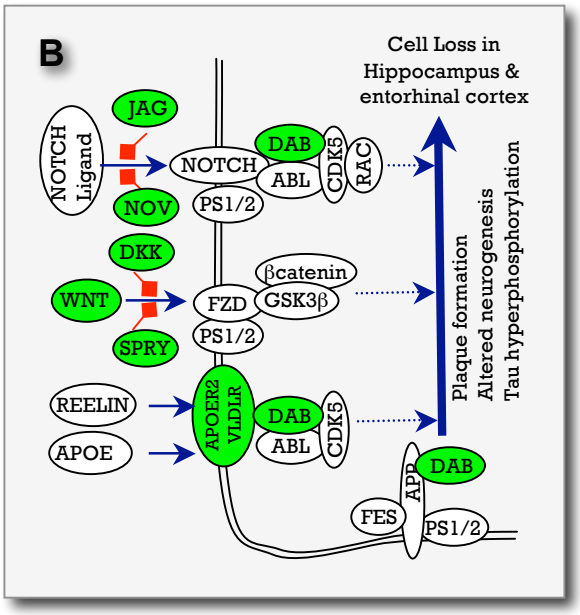

A
Brodman 25
Caudate
Putamen
Prefrontal cortex
Midbrain
Working memory
Episodic memory
Orbitofrontal cortex
Amyg dala
Hippocampal formation
Glutamate/glutamine depletion
Presynaptic
Postsynaptic
GABA
GAT
GABA$_{A/B/C}$R
Gln
PAG
Glu
mGluR1/5,2/3,4/7
NMDA/AMPA/ Kainate
Gln
B
GS
GAD
Gln
Glu
GABA
Glucose
Glia
Temporal cortex (Hippocampus)
Frontal cortex (Anterior cingulate)
C
A N
GABA
G
GLU
GLU
GABA
A
N
A
A/N
GLU
GABA
G
A AMPA receptor
N NMDA receptor
G GABA$_A$ receptor
Anterior thamalus
Glutamergic/GABAergic dysregulation in Schizophrenia
Prefrontal cortex
D1/2
DA acitivity
Somal size
Py
GABAα_2
GAT1
Ch
GAD$_{67}$
NMDA hypofunction
D
DA output to caudate & putamen
Excitatory activity
DA
GABA
DA
Midbrain DA cell nuclei
Dopaminergic-NMDA dysfucntion in Schizophrenia

Figure 8

Cholesterol & alipoprotein depletion



Alzheimer's disease model



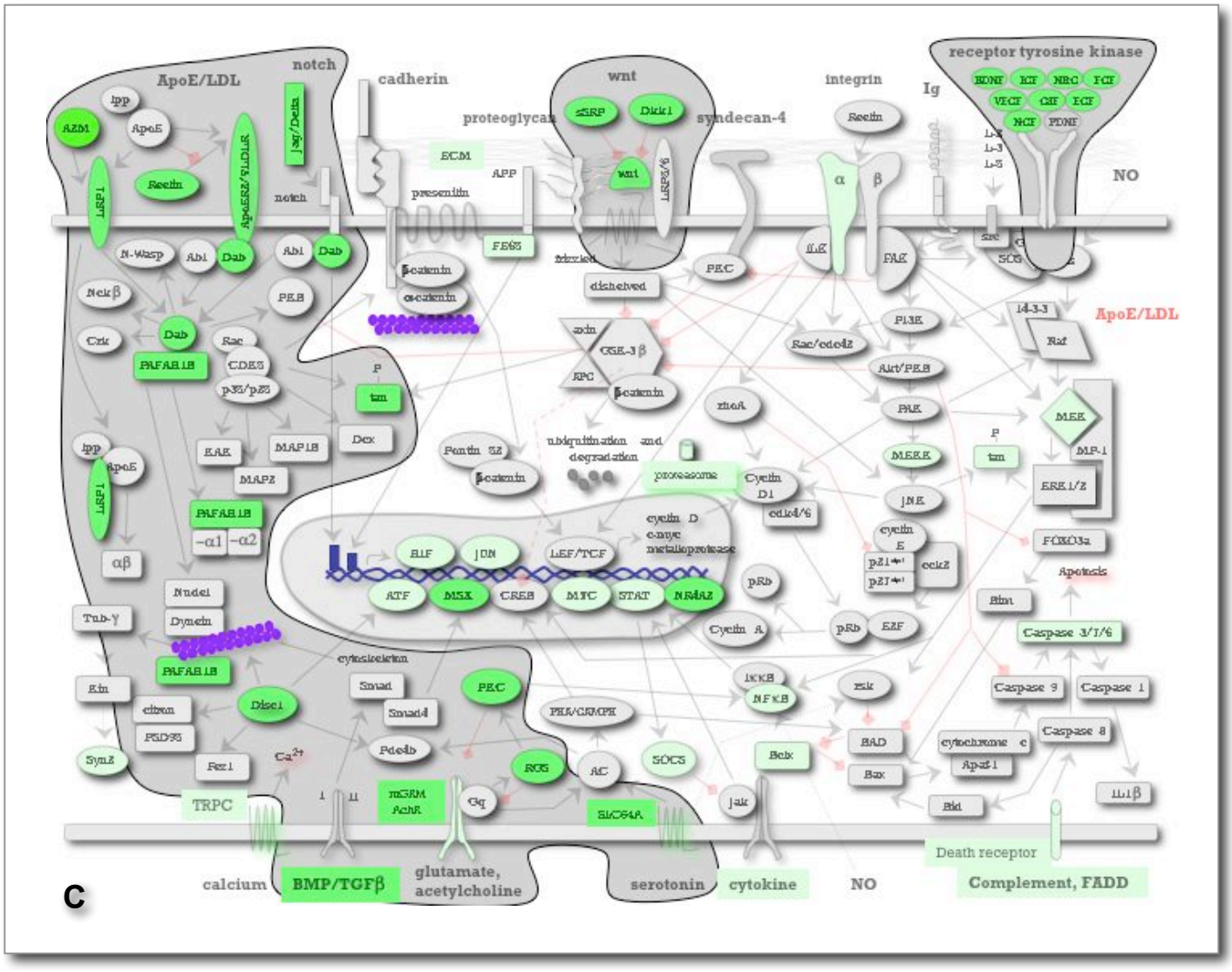
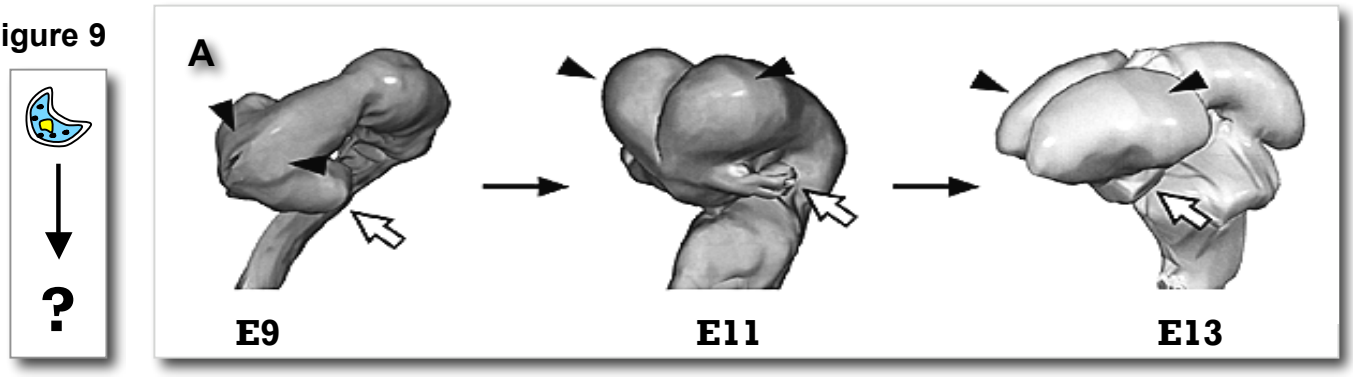
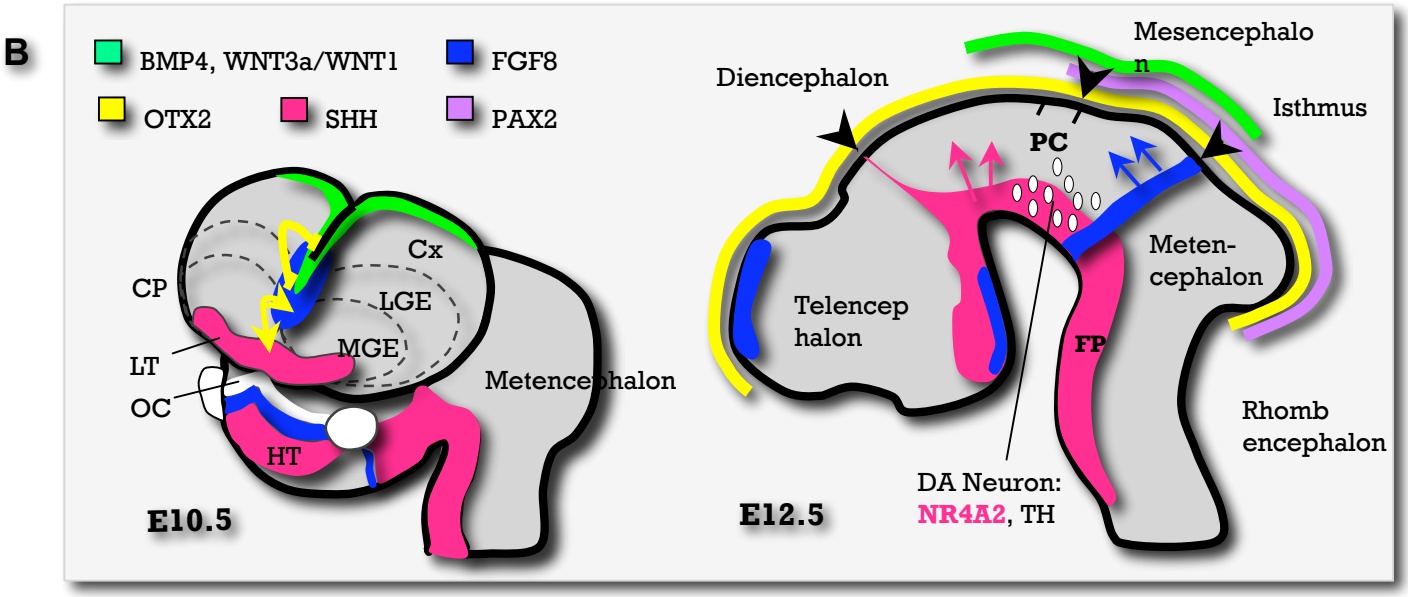
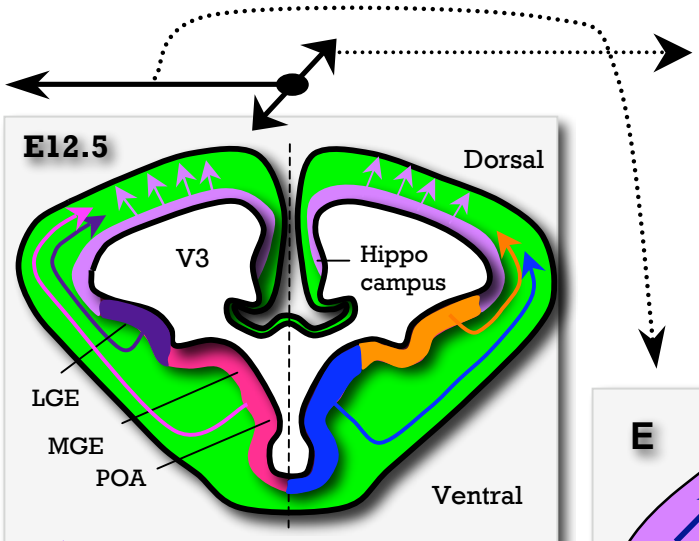
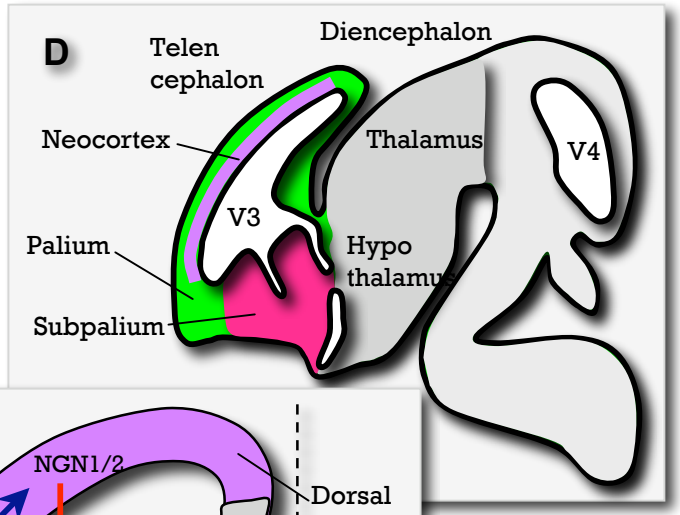


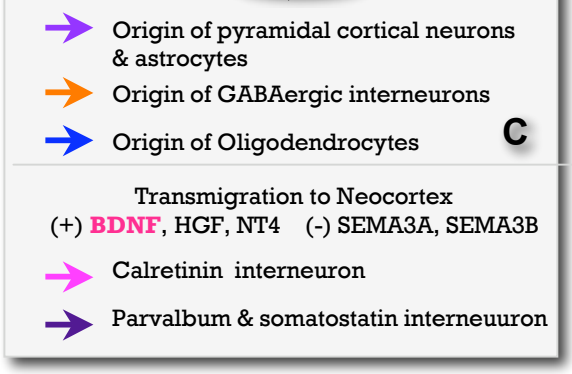
Figure 9


A
?
E9
E11
E13


B
BMP4, WNT3a/WNT1
FGF8
OTX2
SHH
PAX2
CP
Cx
LGE
MGE
LT
OC
HT
Metencephalon
E10.5
Diencephalon
Mesencephalon
Isthmus
PC
Telencephalon
Meten-cephalon
FP
Rhomb encephalon
DA Neuron:
NR4A2, TH
E12.5


E12.5
Dorsal
V3
Hippo campus
LGE
MGE
POA
Ventral


D
Telen cephalon
Diencephalon
Neocortex
Thalamus
V4
V3
Palium
Hypo thalamus
Subpalium


Origin of pyramidal cortical neurons & astrocytes
Origin of GABAergic interneurons
Origin of Oligodendrocytes
C
Transmigration to Neocortex
(+) BDNF, HGF, NT4 (-) SEMA3A, SEMA3B
Calretinin interneuron
Parvalbum & somatostatin interneuuron

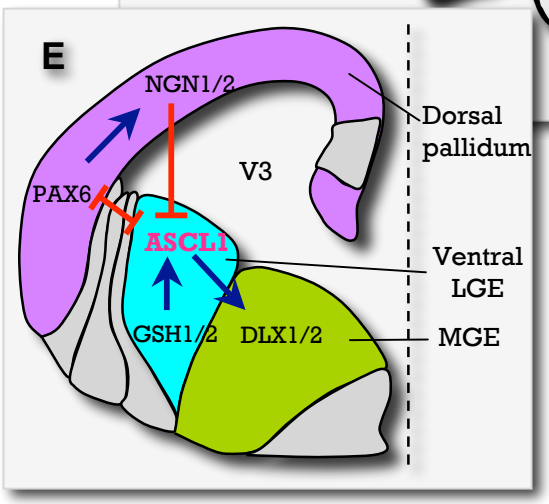

E
NGN1/2
Dorsal pallidum
V3
PAX6
ASCL1
Ventral LGE
GSH1/2
DLX1/2
MGE

Figure 10

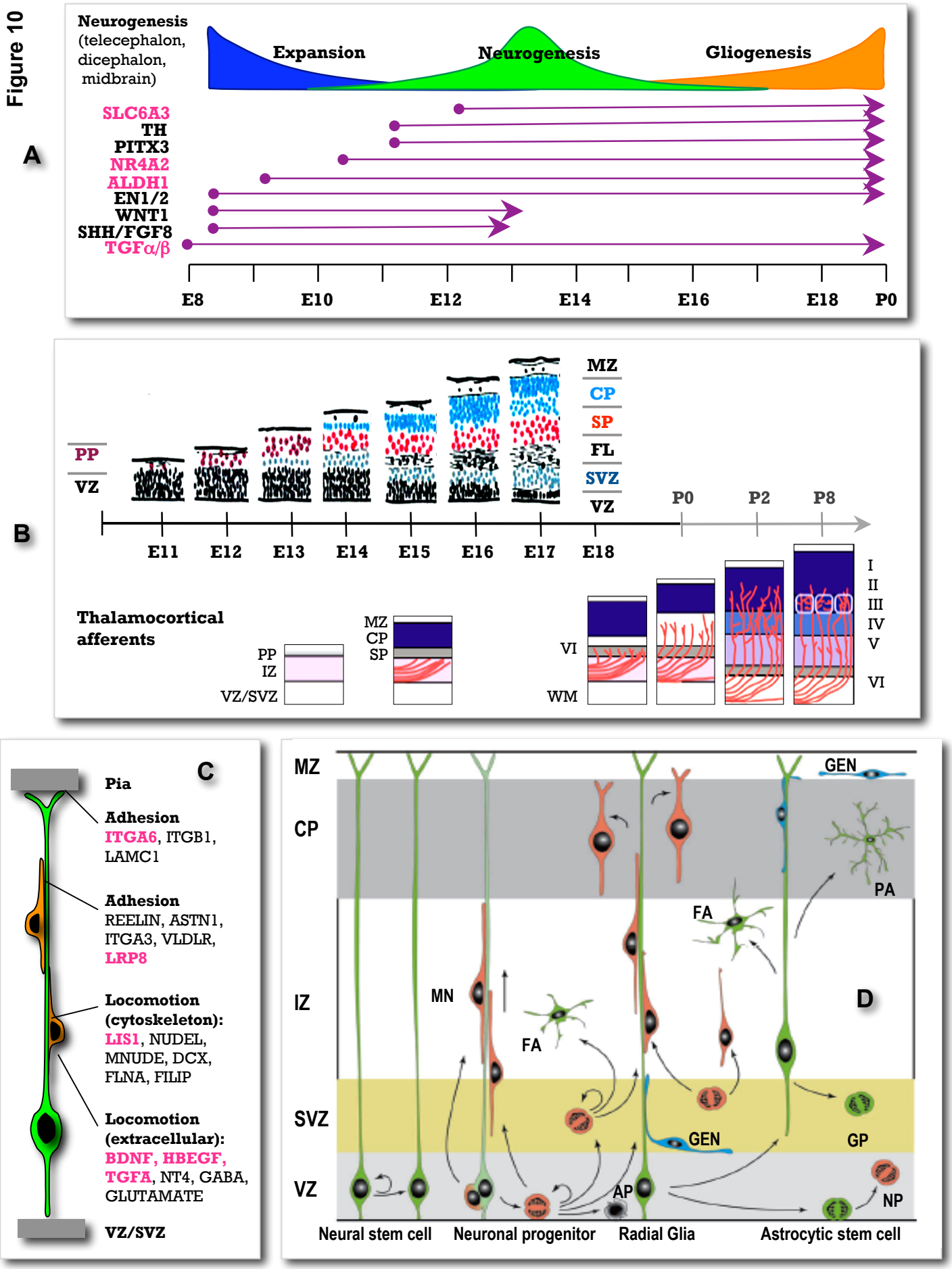

A
Neurogenesis
(telecephalon,
dicephalon,
midbrain)
Expansion
Neurogenesis
Gliogenesis
SLC6A3
TH
PITX3
NR4A2
ALDH1
EN1/2
WNT1
SHH/FGF8
TGFα/β
E8
E10
E12
E14
E16
E18
P0
B
PP
VZ
MZ
CP
SP
FL
SVZ
VZ
E11
E12
E13
E14
E15
E16
E17
E18
P0
P2
P8
Thalamocortical
afferents
PP
IZ
VZ/SVZ
MZ
CP
SP
VI
WM
I
II
III
IV
V
VI
C
Pia
Adhesion
ITGA6, ITGB1,
LAMC1
Adhesion
REELIN, ASTN1,
ITGA3, VLDLR,
LRP8
Locomotion
(cytoskeleton):
LIS1, NUDEL,
MNUDE, DCX,
FLNA, FILIP
Locomotion
(extracellular):
BDNF, HBEGF,
TGFA, NT4, GABA,
GLUTAMATE
VZ/SVZ
MZ
CP
IZ
SVZ
VZ
GEN
PA
FA
MN
FA
D
GEN
GP
AP
NP
Neural stem cell
Neuronal progenitor
Radial Glia
Astrocytic stem cell

Figure 11

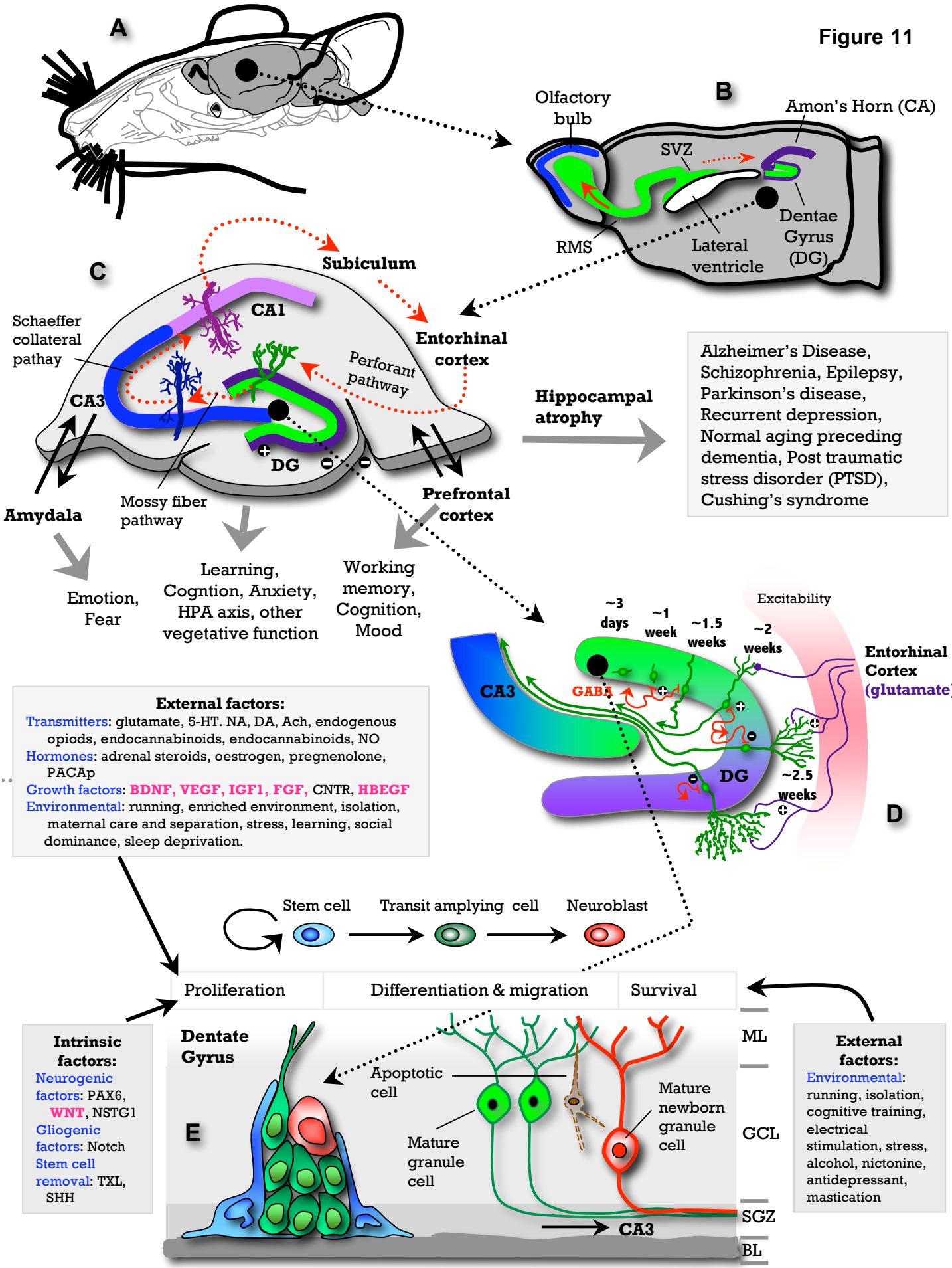

A
B
Olfactory bulb
Amon's Horn (CA)
SVZ
RMS
Lateral ventricle
Dentae Gyrus (DG)
C
Subiculum
Entorhinal cortex
Schaeffer collateral pathay
CA1
Perforant pathway
CA3
DG
Amydala
Mossy fiber pathway
Prefrontal cortex
Hippocampal atrophy
Alzheimer's Disease, Schizophrenia, Epilepsy, Parkinson's disease, Recurrent depression, Normal aging preceding dementia, Post traumatic stress disorder (PTSD), Cushing's syndrome
Emotion, Fear
Learning, Cogntion, Anxiety, HPA axis, other vegetative function
Working memory, Cognition, Mood
External factors:
Transmitters: glutamate, 5-HT. NA, DA, Ach, endogenous opiods, endocannabinoids, endocannabinoids, NO
Hormones: adrenal steroids, oestrogen, pregnenolone, PACAp
Growth factors: BDNF, VEGF, IGF1, FGF, CNTR, HBEGF
Environmental: running, enriched environment, isolation, maternal care and separation, stress, learning, social dominance, sleep deprivation.
~3 days
~1 week
~1.5 weeks
~2 weeks
Excitability
Entorhinal Cortex (glutamate)
CA3
GABA
DG
~2.5 weeks
D
Stem cell
Transit amplying cell
Neuroblast
Proliferation
Differentiation & migration
Survival
Intrinsic factors:
Neurogenic factors: PAX6, WNT, NSTG1
Gliogenic factors: Notch
Stem cell removal: TXL, SHH
Dentate Gyrus
E
Apoptotic cell
Mature granule cell
Mature newborn granule cell
ML
GCL
SGZ
BL
CA3
External factors:
Environmental: running, isolation, cognitive training, electrical stimulation, stress, alcohol, nictonine, antidepressant, mastication

Figure 12 Allen Brain Atlas Mouse Brain

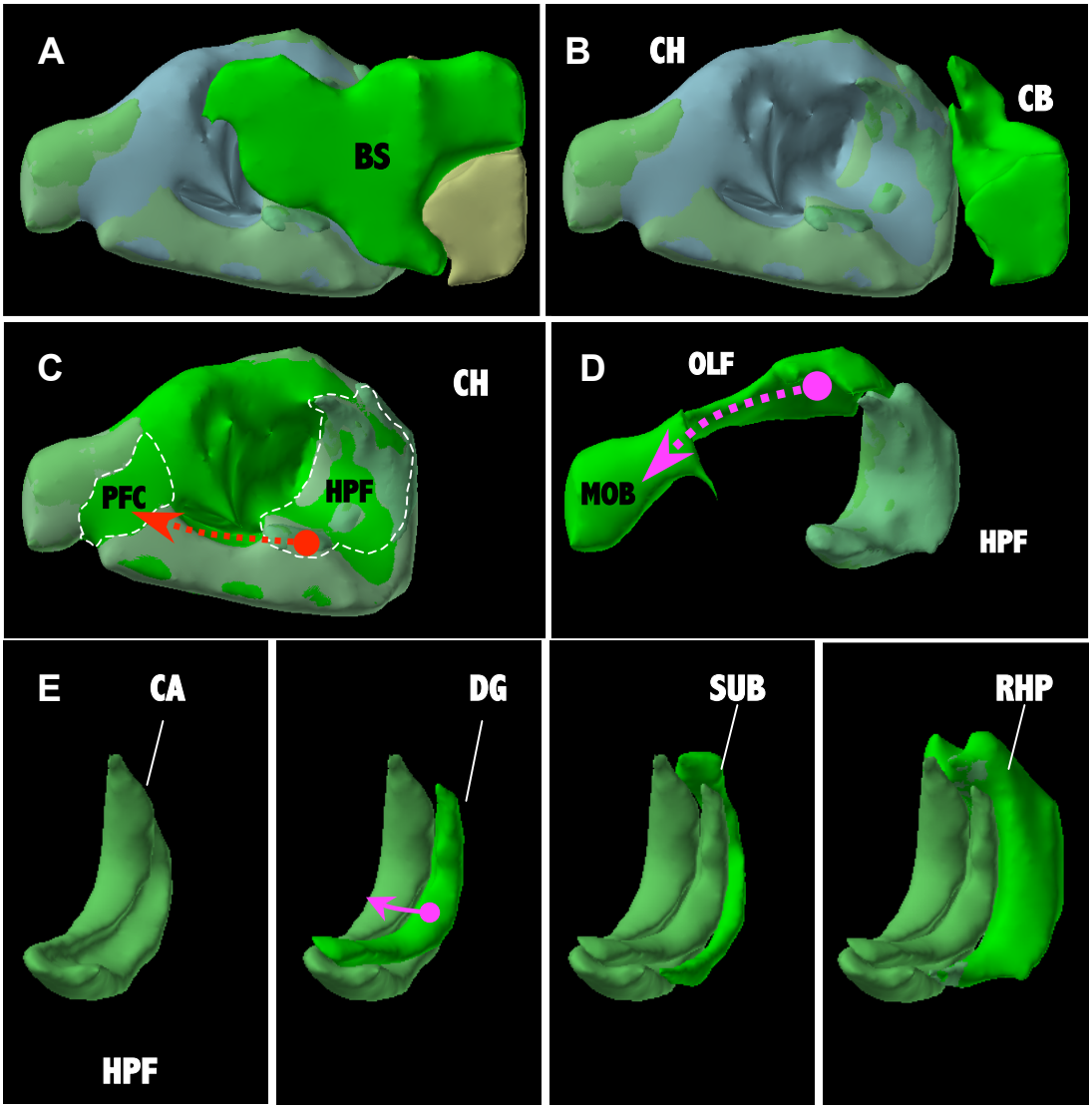


Figure 13

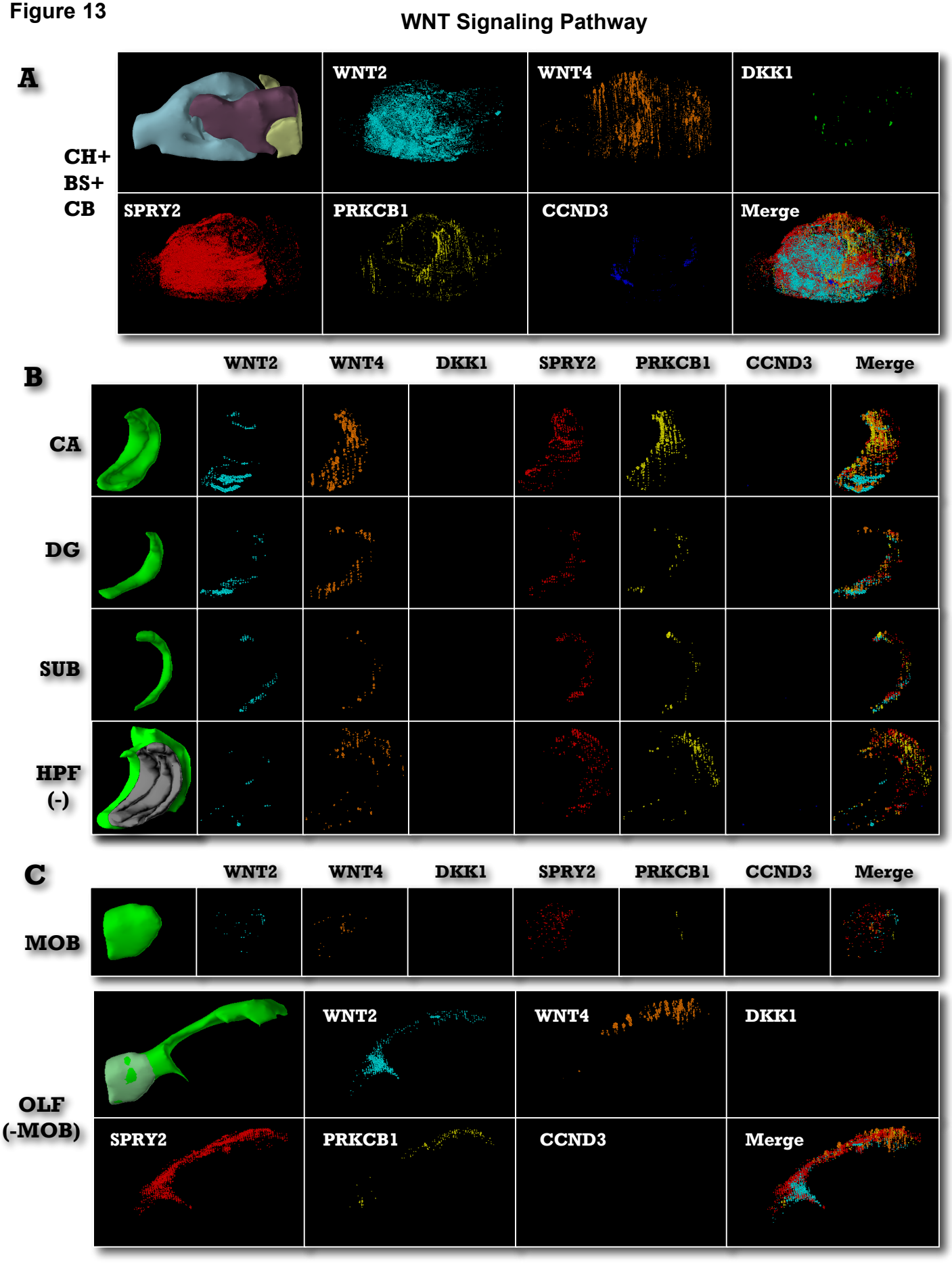

WNT Signaling Pathway
A
CH+
BS+
CB
WNT2
WNT4
DKK1
SPRY2
PRKCB1
CCND3
Merge
B
WNT2
WNT4
DKK1
SPRY2
PRKCB1
CCND3
Merge
CA
DG
SUB
HPF
(-)
C
WNT2
WNT4
DKK1
SPRY2
PRKCB1
CCND3
Merge
MOB
OLF
(-MOB)
WNT2
WNT4
DKK1
SPRY2
PRKCB1
CCND3
Merge

Figure 14 Cholesterol Metabolism, Cholesterol & Notch Signaling Pathway

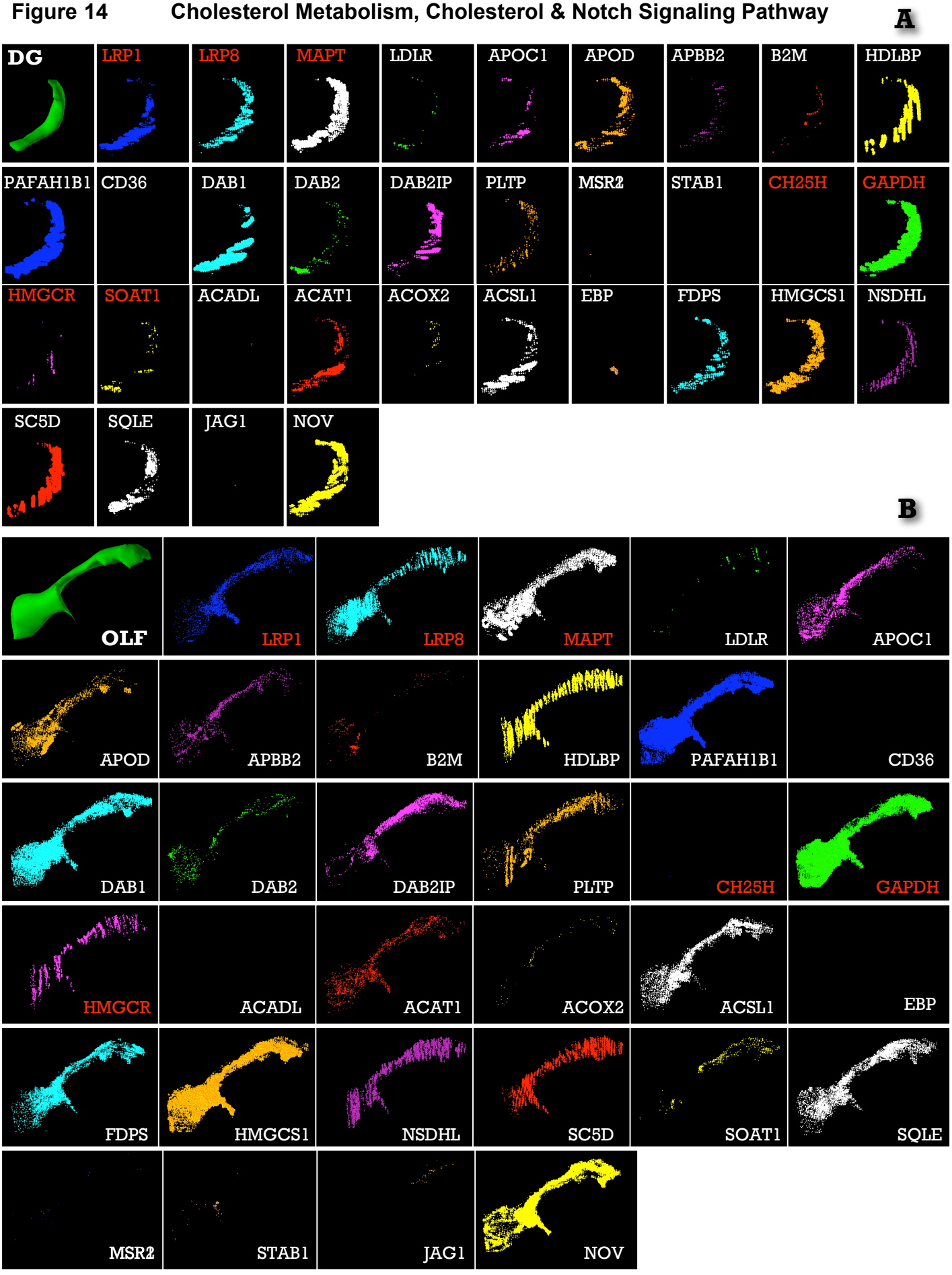
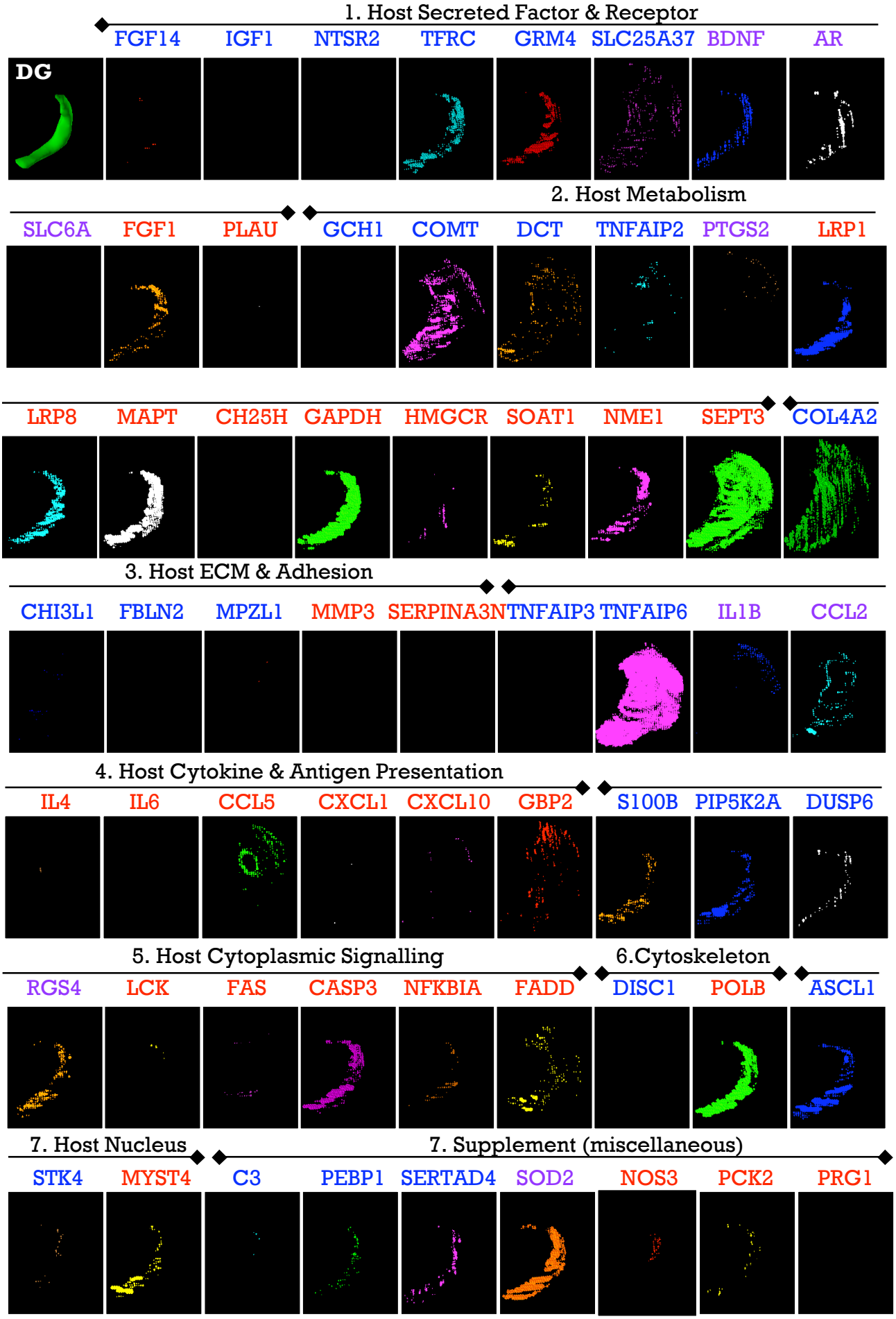


Figure 15


1. Host Secreted Factor & Receptor
FGF14
IGF1
NTSR2
TFRC
GRM4
SLC25A37
BDNF
AR
DG
2. Host Metabolism
SLC6A
FGF1
PLAU
GCH1
COMT
DCT
TNFAIP2
PTGS2
LRP1
LRP8
MAPT
CH25H
GAPDH
HMGCR
SOAT1
NME1
SEPT3
COL4A2
3. Host ECM & Adhesion
CHI3L1
FBLN2
MPZL1
MMP3
SERPINA3N
TNFAIP3
TNFAIP6
IL1B
CCL2
4. Host Cytokine & Antigen Presentation
IL4
IL6
CCL5
CXCL1
CXCL10
GBP2
S100B
PIP5K2A
DUSP6
5. Host Cytoplasmic Signalling
6.Cytoskeleton
RGS4
LCK
FAS
CASP3
NFKBIA
FADD
DISC1
POLB
ASCL1
7. Host Nucleus
7. Supplement (miscellaneous)
STK4
MYST4
C3
PEBP1
SERTAD4
SOD2
NOS3
PCK2
PRG1