Université de Montréal

METABOLOMICS ANALYSIS IN RATS WITH THIAMINE DEFICIENCY IDENTIFIES KEY METABOLITES IN VULNERABLE BRAIN REGIONS AND SUGGESTS NEURAL STEM-PROGENITOR CELLS PLAY A ROLE IN AMELIORATING METABOLIC DYSFUNCTION

par

Ashraf Azar

Programme de Sciences biomédicales

Faculté de médecine

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RÉSUMÉ

La documentation scientifique fait état de la présence, chez l'adulte, de cellules souches et progénitrices neurales (CSPN) endogènes dans les zones sous-ventriculaire et sous-granulaire du cerveau ainsi que dans le gyrus denté de l'hippocampe. De plus, un postulat selon lequel il serait également possible de retrouver ce type de cellules dans la moelle épinière et le néocortex des mammifères adultes a été énoncé. L'encéphalopathie de Wernicke, un trouble neurologique grave toutefois réversible qui entraîne un dysfonctionnement, voire une défaillance du cerveau, est causée principalement par une carence importante en thiamine (CT). Des observations récentes laissent envisager que les facteurs en cause dans la prolifération et la différenciation des CSPN pourraient également jouer un rôle important lors d'un épisode de CT.

L'hypothèse, selon laquelle l'identification de nouveaux métabolites entrant dans le mécanisme ou la séquence de réactions se soldant en une CT pourraient en faciliter la compréhension, a été émise au moyen d'une démarche en cours permettant d'établir le profil des modifications métaboliques qui surviennent en de telles situations. Cette approche a été utilisée pour constater les changements métaboliques survenus au niveau du foyer cérébral dans un modèle de rats déficients en thiamine (rats DT), particulièrement au niveau du thalamus et du colliculus inférieur (CI). La greffe de CSPN a quant à elle été envisagée afin d'apporter de nouvelles informations sur la participation des CSPN lors d'un épisode de CT et de déterminer les bénéfices thérapeutiques potentiels offerts par cette intervention.

Les sujets de l'étude étaient répartis en quatre groupes expérimentaux : un premier groupe constitué de rats dont la CT était induite par la pyrithiamine (rats DTiP), un deuxième groupe constitué de rats-contrôles nourris ensemble (« pair-fed control rats » ou rats PFC) ainsi que deux groupes de rats ayant subi une greffe de CSPN, soit un groupe de rats DTiP greffés et un dernier groupe constitué de rats-contrôles (rats PFC) greffés. Les échantillons de foyers cérébraux (thalamus et CI) des quatre groupes de rats ont été prélevés et soumis à des analyses métabolomiques non ciblées ainsi qu'à une analyse visuelle par microscopie à balayage électronique (SEM). Une variété de métabolites-clés a été observée chez les groupes de rats déficients en thiamine (rats DTiP) en plus de plusieurs métabolites dont la documentation ne faisait pas mention. On a notamment constaté la présence d'acides biliaires, d'acide cynurénique

et d'acide 1,9— diméthylurique dans le thalamus, alors que la présence de taurine et de carnosine a été observée dans le colliculus inférieur.

L'étude a de plus démontré une possible implication des CSPN endogènes dans les foyers cérébraux du thalamus et du colliculus inférieur en identifiant les métabolites-clés ciblant les CSPN. Enfin, les analyses par SEM ont montré une amélioration notable des tissus à la suite de la greffe de CSPN. Ces constatations suggèrent que l'utilisation de CSPN pourrait s'avérer une avenue thérapeutique intéressante pour soulager la dégénérescence symptomatique liée à une grave carence en thiamine chez l'humain.

ABSTRACT

Endogenous neural-stem progenitor cells (NSPC) have been documented to be found in the subventricular and subgranular zones, the dentate gyrus, and suggestions of the possibility of these cells being found in the spinal cord and neocortex in adult mammalian brain have been postulated. Thiamine deficiency (TD) is the major cause of Wernicke's Encephalopathy, a reversible neurological disorder that results in cerebral dysfunction and impairment. Recent evidence suggests factors involved in neural NSPC proliferation and differentiation are involved during TD.

By means of a current approach for profiling metabolic changes occurring in focal areas of the TD rat brain, specifically the thalamus and the inferior colliculus (IC), it was hypothesized that new metabolites that might offer a better understanding into the sequel and/or mechanism of TD could be identified. It was also considered that the use of NSPC transplantation could offer new information into the involvement of NSPC and potential therapeutic benefit in TD.

Non-targeted metabolomics analysis, fluorescences microscopy, and scanning election microscopy (SEM) analysis visualization was performed on samples of the focal areas (thalamus and IC) of pyrithiamine induced TD rats (PTD), pair-fed controls (PFC) rats, and NSPC transplanted TD and PFC rats. Various key metabolites were identified in rats with TD, including previous undocumented metabolites such as bile acids, kynurenic acid, and 1,9-dimethyluric acid in the thalamus and taurine and carnosine in the IC. The study also demonstrated a possible involvement of endogenous NSPC in focal areas of the thalamus and IC identifying key metabolites targeting NSPC and showed tissue amelioration (observed through SEM) following NSPC transplantation. The findings suggested that NSPC could offer a therapeutic alternative to alleviate some of symptomatic degeneration of TD.

KEYWORD

Non-targeted Metabolomics, Neurochemistry, Thiamine Deficiency, Wernicke's Encephalopathy, Neural Stem-Progenitor Cells, Transplantation

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LIST OF ABBREVIATIONS

AD	:	Alzheimer's disease
AMPA	:	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
BBB	:	Blood-brain barrier
BCKDHC	:	Branched-chain α-ketoacid dehydrogenase
BDNF	:	Brain-derived neurotrophic factor
bFGF	:	Basic fibroblast growth factor
BMEC	:	Brain microvascular endothelial cells
CFSE	:	Carboxyfluorescein diacetate succinimidyl ester
COX	:	Cyclooxygenase
COX-2	:	Cyclooxygenase-2
DHA	:	Docosahexaenoic Acid
EAAT-1	:	Excitatory amino acid transporter-1
EAAT-2	:	Excitatory amino acid transporter-2
ESC	:	Embryonic stem cells
GABA	:	γ-aminobutyric acid
GFAP	:	Glial fibrillary acidic protein
GLT-1	:	Glutamate transporter-1
GLT-1a	:	Glutamate transporter-1 splice variant a
GLT-1b	:	Glutamate transporter-1 splice variant b

GLAST	:	Glutamate/Aspartate transporter
Gro1	:	Growth-regulated oncogene-1
GSH	:	Glutathione
GUDCA	:	Glycoursodeoxycholic acid
IC	:	Inferior colliculus
IL-6	:	Interleukin-6
iPSC	:	Induced pluripotent stem cell
iNOS	:	Inducible nitric oxide synthase
KEGG	:	Kyoto Encyclopedia of Genes and Genomes
KGDHC	:	α-ketoglutarate dehydrogenase complex
Klf4	:	Krüppel-like factor 4
KYNA	:	Kynurenic Acid
LRR	:	Loss of righting reflex
MCP-1	:	Monocyte chemoattractant protein 1
MMP-9	:	Matrix metalloproteinase-9
MPT	:	Mitochondrial permeability transition
MS	:	Mass spectroscopy
MSC	:	Mesenchymal stem cell
mtDNA	:	Mitochondrial DNA
NeuN	:	Neuronal nuclear antigen protein/neuronal marker
NGF	:	Nerve growth factor

NMDA	:	N-methyl-D-aspartate
NSC	:	Neural stem cells
NSPC	:	Neural stem-progenitor cells
Oct4	:	Octamer-binding transcription factor 4
PD	:	Parkinson's disease
PET	:	Positron emission tomography
PFC	:	Pair-fed control
PLS-DA	:	Partial least squares discrimination analysis
PTD	:	Pyrithiamine induced thiamine deficient
ROS	:	Reactive oxygen species
SEM	:	Scanning electron microscopy
SOD	:	Superoxide dismutase
STAT-3	:	Signal transducer and activators of transcription 3
TCA	:	Tricarboxylic acid cycle
TD	:	Thiamine deficiency
TDP	:	Thiamine diphosphate
TGF- α	:	Transforming growth factor α
TK	:	Transketolase
TNF-α	:	Tumor necrosis factor- α
TPP	:	Thiamine pyrophosphate
TSPO	:	Translocator protein

TUDCA	:	Tauroursodeoxycholic
UCB	:	Unconjugated bilirubin
VIP	:	Variable Importance in Projection
WE	:	Wernicke's encephalopathy
WKS	:	Wernicke-Korsakoff syndrome

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1. GENERAL INTRODUCTION

Thiamine deficiency (TD) is a classic model of cellular death often documented in cases of metabolic encephalopathy. Wernicke Encephalopathy (WE), part of the Wernicke-Korskoff syndrome, is a major consequence of TD and is associated with impaired oxidative metabolism. Understanding the mechanisms underlying TD can allow for a better understanding of several diseases including, but not limited to, ischemia (stroke), hematologic tumors, diabetes, Alzheimer's disease (AD) and several other neurodegenerative disorders.

Our understanding of the mechanisms that initiated, propagated, and maintained these disease states have mostly yet to be realized. One proposal to gain further insight into how to treat these disorders favourably has come through the use of stem cells. Stem cells have offered voluminous libraries of information concerning various diseases including neurological disorders. As a result, they are a potential tool for therapeutic treatment.

1.1 Neuropathology in Thiamine Deficiency

The pathophysiological mechanisms of TD, due to its complexity, remains under debate and still unclear. There are four major thiamine-dependant enzyme systems where thiamine diphosphate (TDP) plays an integral role. Also known as thiamine pyrophosphate (TPP or TDP), the cofactor functions as a key contributor to the pentose phosphate pathway enzyme, transketolase (TK). TPP is also a coenzyme for the mitochondrial enzymatic complexes of pyruvate dehydrogenase (PDHC, an essential connector of glycolysis and the TCA cycle), ketoglutarate dehydrogenase (KGDHC, which contributes to the TCA cycle as a rate limiting enzyme), and branched-chain α-ketoacid dehydrogenase (BCKDHC, playing a role in the accumulation of branched chain amino acids leucine, isoleucine, and valine) (Wendel et al., 1983), associated with maple syrup urine disease a rare inborn error of metabolism, heart failure (Sun et al., 2011), obesity, and type 2 diabetes (Wang et al., 2011, Wurtz et al., 2013).

A regionally selective reduction in the levels of the known thiamine-dependant enzymes in the brain are observed with chronic thiamine deprivation(Butterworth, 1986), typically KGDHC and TK. Apart from KGDHC, which is believed to be responsible for the numerous reversible changes associated with TD, the roles of the remaining enzymes are unclear (Gibson et al., 1984). This involvement is apparent in many identified experimental TD cases where the subsequent reduction in TPP (in some cases below 15% of the normal) is associated with activity reduction of KGDHC. This is observed in both human brain tissue (autopsied) of alcoholic patients with confirmed Wernicke-Korsakoff syndrome WKS (Butterworth et al., 1993) and animals with experimental WKS (Heroux and Butterworth, 1995).

The observed histological lesions reflect regional localization of increased lactic acidosis (McCandless, 1982, Hakim, 1984, Munujos et al., 1993). This is a consequence of the production of lactate, a decrease in ATP production, and pyruvate accumulation which follow the inhibition of the oxidative decarboxylation of pyruvate and α-ketoglutarate (Aikawa et al., 1984, Navarro et al., 2005). This occurrence is usually only observed experimentally if the TD is not supplemented with thiamine within the first 24 hours of the onset of the identifiable symptoms.

WE is characterized by cerebral damage and is a direct consequence of thiamine deficiency. Selective histological lesions due to cerebral vulnerability, a major consequence of WE

associated with neuronal loss in diencephalic regions, are common in the thalamus (Victor, 1989). Areas in the midbrain such as the inferior colliculus and other periventricular brainstem regions (including the vestibular nuclei and inferior olivary complex in particular) also exhibit lesions caudally. Several conditions are associated with the nutritional depletion linked to WE and they include systemic diseases (AIDS, renal diseases, thyrotoxicosis, etc.), several types of cancer and their respective chemotherapeutic treatments, gastrointestinal procedures, intestinal obstruction, magnesium metabolic reductions, and the usage of various chemical drugs or compounds (Donnino et al., 2007, Sechi and Serra, 2007). Several mechanisms have been proposed to explain the pathophysiology of the encephalopathy associated with TD (Hazell, 2009a). Some of these include acetylcholine synthesis shifts along with gamma-aminobutyric acid (GABA) level changes (Heroux and Butterworth, 1988), oxidative stress (Hazell and Wang, 2005), alteration in cerebral glucose use, cerebral blood flow changes (Hakim and Pappius, 1983), lactic acidosis and decreased brain pH (Navarro et al., 2005), inflammation (Vemuganti et al., 2006), apoptosis (Matsushima et al., 1997), and excitotoxicity (Hazell, 2009a). Mitochondrial oxidative metabolism, oxidative/nitrosative stress, and the release of proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α(TNF-α) (Gu et al., 2008), have become areas of particular interest in recent research efforts to understand the underlying mechanisms. Hypotheses have emerged concerning the cyclooxygenase (COX) pathway and its involvement in the selective brain damage in WE. The isoform of cyclooxygenase-2 (COX-2) has been observed to selectively increase in specific areas at symptomatic stages of experimental WE compared to pair-fed controls or presymptomatic rats (Gu et al., 2008). This suggested that (COX-2) could play a major role in brain damage, and the accompanying regulation and inflammation. The proposed mechanism is believed to be accomplished through the COX-2 rapid induction of activated glutamatergic N-Methyl-d-Aspartate (NMDA)-dependent synaptic activity (Marcheselli and Bazan, 1996). These findings were confirmed via immunofluorescence analysis (microscopy confirmed immunoreactivity results in the WE brain) and quantified by western blot analysis (Neri et al., 2011).

1.2 Mitochondrial Dysfunction and Neurodegenerative Disease

Mitochondrial dysfunction relates to a broad number of metabolic and degenerative diseases, cancers, and aging. This dysfunction, often disease related, is studied and analyzed from a genetics perspective due to the inherent genetic basis of the disorder. Consequently, gene target therapeutics leads the research on the disorder of the organelle. However, energetic and metabolic dysfunction can play an integral role in the understanding of the overall function the mitochondria plays in the related diseases. For instance energetic dysfunction has been demonstrated to play a role in metabolic and degenerative disease, cancer, and aging (Wallace, 2005, Wallace et al., 2007, Wallace, 2008). Mutations in the genes of mitochondrial DNA (mtDNA) have also shown that degenerative diseases are caused by energy deficiency (Holt et al., 1988, Wallace et al., 1988a, Wallace et al., 1988b, Shoffner et al., 1990). However, metabolic therapies remain mostly inadequate for treatment of the symptoms of sufferers of the disorders (Wallace et al., 2010).

There are four main cellular functions that mitochondria are primarily involved with, (i) supplying energy to the cell as adenosine triphosphate (ATP), (ii) the generation and regulation of reactive oxygen species, (iii) the buffering of cytosolic calcium ions, (iv) and the regulation of apoptosis through the mitochondrial permeability transition pore (Wallace et al., 2010, Meyers et al., 2013). It also plays an integral role in protecting cellular metabolism by countering ROS.

As the mitochondria is the main supplier for energy to cells it should therefore hold that the disorders/dysfunction related to it would involve tissues of high orders for energy, for example, the heart, brain, muscle, and endocrine system, but there is no tissue or organ excluded from mitochondrial dysfunction. It therefore follows that if energetic deficits have been associated with other disorders such as renal dysfunction, myopathy, dementia blindness, deafness, cardiomypathy, movement disorders, and aging, that the mitochondrion is probably the link between the cell and the energetics depletion of most metabolic disorders (Wallace et al., 2010), and more importantly brain metabolic disorders such as TD.

The mitochondria is targeted by and a major source of ROS, inevitably structural damage and activation of signaling pathways may result. The activation of the mitochondrial permeability

transition (MPT) pore is pursuant to the loss of mitochondrial membrane potential, hence releasing in the cytoplasm mitochondrial contents, leading to the apoptosis execution phase (Coskun and Busciglio, 2012). It was previously shown that the binding site densities for the translocator protein (18 kDa) (TSPO), also known as the peripheral-type benzodiazepine receptor, are elevated in the brains of TD animals (Leong et al., 1994). Possibly, these receptors are activated with the opening of the MPT pore, providing a useful marker for mitochondrial dysfunction. TSPO, centralized in microglia and astrocytes, functions in the synthesis of neurosteroids (Papadopoulos, 1993). TSPO has also demonstrated several uses for imaging of inflammatory processes in different neurological disorders in humans (Cagnin et al., 2001, Gerhard et al., 2003, Ouchi et al., 2009) using PET. Elevated binding of PK11195, a first generation low-affinity ligand for the TSPO with suboptimal specific/nonspecific binding ratio, was shown in post mortem brain tissue from patients with Huntington's Disease (Schoemaker et al., 1982), AD (Owen et al., 1983, Diorio et al., 1991), and cerebral ischemia (Myers et al., 1991) where both gliosis and neuronal cell loss occur. It was postulated that TSPOs can be used as an index for the measurement of neuronal loss in such disorders (Benavides et al., 1987). Further studies are required, with better ligands, to conclude the application of the protein for targeting use in imaging techniques.

A consequence of elevated levels of intracellular Ca2+ is mitochondrial dysfunction, which affects the opening of the MPT pore, and can cause a complete loss of cellular respiration along with NADH and cytochrome release, the effects of which can be devastating (Hazell et al., 2013). Although the role of the mitochondria has been considered in the pathogenesis of neurodegenerative diseases the involvement of oxidative stress in the commencement and the further development of neurodegenerative diseases remains unclear, specifically Parkinson's disease (PD), AD, Huntington's disease (HD) and amyotrophic lateral sclerosis. It remains plausible that the mitochondrial dysfunction is a consequence of neurodegeneration. It is however worth noting that several mitochondrial DNA mutations have demonstrated that the proteins associated with the mutation lead to the development of neurodegenerative diseases (Federico et al., 2012). It is believed that mitochondrial dysfunction can provide a useful model for the studying of TD as several characteristic features of the dysfunction are also characteristic of TD. An example of this is the reduction in thiamine diphosphate and amyloid-b peptide plaque formation (Calingasan et al., 1995, Zhang et al., 2011, Zhao et al., 2011).

Figure 1 provides a brief overview of the biochemical metabolism associated TD and the attributed mitochondrial dysfunction.

1.3 Astrocytes, Glutamate Transporters, and Excitotoxicity in TD

Astrocytes are important glial cells of the central nervous system. They play integral roles in potassium homeostasis, the uptake of neurotransmitters, synaptic formation, blood–brain-barrier (BBB) regulation, and developmental maintenance of the nervous system (Zhang and Barres, 2010). Also, in the brain, astrocytes help regulate the potassium levels (Gardner-Medwin et al., 1981, D'Ambrosio et al., 2002), inactivate neurotransmitters released (Schousboe, 1981), serve as metabolite communicators (Hertz et al., 1999), and help regulate homeostasis of brain water (Walz, 1987). The involvement of glutamate was identified in the development of brain lesions, specifically thalamic lesions associated with TD (Hazell, 2009b), in whole animal brains (Gubler et al., 1974), which demonstrated a reduction in the conversion of glucose to glutamate in TD rats (Gaitonde et al., 1975). Glutamate involvement was also shown through a decrease in levels of calcium dependent release of glutamate in slices of symptomatic TD hippocampus (Le et al., 1991).

Glutamate has been determined to be the most prevalent inducible neurotransmitter in the brain and extracellularly and is not enzymatically metabolized. Rather, glutamate is removed by reuptake, through the intrinsic function of glutamate transporters. In this regard, GLT-1 and GLAST (transporters) are known crucial removers of cellular glutamate (Rothstein et al., 1996, Tanaka et al., 1997, Danbolt, 2001), being localized within the astrocytic compartments of the brain (Lehre et al., 1995). Maintenance of glutamate levels are important as elevations can lead to excitotoxic injury at the glutamate receptor level, leading to overstimulation, e.g.in ischemic stroke, and brain trauma.

Five glutamate transporter subtypes have been cloned GLAST (EAAT1), GLT-1 (EAAT2), EAAC-1 (EAAT3), EAAT4 and EAAT5 (Danbolt, 2001). Apart from GLAST, and GLT-1 mostly located in astrocytes (Danbolt et al., 1992, Rothstein et al., 1994, Lehre et al., 1995, Reye et al., 2002), EAAC-1, EAAT4, and EAAT5 are localized in neurons (Arriza et al., 1997, Berger and Hediger, 1998, Dehnes et al., 1998, Kugler and Schmitt, 1999). Over 90% of total glutamate

transport activity in the forebrain is attributable to GLT-1 (Danbolt et al., 1992, Haugeto et al., 1996, Tanaka et al., 1997) . It remains unclear as to what level the remaining glutamate transporters interact and play roles with the cellular functions of astrocytes and neurons, specifically with regards to TD (Yi and Hazell, 2006).

In homeostasis, EAAT1 and EAA2 are involved in removing extracellular space glutamate. Glutamate can then be converted to glutamine by glutamine synthetase (GS), but TD causes a decrease in the oxidative metabolism impairing this ATP-dependent process. Cell death due to excitotoxicity is usually the consequence of TD following astrocytic loss of EAAT1 and EAAT2 transporters.

Figure 2 provide a global overview of the cell death indicators associated with TD.

1.4 Therapeutics

The past 50 years have seen many novel developments in stem cells but treatments for numerous neurodegenerative disease including strokes, amyotrophic lateral sclerosis, AD, and PD, primary medical approaches inadequately alleviate the symptomatic manifestations of these diseases. A therapeutic application widely recognized is the use of hematopoietic stem cells (HSCs) extracted from bone marrow transplants often used to treat leukemia, hemophilia, and anemia. Recently mesenchymal stem cells (MSCs) have been studied in ischemia, and the role they can play in tissue repair, and revascularization (Liu et al., 2009). Stem cell transplantation is still in its early stages, although increasing numbers of clinical trials are now ongoing.

Four stem cell types provide promise in neurodegenerative disorder therapy applications. Firstly MSCs, which are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and stroma cells, are being used for cell replacement therapy (Bilic et al., 2008, Hunt et al., 2008). Several studies have looked at ways that MSCs can support hematopoiesis from bone marrow, synovial fluid, umbilical cord blood, amniotic fluid, deciduous teeth, and placental, adipose, and dermal tissues (Lakshmipathy and Hart, 2008, Sorrentino et al., 2008). Others have looked into the differentiability of MSCs into several connective tissue cell types (Bruder et al., 1997, Bruder et al., 1998, Dennis et al., 1999).

The second type are neural stem cells (NSCs). Their capacity to differentiate into neuronal and glial populations is of primary use (Namihira et al., 2008). Flow cytometry has allowed for the isolation of NSCs based on physical properties, including granularity (McLaren et al., 2001, Murayama et al., 2002), size (measure via forward scattering), and surface antigens (cluster of differentiation 34 (CD34), CD133) (Johansson et al., 1999, Uchida et al., 2000). NSCs are prime candidates for responsive neural transplantation for neurological disorders (Trujillo et al., 2009).

Thirdly are embryonic stem cells (ESCs), pluripotent cells from the inner cell masses of mammalian blastocysts differentiating to three embryonic germ layers (endoderm, mesoderm, and ectoderm) (Blakaj and Lin, 2008). Their self-renewability and differentiability into various specialized cells types has made it a primary focus in clinical therapies. Functional dopamine neurons are currently being studied and their uses for various cell therapies (Kim et al., 2007).

Finally induced pluripotent stem (iPS) cells, which are generated using fibroblasts transfected with the transcriptional factors octamer-binding transcription factor 4 (Oct4), sex-determining region Y Box 2 (Sox2), v-myc myelocytomatosis viral oncogene homolog (c-Myc), and Krüppel-like factor 4 (Klf4) (Takahashi and Yamanaka, 2006). Human iPS cells share similarities with human ESCs with regard to proliferation, morphology, gene expression, surface antigens, epigenetic status of pluripotent cell-specific genes, and telomerase activity (Takahashi et al., 2007). iPS cells appear to offer alternatives to ESCs research application difficulty areas (Liu et al., 2013).

While the mechanism(s) that contribute to the selective histological lesions characterizing TD remain unclear, oxidative stress, however, has been demonstrated to be a major contributing factor to its pathophysiology. It is believed that several prominent neurodegenerative diseases, including AD and PD, can be triggered by oxidative stress. TD oxidative stress can be useful in modelling the dysfunction and project the understanding on to other neurodegenerative diseases. Current therapies targeting oxidative stress production have remained inadequate, providing strong indication that the sequence of event for these maladies have not been clearly identified. Pharmacological approaches have shown little in translational clinical efficacy and also remain unproven (Melo et al., 2011).

The multifactorial factors concurrent with oxidative stress contributing to cellular dysfunction suggest that once initiated, net production of oxidative stress, is difficult to control. This would indicate that more complex and intricate therapeutics may be necessary, requiring a deeper understanding into various neurodegenerative disorders (Hazell et al., 2013).

1.4.1 Stem Cell Strategies in Neurologic Disease

Stem cells are undifferentiated cells that are capable of differentiation into various mature cells. Three major categorizations, according to degree of differentiability of stems cells, are usually applied respectively classified according to their order of differentiation, totipotent, pluripotent and multipotent cells (Scholer, 2007). There remain other classifications with limited abilities of differentiation, oligopotent and unipotent stem cells.

Research on these highly complex cells has grown in recent years due to the treatment opportunities they present. Where they offer particular interest, however, is their feature as a tool in assessing control mechanisms of differentiation. They can offer insight into possible plaguing disease mechanisms that to this day, due to their complexities and multiorgan targeting and dysfunction remain difficult in assessing and treating. Diabetes, vascular diseases, and neurodegenerative diseases present some interesting uses for stem cells (Şimşek and Şimşek, 2012). The more fascinating functionality of the cells is their recently found properties to differentiate into varying cells apart from their source of origin, for instance bone marrow cells have been shown to differentiate into liver, muscle, kidney, cardiomyocytes, new blood vessels, and even neuronal cells (Malcolm et al., 2002). This feature of transdifferentiation has allowed researchers to model in vitro cell growth to that of in vivo application (Şimşek and Şimşek, 2012).

The use of neural progenitor cells (NSCs) was used in identifying some possible roles of O2/ROS in neurogenesis, oxidative stress, and its involvement in a neurodevelopmental disorder, schizophrenia (Paulsen Bda et al., 2013). Interestingly the obtention of live neurons from schizophrenic patients has been very difficult and has led to most information considered to be based on brain images or post-mortem neural tissues (Urbanek et al., 2005). The latter is

consequentially inadequate in assessing the primary mechanism and functions of the disorder and is similar to chronically medicinally treated patients and aged brains (N et al., 2007). In fact, post-mortem brains offer little to explanatory descriptions of neurodevelopmental disorders (Strauer et al., 2009).

Somatic cells have demonstrated that through reprogramming there is great potential for human induced pluripotent stem cells (hiP-SCs) uses in modeling human neurological diseases. It has been used in patients with Amyotrophic Lateral Sclerosis (Dimos et al., 2008), Spinal Muscular Atrophy (Ebert et al., 2009), Familial Dysautonom ia (Lee et al., 2009), Fragile XSyndrome (Urbach et al., 2010), AD (Yagi et al., 2011, Yahata et al., 2011), Timothy Syndrome (Yazawa et al., 2011), PD (Hargus et al., 2010, Byers et al., 2011, Devine et al., 2011, Nguyen et al., 2011). The general principle is to differentiate reprogrammed cells into ones that are identical to the disease being identified, characterize the phenotypes associated with the pathological manifestation and biochemically reverse the effects as proof of functionality. This is especially useful as modeled diseases using hiPSCs allow for possible minute monitoring of differentiation in varying cells, inclusive of neurons. Reprogramming however remains an expensive technique, due to the complexity of the techniques involving transduction of e.g. human dermal fibroblasts into pluripotent stem cells with modified retrovirus, followed by the actual reprogramming. Another limitation which remains an issue with most stem cell usage, is the lack of a robust and efficient protocol for cell-line generation that are disease-specific (Kunkanjanawan et al., 2011). Several other issues arise, including the number of uncharacterized cells (also contaminated cells) that have originated from the differentiable cell. Although measures are often taken to ensure that homogeneous populations are purified and collected, certain subtype protocols are adhered to, as in the case of neurons. This will inevitably hinder the modelling of the disease especially in vitro. This leaves hiPSCs as the leader for analogous modelling of neurological disease in vitro owing to its ability to obtain disease specific portions of brain development (Dolmetsch and Geschwind, 2011). The former strategy differentiating reprogrammed cells into cells identical to the disease desired for analysis, is the method of choice for studying and modelling of long-latency disease such as PD, AD, and/or multifactorial disorders, such as TD.

1.4.2 Stem cells and TD

In adult mammalian brain it has been well documented that there exists a repository of neural stem-progenitor cells. Known areas with significant levels of neural stem-progenitor cells (NSPC), known as neurogenic (neuron-generating) zones include the subventricular zone and dentate gyrus in rats, primates, and humans (Temple, 1999). Further evidence suggesting other areas maintaining the presence of NSPC have emerged including the spinal cord (Weiss et al., 1996, Shihabuddin et al., 1997), and neocortex (Gould et al., 1999).

Vitamin B1 (thiamine) deficiency (TD) is the major cause of Wernicke's encephalopathy (WE) where cerebral function is impaired due to vulnerability (Hazell, 2009b). The underlying mechanism remains unclear, but the result is reduction in neurogenesis due to impaired NSPC function (Hazell et al., 2014). First evidence of the existence of factors related to NSPC proliferation and differentiation during TD were reported in Vemeganti and colleagues (Vemuganti et al., 2006), citing many identifiable transcripts that would later be found to be implicated in the signaling and function of NSPC. In 2000 human neural stem cells (NSC) were transplanted and reported to have "homing in" capabilities in intracerebral xenogeneic brain tumors deposited into rodent brains over long distances (Aboody et al., 2000). The tracking ability of NSC was also demonstrated of tumor cells for the original tumor mass to the localized areas in normal brain. These NSC were found in exact proximity of the tumor cells.. The NSCs homing capability was not specific to the methodology of ventricular introduction. Tail vein injection demonstrated the same homing capability as intracranial ventricular injection, and although a limited number of cells and compounds are capable of crossing the tight junctions that form the blood-brain barrier, the migration and homing ability of NSCs towards the brain areas did not appear to be affected (Pluchino et al., 2003). Thus NSPC can offer an important tool to examine damage in TD.

Studies indicate that critical areas involved in TD include the thalamus and inferior colliculus (Butterworth, 1989). Detailed investigation of the molecular sequence can offer a better understanding into some of the underlying mechanisms. One strategy more readily employed for molecular changes involves the "omics" technologies, referring to simultaneous determination and identification of thousands of molecules (including metabolites, proteins, transcripts and genes) in various cells, tissues, or organs (Meinhardt et al., 2015).". Using comprehensive

statistical methods, global molecular profiles can be analyzed. Transcriptome profiling is more commonly used in the studying of postmortem mammalian brain samples, but metabolite profiling is less frequently used. Metabolites can be the by- and end- products of various biological systems pathways and can offer a much better indication of the phenotypic manifestation (Dettmer et al., 2007, Kaddurah-Daouk and Krishnan, 2009). The metabolic profiles of CNS diseases/disorders have been made more readily achievable with the advances of analytical platforms and bioinformatics methods (Bogdanov et al., 2008, Gika et al., 2012). Nontargeted mass spectrometry-based metabolomics can be used for specific brain regions and provides a global neurometabolic profiling of the subject. It can provide high selectivity and sensitivity for large numbers of metabolites. The technique can potentially allow for the identification of novel pathophysiological mechanisms and, with regard to the aforementioned usage of NSPC, can further identify key contributors in TD that share mechanisms of dysfunction with a number of other neurological disorders, including ischemic stroke, traumatic brain injury, and amyotrophic lateral sclerosis (Rao et al., 2001, Allen et al., 2004, Maragakis and Rothstein, 2004).

1.4.3 Hypothesis

This study aimed at providing a metabolic profile of areas of localized dysfunction in the thalamus and the IC of rats with TD, identifying new metabolites that could offer a better understanding into the pathophysiology of this disorder. Following thiamine replenishment of rats with TD at the loss of righting reflex stage, animals transplanted with and without NSPC would be analyzed using a metabolic profiling approach. It was also expected that the transplantation would confirm and provide a better visualization of areas affected in TD showing a different metabolic profile, revealing possible key pathways associated with a role for NSPC in this disorder.

Hypothesis: ***Induction of key metabolites in vulnerable brain regions is a feature of TD, with transplantation of NSPC producing changes consistent with an attempt to ameliorate metabolic dysfunction and structural damage in these areas.***

2. METHODS

2.1 TD Model

A well-established rodent model of TD was used (Troncoso et al., 1981, Butterworth et al., 1985, Heroux and Butterworth, 1988, Hazell et al., 2014). Briefly, 6 -7 week Male Sprague–Dawley rats (n=5), weighing 225-250 g were fed a TD diet* (MP Biomedicals, LLC, Solon, OH). The rats were injected intraperitoneally for a period of 12-13 days consecutively with pyrithiamine hydrobromide (0.5 mg ⁄ kg body weight) (Sigma-Aldrich Canada). Pyrithiamine acts as an antagonist to thiamine inhibiting TDP phosphokinase. Injections were stopped when TD symptoms were identified (at the first signs of ataxia). TD symptoms included movement impairments, ataxia, opisthotonus, and the loss of righting reflex (LRR).

Animals were sacrificed via decapitation following pentobarbitol administration (65 mg/kg) at i) the immediate LRR or ii) within 6 h of the appearance of LRR. A pair fed control (PFC) group was placed on a TD diet of equal quantity to an assigned TD rat (PFC consumed equal amounts to its paired TD). To avoid TD onset the PFC animals were supplemented with thiamine (100 µg /0.2 ml saline, I.P.). All animal procedures were conducted in accordance with guidelines set out by the Canadian Council on Animal Care and were approved by the CRCHUM Animal Ethics committee(CIPA).

2.2 Neurospheres

Primary culture neurospheres were prepared from the cerebral cortex of embryonic rats at the E18 stage and growing in NeuroCult® NS-A Complete Proliferation Medium (Rat) (as per manufactures instruction StemCell Technologies Inc, Vancouver, BC). Briefly, the proliferation medium was prepared with NeuroCult serum-free medium with NeuroCult® NS-A Proliferation Supplement (Rat) containing EGF, FGF-2 and heparin to NeuroCult® NS-A Basal Medium (Rat) (StemCell Technologies, Vancouver, BC). Following 5 min of centrifugation of the collected tissue the pellet was resuspended in fresh NeuroCult® NSA Complete Proliferation Medium. Cells and seeded at 1.2×10^5 viable cells/ml in 25 cm^2 culture flasks and maintained in a 5 % CO2 incubator at 37 °C. Neurospheres subcultures were passaged every 4 days and medium change was performed every 2 days.

*Nutritional information of the rodent diet: Crude protein: 18.9%; Crude oil: 6.0 %; Carbohydrate: 57.33 %; Sugar: 4.93 %; Starch: 41.24 %; Vitamin A: 15.4 IU/g; Vitamin E: 101 mg/kg; Vitamin K (menadione): 51 mg/kg; Vitamin B1: <0.1 mg/kg; Vitamin B2: 14.9 mg/kg; Niacin: 41.2mg/kg;Vitamin: B6 18.5mg/kg; Pantothenic acid: 33 mg/kg; Biotin: 0.3 mg/kg; Folate: 3.34 mg/kg; Vitamin D3: 1.5 IU/g; Vitamin B12:0.08 mg/kg.

Neurospheres are known to contain neural stem and progenitor cells. They are also known to target areas of dysfunction. Neurospheres were used to monitor transplantation, identified in the following section, and to observe where they would localize in hopes of offering further insight into some of the mechanisms involved in TD.

2.3 Transplantation

PFC and TD rats were tail-vein transplanted with P4-P6 subcultures of dissociated neurospheres incubated with 5μM of CFSE medium (prepared according to manufacturer, Life Technologies) for 30 min at 37 °C and then retitrated prior to tail vein injection. Confirmation of CFSE labelling was performed with a fluorescence microscope using a FITC filter.

Neural stem cells (NSC) have been reported to have "homing in" capabilities over long distances in areas affected by disease/disorder (Aboody et al., 2000). In Aboody et al. (2000) neural stem cells were demonstrated to have tracking ability to home in to tumor cells to the localized areas in normal brain, found in proximity of the tumor cells. This homing ability was also seen in tail vein injection without affecting the migration. This suggested that the blood-brain barrier crossing was not an issue. (Pluchino et al., 2003). This phenomenon allowed for neurosphere transplantation to be an important and useful tool in examining damage and affected areas in TD.

2.4 Metabolite Extraction

The metabolite extraction methods used are those described by Meinhardt (Meinhardt et al., 2015). Briefly, rats were decapitated and brains immediately snap frozen in 2-methylbutane at -40ºC and then quickly transferred to -80 ºC. Bilateral sections were dissected of the thalamus and the IC using anatomical identifiers from the rat brain atlas of Paxinos and Watson (1998). Macropunchs of 2mm samples of each section were obtained to extract approximately 10 mg of metabolites. The extraction was performed with 1mL of preheated 70% ethanol in water (v/v) at 80 ºC (2 minutes) and vortexing every 30 seconds. Following centrifugation for 10 min at 0 ºC (20,800 g), the supernatants was evaporated to dryness via vacuum. The remaining pellets were resuspended in 40 μL H_2O and stored at -80 ºC before mass spectrometry analysis.

2.5 Nontargeted Mass Spectrometry

The mass spectrometric analysis was performed on a platform with a Agilent 6520 liquid chromatography mass spectrometry (LC-MS) system for the MS analysis and using Agilent 6520 IonFunnel QTOF (Agilent, Santa Clara, CA), operating with the published settings described in the negative ionization mode (Fuhrer et al., 2011) for the automated isocratic flow injection without chromatographic separation. Briefly, the flow rate was set at 150 mL/min of the mobile phase (60% isopropanol in water (v/v) and 5mM ammonium fluoride at pH 9 as a buffer. Online mass axis correction was performed by the use of an electrospray ionization (ESI) tuning mix consisting of the mobile phase, taurocholic acid, and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (HP-0921, Agilent Technologies) being added to the mobile phase. Measurements were performed in technical duplicates. Recordings were profiled in m/z mode at 50-1000 with a scan rate of 1.4 spectra/s for 0.48 minutes under the highest resolving power (4GHz HiRes). Electrospray source temperature was set at 325 ^{o}C, with 5 L/min drying gas and a nebulizer pressure of 30 psig.

This method allowed for quick unprocessed metabolite detection. Although further MS-MS, GC-MS, and NMR optimized detection is required in the process of confirming the metabolites, the technique retains an advantage over many other separation and detection techniques in that for every separation or processing technique metabolites or part of the metabolite are lost.

2.6 Statistical Methods and Interpretation

Spectral processing was performed using MetaboAnalyst 3.0 (found at http://www.metaboanalyst.ca) with ions and compounds being identified via MassBank (found at http://www.massbank.jp/index.html). Both are online free public repositories of statistical analysis tools for NMR and mass spectrometry spectras and spectral peak identifiers linked to compounds listed and identified in the Kyoto Encyclopedia of Genes and Genomes (KEGG, found at http://www.genome.jp/kegg/) Rattus norvegicus metabolite database (Kanehisa, 2002) and Genbank (found at http://www.ncbi.nlm.nih.gov/genbank). Deprotonation $[M-H]^-$ or fluoride addition $[M+F]^-$ and zero or one ^{12}C->^{13}C exchange as possible electrospray-derived species were assumed.

As previously stated all statistical analysis was performed using MetaboAnalyst. Briefly, peak lists of m/z and ion signal intensity were extracted from the raw data, taken from MS spectras generated. The peak lists and intensity data were uploaded to the online database as a zip file. The zip contained subfolders for the different groups being analyzed saved as .csv and comma separated values formatted files. To conduct analysis the relative m/z peaks to be analyzed are matched and adjusted to the median values. An average of 100 peaks per sample were detected for analysis. Missing values and data integrity checks were performed by the software.

The main statistical analysis methods used, which provides a summary of clustering patterns in references to the samples being studied, were the principal component analysis (PCA) and the partial least square - discrimination analysis (PLS-DA). These methods provide a graphical representation of metabolomics raw data into 2 or 3 dimensions, illuminating respectively, the maximal variance and co-variance. PCA was performed on annotated ion intensities, cluster departure was assessed according to 95% confidence calculated using X^2 tests. The PCA method was also performed for missing values or values detected as zeros (in the case of sample outputs with too many zeros). The software assumes in this specific case of zeros that the missing value or redundancy in zeros is due to the low abundance of the metabolite, usually a condition of metabolites under the detection limit. This is done to avoid statistical normalization problems.

Unique metabolites were identified, along with several unknown metabolite peaks (possibly from novel metabolites) through data mining in MetabolAnalyst and pathway relationships were provided via the Pathway Analysis tools. Of the metabolites identified only the ones of significance were used. Significance was established using the partial least squares discrimination analysis (PLS-DA), a multivariate regression technique for extrapolation of linear combinations of original variables against the predictors of membership classification, which is performed on using the PLSR function provided by R PLS package (Eriksson et al., 2009). Important features using PLS-DA were assessed via Variable Importance in Projection (VIP) and a weighted sum of squares of the PLS loadings taking into account the amount of explained Y-variation in each dimension. Metabolites attaining a VIP score of 1.0 or higher were considered as the primary driving features of the calculated discrimination and used for analysis (Bijlsma et al., 2006, Witowski et al., 2015).

Metabolic pathway enrichment analysis was performed via MetaboAnalyst using metabolites clusters based on the KEGG R. norvegicus metabolic pathway definitions (Kanehisa, 2002), and Integrative Anaylsis of Gene-Metabolite additionally used genes defined in GeneBank, which were identified with the accession numbers highlighted in the previous study (Vemuganti et al., 2006). These methods of analysis used models from KEGG metabolic pathways underlies the assumption behind these modules and combines the evidence based on changes in both gene expression and metabolite representative concentrations. The pathways involved in the underlying biological processes are more likely to be identified using this function with genes and metabolites of interest being recognized from the samples under similar conditions. Following the uploading of genes and metabolites desired for analysis a pathway topology analysis using maps from KEGG metabolic pathways for over-representation analysis was performed. Topology analysis is an evaluation based of the structure of the given pathways of the importance and correlating it to the genes-compounds with regards to their relative pathway locations. A common interpretive problem with metabolomics technologies is that unlike transcriptomics (routine mapping of entire transcriptome), current metabolomic technologies allow for a limited assessment of the metabolome, which leads to the possibility of potentially biased results. The aforementioned methods provided by the Metabolic Pathway enrichment and Integrative Analysis function (MetaboAnalyst) allows for exploration of different platforms of this "omic" and a comparative perspective for assessing the results through the enriched pathways based on the joint evidence or on the evidence obtained from another platform (Xia et al., 2015).

2.7 Immunohisto/cytochemistry

Immunohistochemistry was performed on fix-perfused sectioned rat brains 48 hours post-transplantation of CFSE labelled NSPC, as per Hazell et al. (2001). Briefly, rats were anesthetized with pentobarbital (65 mg/kg, i.p..) and fix-perfused transcardially as described (Hazell et al., 2001). Brains were post-fixed in neutral-buffered formalin containing 4% formaldehyde, 0.5 % sodium phosphate buffer, and 1.5 % methanol, pH.7.0 overnight. Corona1 sections of 40-80 μm thickness were obtained at the medial posterior thalamus and the inferior colliculus, using a vibrotome and identified using the rat brain atlas of Paxinos and Watson (1998). The sections were coverslipped with Prolong Gold antifade reagent (Invitrogen, Burlington, ON) and imaged. CFSE maintains its fluorescence ability for several days following

transplantation. The sections were therefore directly imaged for confirmation of CFSE fluorescence of the labelled NSPC without any further treatment, to confirm transplantation and localization. FITC filtered fluorescence microscopy imaging was used and no further analysis was performed.

The immunocytochemistry was performed on formalin fixed NSPC and differentiated NSPC (this involved growing NSPC in NeuroCult® Differentiation media following the procedure describe in section 2.2). The procedure in Hazell et al. (2001) was used to confirm the differentiability of the NSPC cultured into the 3 cells types (neurons, astrocytes, and oligodendrocytes), and to confirm the CFSE labelling of the NSPC used for transplantation. No further analysis was undertaken. Briefly, the technique involved culture cells, being formalin fixed by incubation for 30 min at RT, and subsequently incubated for 10 min in phosphate-buffered saline (PBS) containing 0.3 % hydrogen peroxide to block endogenous peroxidase activity. Following PBS washes (3×10 min) of the cells and blocking with 0.5 % Triton X-100 in PBS and 10 % donkey serum for 30 min, the cells were incubated with 10 % donkey serum in PBS and polyclonal antibodies for GFAP, NeuN, and Tuj-1 were incubated at 4 °C for 12-18 hr. Cells were subsequently incubated with Alexa Fluor 488 and Alexa Fluor 596 secondary antibodies (1:200) (Invitrogen, Burlington, ON) and then coverslipped with Prolong Gold antifade reagent (Invitrogen, Burlington, ON).

2.8 Scanning Electron Microscopy (SEM)

Following transcardial formalin fixation and vibrotome sectioning, sections were dehydrated with different concentrations of ethyl alcohol 50%, 70%, 90%, and 100% for 10 minutes following each step. The sections were then Critical Point Dried by immersing the sections in a cavity that was then placed in liquid CO_2 for rinsing. Drying was performed by raising the temperature of the cavity to 35-45°C, and allowing the CO_2 to change from its liquid phase to a dry gas phase. The sections were then mounted on an adhesive holder and coated with gold particles prior to observing them using a SEM. Morphological confirmation of cells was performed through a comparative research of similar images available in the literature. This method of analysis was the final used and as the previous techniques suggested the involvement and localization of NSPC in the areas that were imaged, literature identifying SEM images of stem-cells were found and morphologies compared. No further analysis was performed.

3. RESULTS

3.1 Metabolites Detected in the Thalamus and IC

A non-targeted metabolomics study was undertaken to obtain a global profiling of the rat brain focal areas, specifically the thalamus and inferior colliculus, in pyrithiamine induced thiamine deficiency in rats. Groups of pair-fed controls (PFC), pyrithiamine induced thiamine deficient rats (PTD) within a few hours (1-6) following the loss of righting reflex, and PTD rats at the moment of the loss of righting reflex (5 rats for each group were used, n=5). A comparative analysis was performed of these groups using Metaboanalyst 3.0 (available at http://www.metaboanalyst.ca/).

Changes identified in some of the most notable (and probable) detected metabolites in the thalamus are outlined in Table 1 extrapolated from Figure 3, and provide insight into various different pathways linked to TD.

Table 1 Most probable compounds
in the Thalamus
based on VIP score for PLSDA

m/z	Compound
111.0066	Histamine
61.9887	1β,3α,7α-Trihydroxy-5β-cholan-24-oic acid (and its isomers, it is a Bile acid)
80.975	3α,4β-Dihydroxy-5β-cholan-24-oic acid (and its isomers, it is a Bile acid)
89.0425	β,3α-Dihydroxy-5β-cholan-24-oic acid
187.0412	Kynurenic Acid
92.007	3β,7β,12β-Trihydroxy-5β-cholan-24-oic acid (and its isomers, it is a Bile acid)
194.9877	1,9-dimethyluric acid
133.0136	Aspartic Acid
169.8335	L-Cysteic acid
192.9283	N-2-Acetylguanine
402.885	Dehydrocholic acid (Bile acid)
233.0285	3β,7α,12α-Trihydroxy-5α-cholan-24-oic acid

Key findings included the detected presence of several bile acids. Another unconfirmed bile acid which is suspected to have produced the m/z readings at 396 and 398, seen in Figure 3, is ursodeoxycholic acid, or Urso. The findings related to the bile acids indicate an accumulation of some bile acids and a depletion of some others at different points of TD. For instance in regards to the peaks that most probably suggest Urso there is an elevation for the PFC group but a decrease with respect to the other groups seeing the greatest decrease once the rats have entered into several hours following the loss of righting reflex. In regards to other bile acids there appears to be very low levels in the PFC group and the highest levels at the moment of the loss of righting reflex, suggesting a neuroprotective function for the bile acids. Another neuroprotective metabolite detected exclusively in the thalamus was kynurenic acid (KYNA). Increased levels were found in the TD groups compared with the PFC group with the highest being found in the LRR TD group. Due to these findings related to metabolic and transcript, detailed in the Vemuganti et al. (2006) study, expressions highlighting a regenerative and protective mechanism in the thalamus, it is believed that this area in particular can be a key area of biomarker discovery.

Another interesting metabolite highlighted in the results is 1,9-dimethyluric acid. Under normal physiological conditions this compound is seldom found in urine, as would be the case with any uric acid derivative. The metabolite is however used as a marker for metabolic disorders or dysfunction. Therefore the presence of this metabolite in the brain is quite unique and could be used in quick clinical analysis settings to detect metabolic neurological dysfunction using urine samples.

The IC exhibit different metabolites showing a variance in function and involvement during TD, outlined in Table 2 and extrapolated from Figure 6. The IC appear to show marked changes in various amino acids, pyrimidines, and/or purines metabolisms. The metabolites are almost all depleted from the IC with the progression of TD. The metabolic and transcript expression and response seem to suggest a more essential cellular function for IC. These metabolites are integral for basic cellular function if any of these fundamental pathways are disrupted major cellular consequences follow.

Table 2 Most probable compounds in the IC based on VIP score for PLSDA

m/z	Compound
172.9852	Arginine
287.0012	Tetrahydropapaveroline
154.9269	Histidine
162.956	Methionine sulfoxide
187.0412	Kynurenic Acid
146.9821	Glutamic Acid
203.0168	Tryptophan
104.0353	Serine
150.9903	Guanine
118.0504	Succinic acid
152.9791	Cysteine Sulfinic acid
187.0003	Kynurenic Acid
119.0355	Threonine and/or homoserine
130.0862	4-Hydroxy-L-proline
132.0514	Ornithine
174.9546	Citrulline
140.9851	Histidinol
133.0135	Aspartic Acid
225.0884	L-Carnosine
226.9779	2'-Deoxycytidine
226.0641	5'-Deoxythymidine
233.0282	3β,7α,12α-Trihydroxy-5α-cholan-24-oic acid
208.9823	Kynurenine
130.0612	Leucine
135.0299	Hypoxanthine
230.9552	D-Ribose 5-phosphate
180.9725	Tyrosine
125.0003	Taurine

From the results 2 metabolites might be more interesting for TD in particular, taurine and carnosine. Taurine is usually obtained from food intake. It is crucial for brain health and function and plays a role in stem cell function. The usefulness however lies in its detectability and how taurine level changes in the body have been linked to various disorders. Taurine is easily detected in blood and could be a measure of TD progression. A relative increase in taurine was observed in the LRR group compared to the PFC group and significant decrease was seen in the

TD group past the LRR. These findings again suggest a neuroprotective mechanism plays a role in trying to salvage the brain during TD, particularly in the IC.

The metabolite carnosine is only found in animals. Taken as a supplement, the compound has been found to have some potency for ameliorating some symptoms of neurological disorders. In high doses, hyperactivity has been noted in patients (McGinnis, 2005, Rossignol, 2009). The highest levels of carnosine were detected in the PFC group and the lowest in the LRR group. Interestingly the PFC group is characterized by hyperactivity. The relative increase in the levels of carnosine in the TD group past the LRR suggests the possibility of a mechanism depleting the muscles or body of the rat of carnosine and sending it to the brain in an attempt to salvage the tissue.

3.2 Results Following Neural Stem-Progenitor Cell Transplantation

This study also sought to answer some questions (on a rudimentary level) such as whether NSPC transplantation could offer a better understanding to the underlying mechanisms of TD, could they localize in affected areas, and do they remain intact as NSPC for the entire time following transplantation or do they differentiate. Rats that underwent TD induced by pyrithiamine treatment were used. The rats were reversed and replenished with thiamine and subsequently (within minutes following the initial replenishment), tail-vein transplanted with NSPC (1×10^6 cells) that were labelled with carboxyfluorescein diacetate succinimidyl ester, CFSE, and sacrificed within 48-72 hours following transplantation. The same metabolic analysis for brains extracted from this group of rats was performed. Interestingly several unidentifiable compounds corresponding to unique peaks were observed, indicating the possibility of several novel metabolites that might play a crucial role in explaining the function between NSPC and the metabolic dysfunction manifested in TD and possibly other mechanistically similar neurodegenerative disorders. Several other metabolite peaks of significance correlated with the PFC group and the other TD groups which were not sufficient for statistical consideration, or not possessing a correlation threshold hold minimum (on the VIP scoring using the PLS discrimination analysis; ≥ 1), were observed when the additional NSPC transplanted groups were added to the statistical analysis. Table 3 provides a list of some different metabolites. Table 3 only highlights the metabolites that differ from the previous method of analysis for the thalamus

and the IC. Noteworthy is the detection of several unknown and unidentified peaks, consistent with the possibility of novel metabolites having been detected. Again, further analysis is required to these unknown metabolites.

A more interesting metabolite detected was arachidonic acid. Arachidonic acid plays a role in the neuroprotection of several parts of the brain and repairing of neurons. The metabolite is found in the highest dose in the thalamus of PFC group and the lowest in the LRR TD group. The levels are increased in the reversed and transplanted TD group but they do not return to the same levels as the PFC group. Noted in the Vemuganti et al. (2006) work in the overexpression arachidonate 5-LOX activator (arachidonate acid is a substrate of this transcript) in the IC in TD animals. This is interesting as arachidonic acid is not significantly present in the IC in TD animals. This highlights the signaling of the brain in the IC during TD, for arachidonic acid, for a protective or regenerative purpose but the metabolite is not significantly present.

Table 3 Most probable significant compounds in the Thalamus and IC following NSPC transplanted based on VIP score for PLSDA

m/z	Compound
132.0293	Asparagine
225.0608	5'-Deoxythymidine
140.0108	Hydroxypicolinic acid*
303.0818	Arachidonic acid
150.9979	Guanine

*Hydroxypicolinic acid has been a topic of pharmacological research due to its wide significance in various biological activities. It is found in many important structural motifs. ___ indicating key identifiable metabolites in the thalamus and ___ indicating key identifiable metabolites in the IC.

3.3 NSPC Confirmation

Reynolds, Weiss and colleagues were the first to demonstrate the isolation of neural stem and progenitor cells (Reynolds and Weiss, 1992). In 1992, they were able to show that cells isolated from the CNS of embryonic and adult mice proliferated with the occurrence of epidermal growth factor and formed large spherical cells they coined "neurospheres". They demonstrated that these neurospheres could be propagated to produce new neurospheres. The functional characterization of neural stem cells have come to be defined as cells that originate from the CNS that have the capacity to proliferate, self-renew, and whose progeny have the same capacity to differentiate into the 3 major cells of the CNS neurons, astrocytes and oligodendrocytes (Kornblum, 2007, Ziegler et al., 2011). Several more rigorous assays exist for stem cell confirmation, but for ease neurospheres propagated from the collection of single cells for the CNS in the presence of appropriate factors, can have subcultures differentiate into the 3 major cells and are referred to as NSPC. This is also because more often than not, neurospheres contain a majority of progenitor cells with a small portion being actual stem cells.

To confirm the presence of NSPC produced from embryonic (E18) rat cortices following the generation of neurospheres (see Figure 11) subcultures (P 1-4) were taken and differentiated into the 3 major cell types. As previously stated, to confirm true stem cell capability the NSPC need to produce all 3 cell types, specifically astrocytes, neurons, and oligodendrocytes. The findings show confirmation that the NSPC used can differentiate into all of the required cell types (Fig 12). Transplantation was performed with CFSE labelled NSPC. To confirm labelling, NSPCs were visualized microscopically under fluorescence illumination following incubation with CFSE (Fig 13). These labelled cells were notably found to localize in the IC (Figure 14).

A further observation of sectioned brain tissue from the various groups using a scanning electron microscope (SEM) was undertaken to see if any different morphological characteristics were identifiable. What was immediately noticeable is the difference in structural integrity of the tissue. The PFC group shows overall intact tissue (Fig 15), whereas the TD (non-transplanted) group shows significant tissue damage possibly associated with lesions (Fig 16), but interestingly the TD group that was reverse and transplanted with NSPC appears to show some return to tissue

consistency that the PFC group exhibited (Fig 19). Also apparent are the presence of some sort of stem cells (from the current study we are suggesting that they are most likely NSPC) seen Fig. 17- 18 (morphologically identical to that reported in the literature) (Feng et al., 2014, Liu et al., 2015, Qin et al., 2015) in the TD un-transplanted group. This is another indication of the involvement of NSPC as a response to TD in these focal areas of TD.

3.4 Integrated Pathway Analysis

The metabolomics data collected in this study and transcripts identified in Vemuganti et al. (2006) for the PFC and TD groups (excluding NSPC transplanted group and the LRR group) were correlated using the function feature of integrated pathway analysis (via MetaboAnalyst 3.0). From the pathways of greatest significance, seen in Figure 20, 2 pathways appear to be of greatest significance (statistically) the VEGF signalling pathway and the Jak-STAT signalling pathway. The VEGF signalling pathway is involved angiogenesis, and the Jak-STAT signalling pathway is involved neurogenesis and astrogenesis. These results again strongly suggest a regenerative and protective mechanism triggered during TD.

4. DISCUSSION

4.1 Non-targeted Metabolomics

A more recently used member of the 'omics' research, metabolomics is primarily focused on characterizing large numbers of metabolites using NMR, chromatography and/or mass spectrometry. Its applicative purposes has been more readily used in metabolic profiling of cells, tissues or organisms. Data processing of metabolomics is quite specialized, and more often than not specialized data analysis software is required along with a background in cheminformatics, bioinformatics and statistics. MetaboAnalyst is a freely accessible, easy-to-use web server specifically designed for metabolomic data analysis with the same principle approaches to web-based microarray analysis packages. Raw input data from the NMR, chromatography, and/or MS devices is used and data processing can be performed with data normalization, multivariate statistical analysis, graphing, metabolite identification and pathway mapping (Xia et al., 2009). There exist two primary approaches to analyzing metabolomic data, chemometric and quantitative approaches. For unidentified compounds and nontargeted profiling, chemometric is the method of choice for sample analysis of distinguishing features. Quantitative metabolomics however is focused on a clear identification of all detectable metabolites or desired metabolites for specialized analysis (usually used for targeted profiling). Metabolites need to be known prior to analysis and internal standards, whether through in-house synthesis or commercial manufacturing, provide spectral comparisons that are then used to authenticate the compounds of interest.

The findings highlighted in table 1, outlining the most probable metabolites in the thalamus, show a number of bile acids prevalent in the thalamus as opposed to the metabolic significance provided from the data in the IC. Although bile acids have been documented to have neuroprotective properties and help reduce neuronal loss, so much so that they and their synthetic derivatives have been the main targets of therapeutic use for new treatments of various neurodegenerative disorders (AD, HD and PD, and also in humans with amyotrophic lateral sclerosis)(Cortez et al., 2015, Dionisio et al., 2015), this is the first known reporting of these bile acids in TD.

Bile is composed of 4 main components, bile acids, cholesterol, phospholipids and bilirubin. Bile acids (more appropriately bile salts but for general uses bile acids are interchangeable with salts) more specifically are water-soluble, cholesterol derived, hydrophilic molecules formed in the liver as primary bile acids transformed in the gut to secondary bile acids. This transformation offers the ability for diverse chemical interactions. Generally, bile acids conjugate with either glycine or taurine, and are negatively charged. A cell-surface receptor activated by bile acids is TGR5 (also referred to as G-protein coupled bile acid receptor 1) and interestingly does not depend on transport systems for cellular ligand uptake. TGR5 is located in a variety of cells and cell types including intestinal epithelium, Kupffer cells, sinusoidal endothelium and bile duct cells. It is also in tissues not involved in the enterohepatic circulation. This relatively new concept brings the attention to various tissues (more specifically related to this report, the brain) not involved in the classic understanding of bile acid cycling as possible targets of bile acid signalling (Schaap et al., 2014).

The biosynthesis of bile acids is the primary pathway for cholesterol catabolism. Briefly, it involves modification of the ring structure of cholesterol, shortening and oxidizing the side chain, and finally conjugating the bile acid with an amino acid (Russell, 2003). Outlined in Figures 4 and 5 are the pathway overviews (KEGG Pathways)

While cholic acid and chenodeoxycholic acid are the most abundant primary bile acids in humans and deoxycholic acid and lithocholic acid respective as secondary bile acids, in mice and rats, chenodeoxycholic acid is converted into muricholic acid (Botham and Boyd, 1983). Prior to bile acids being transported out of the hepatocytes, most are conjugated to taurine (mice/rats) or glycine (humans). The normal pathophysiology of mammals involves the use of bile-acid transporters as adaptors to minimize any deleterious manifestations of bile-acid accumulation (Thomas et al., 2008). The regulation of transporters to maintain homeostasis is mainly controlled at the transcriptional level and following our groups 2006 study (Vemuganti et al., 2006) several transcripts were identified in TD in the affected areas of the thalamus and IC. A key indicator to corroborating the current findings of this bile accumulation was reported in the 2006 study identifying Kruppel-like factor 4 (Klf4). Klf4 are zinc finger-containing transcription factors regulating several biological processes, including proliferation, differentiation,

development and apoptosis. During a morphogenesis of the gut this transcript is overly expressed and along with bile acids producing the same effect on Klf4. This Klf4 overexpression is a mechanism used to initiate the reversible cell replacement process referred to as metaplasia. Noteworthy is that Klf4 is a key factor in embryogenesis and one of the driving participators in embroyonic cell differentiation (Kazumori et al., 2011)

To look into the possible functional mechanisms of these detected bile acids in the brain, comparative investigation into other known neurological disorders and dysfunction related to bile or any of its known components was performed and reports related to increased levels and prolonged exposure to unconjugated bilirubin (UCB) at the neonatal stage (observed in prolonged jaundice) suggest it may induce neurological dysfunction (Cohen et al., 2010). Neuronal oxidative stress, pro-inflammatory cytokines released via glial cells, was observed (Silva et al., 2002, Falcao et al., 2006, Fernandes et al., 2006, Brito et al., 2008, Silva et al., 2010, Vaz et al., 2010, Barateiro et al., 2014). One of the major functional relationships shared with UCB and TD is the role of BBB disruption and brain microvascular endothelial cells (BMEC)(Ham and Karska-Wysocki, 2005) leading to neurological dysfunction. UCB influence in porcine and rat on BMEC (Akin et al., 2002, Cardoso et al., 2012) suggested altered endothelial cell viability due to UCB. Conversely, in human BMEC (HBMEC) it was demonstrated that a decrease in UCB levels had similar results with regard to endothelial cell survival and induced cytokine release, notably interleukin-6 (IL6) (Palmela et al., 2011). IL6 has been known to be involved in BBB disruption in several pathological disorders (Kaur and Ling, 2008, Carvey et al., 2009). UCB exposure was also shown to affect endothelial junctions and leading to considerable compromise to the integrity of the barrier (Palmela et al., 2012). In cases of bilirubin encephalopathy UCB toxicity has been found in focal regions of the cerebellum, hippocampus, and basal ganglia, with distinct signs of BBB dysfunction (Palmela et al., 2015). The aforementioned suggests bile acids, may be involved in the dysfunction of and disruption of the BBB and BMEC in TD.

A bile acid of interest that was not confirmed using our method, of single MS and LC-MS analysis (further experimental analysis by MS-MS to confirm the product is currently pending) but is suggestive by the peaks at 396 and 398 m/z (could include some impurities which could

account for the discrepancy in the theoretical mass) is ursodeoxycholic acid, or Urso. The compound under normal conditions exists in trace amounts in the circulation in humans, and is widely used in chronic liver diseases as a therapeutic involving cholestasis (Poupon et al., 1994, Brites et al., 1998, Lazaridis et al., 2001). Urso and its conjugates (originating in the liver), tauroursodeoxycholic acid (TUDCA), and glycoursodeoxycholic acid (GUDCA), have been reported to account for up to 80% of the bile acid conjugates in patients undergoing therapy (Rudolph et al., 2002). There have been numerous studies outlining the potential role of Urso in the treatment of non-liver diseases involving elevated levels of apoptosis (Keene et al., 2002, Rodrigues et al., 2003) mainly as the bile acid has been reported to have anti-apoptotic properties (Amaral et al., 2009). Another study has demonstrated that Urso can protect astrocytes from apoptosis and cause the suppression of the production of pro-inflammatory cytokines (Rodrigues et al., 2000, Silva et al., 2001, Fernandes et al., 2007) in conjunction with the counteracting of UCB-induced neuronal death and synaptic alterations (Silva et al., 2012). GUDCA has also been shown to nullify alterations in mitochondrial dysfunction and energy impairment in neurons seen by UCB (Brito et al., 2008, Vaz et al., 2010). The suggested protecting mechanism of Urso and its conjugates has been inferred to be based on the stabilization of the cell membrane structure, the maintenance of its properties, and prevention of the alterations in membrane lipid keeping polarity, fluidity, protein order, and the redox status in tact (Rodrigues et al., 2001, Rodrigues et al., 2002, Rodrigues et al., 2003, Sola et al., 2003).

Although little has been investigated or known in terms of the actual benefits of bile acids on endothelial cells it has been demonstrated that TUDCA can protect against amyloid-β-induced apoptosis, hallmarks of AD (Viana et al., 2009), leukocyte adhesion and movement along the endothelium (caused by lipid peroxidation products)(Vladykovskaya et al., 2012), and promote vessel repair (Cho et al., 2015). The endothelial cell interaction appears to be a more complex one exhibited by the anti-angiogenic capacity of Urso on these cells (Suh et al., 1997, Woo et al., 2010). The protective cell characteristics of Urso and its derivatives cells have not yet been proven to be exerted on the endothethial cells of the BBB. Recent only Urso has been shown to maintain the BBB integrity in elevated conditions of UCB in-vitro on endothelial cells and abbrogate barrier permeability (Palmela et al., 2015).

The accumulation of some bile acids and a reduction of some others at different points of TD progression suggest the involvement of a protective mechanism. The peaks suspected to be

produced by Urso show a relative increase for the PFC group but a decrease with respect to the other groups and the most significant decrease at the loss of righting reflex. In regards to other bile acids there appears to be very low levels in the PFC group and the highest levels following the loss of righting reflex. This along with much of the literature cited seems to indicate that a protective mechanism is at play to try and maintain the functional integrity of the thalamus not seen in the IC. Due to its position, lying deep within the brain, and playing vital roles in various neurological functions it can be understood that structural, functional, and chemical integrity needs to be maintained at all costs, this protective mechanism with the presence of crucial regenerative transcripts being identified in the thalamus exclusively rather than the IC further elaborates the requirement to target this area for possible biomarkers for TD.

Other key findings from the thalamus metabolites are increased levels of Kynurenic Acid (KYNA), which has been documented to have neuroprotective and anticonvulsive characteristics. The compound is well known to be an antagonist to NMDA, and several other neuro-receptors. An abnormal metabolism of the compound has been observed in AD, PD and HD. Like many of the detected metabolites and transcripts, KYNA appears to play a vital role during embryogenesis showing an increase in the CNS prior to birth with a significant decrease after birth. Profiling of KYNA metabolism in the brain has suggested it plays a role in of the organisation of neuronal connections and synaptic plasticity, also playing roles in aging and maturation. The observed elevations and/or reductions of KYNA during cognitive functional impairment accompanying many neurodegenerative disorders, have led researchers to believe that the compound plays a role in cognitive function and is further corroborated by the increased metabolism of KYNA observed with the cognitive progressive impairment in AD, in the early stages of HD, and in down's syndrome. KYNA has been identified as the only N-methyl-D-aspartate (NMDA) receptor antagonist that occurs endogenously, mediating glutamatergic hypofunction. In low dosage, KYNA has also been noted to block the nicotinergic acetycholine receptor providing an explanation into why with elevated levels psychosis and cognitive deterioration is observed (also noted in Schizophrenia). (Kepplinger et al., 2005, Muller and Schwarz, 2006, Wennstrom et al., 2014, Blanco Ayala et al., 2015). The current experiments show the highest levels of KYNA in the TD LRR group and the lowest in the PFC group. The findings are consistent with what is known about KYNA the elevated levels being observed at peak onset of neurological impairment and KYNA along with several other mechanisms are at work trying to preserve the integrity of the thalamus the chemical influx is not available and the

depletion of KYNA already began as short as an hour following the LRR. A possible explanation for the sudden increase from the PFC to the LRR reflex groups could be the loss and damage to many neuroreceptors, and finally at the more advanced stage a complete inutility for needing or using KYNA as the more permanent neurological impairments set in. It is worth noting that KYNA has been the source of recent pharmacological targeting for various neurological disorders (Mackenzie and Milligan, 2015, Toledo-Sherman et al., 2015)

Finally although several other metabolites were detected and can be further discussed, their functional purposes are very similar in the overall inferred internal regulatory mechanism of neuroprotection and neurodegeneration. One metabolite however, 1,9-dimethyluric acid, is noteworthy in its possible candidacy in clinical detection readily and easily measurable in human (and in this case rat) urine. The metabolite is rarely found in urine under normal conditions and is a methylated derivative of uric acid. As a product of purine nucleotide metabolism, uric acid and its derivative are often used as markers for various disorders mainly identifiable by their abnormal purine metabolism. The issue however is that many of the uric acid derivatives are not discernible from uric acid analytically and require further separation, but there are several simplified techniques available including a method using HPLC to distinguish between the different compounds (Morris et al., 1986, Safranow, 2000, Safranow et al., 2000). The highest levels of this metabolite were observed in the PFC group and the lowest in the advanced stage TD group. This finding would suggest that this marker begins to show very early in the stages of TD when nutritional irregularities occur. This also suggests its involvement in purine metabolism is still functional up until TD begins its metabolic manifestations. Although the PFC group (by the time the TD group has reached LRR the PFC group will have not had any food for several days) is supplemented with thiamine, if left long enough it will undergo TD and thus the precursor indications of such seem to be exhibited in the elevated levels of this dimethyl uric acid.

The IC highlights very different functional characteristics and applications by the repertoire of metabolites detected, showing marked differences in different metabolites of significance for the PFC group, the TD group during the LRR, and the more advanced stage TD group, seen in Figure 6 (metabolites annotated in Table 2). Noticeably is the involvement of metabolic

pathways involved in the metabolism of various amino acids, pyrimidines, and/or purines (of statistical significance based on the metabolites identified, seen in Figure 7 taken from the analytical report performed through Metabolanalyst 3.0 via the Pathway Analysis). One clear feature is that most detected metabolites are non-existent or non-functional in the advanced TD group in contrast with the PFC group or the TD at the LRR group. The IC seems to exhibit, metabolically, a more basic biological cellular feature of significance shown by the significant detection of amino acids, pyrimidines, and/or purines, suggesting a more fundamental role for the IC in providing necessary compounds for cell growth and function. Once clear histological damage to this area has been demonstrated in the sequel of TD, the most profound clinical signs of neurological impairment have been observed (Hazell et al., 2014).

Only 2 metabolites, taurine, and carnosine will be discussed briefly as most of the data results are self-explanatory, in regards to the pathway interconnections, but for further discussion more elaborate analysis is required, perhaps quantitative analysis of specific compounds and their chemical changes during different stages of the disorder.

Taurine is a sulfonic acid derived from cysteine and is an amino acid but not in the traditional meaning as it does not contain the usual carboxylic acid groups and does not incorporate into the structural configuration of proteins. Although in adult mammals, taurine can be endogenously synthesized, it is usually more reliant on the nutritional source for production. It plays a fundamental role in fetus and infancy development (Hernandez-Benitez et al., 2013). The brain is rich in the compound, and it plays a crucial role in the overall homeostasis of many different organs including the brain. It also plays a role in stem cell homeostasis and neurodegenerative disorders (Yenkoyan et al., 2011, Inoue et al., 2012). Next to GABA, taurine as an inhibitory neurotransmitter, is second in functional importance (Schober and Mongin, 2015). The anticonvulsant and antianxiety characteristics are attributed to this inhibition property. Also known to lower glutamate levels in the brain, clinical trials targeting the uptake and interaction of taurine have suggested positive results in the treatment of a number of forms of epilepsy (Plum et al., 2014). Taurine has been linked to alanine, glutamate (inhibitory effect) while vitamins A, B1, B6, zinc, and manganese have been found to have the opposite effect (Liu et al., 2010). A brief overview has been provided in Figure 8 (KEGG). Although taurine is often

elevated in the blood plasma of epileptics to distinguish between a chemical supplementation requirement rather than a disorder or disease manifesting itself via a taurine dysregulation. However taurine is readily detectable in blood plasma and offers an easy and invaluable method to quickly assessing the possibility of TD (in conjugation with currently available assays, and possibility of the aforementioned uric acid derivative from urine). The PFC group showed a stable content of taurine, while the LRR group exhibited marked increases, suggestive of a mechanism attempting to inhibit opisthotonus and an attempt to stabilize the cell membranes' ion transportation (another integral function of taurine), once TD has progressed past the LRR the levels quickly drop below those of the PFC group, perhaps indicating that in this area there is no more ability to salvage the cell membranes integrity nor can it function effectively any longer as an inhibitory neurotransmitter. Noteworthy is that some genetic disorders causing taurine metabolism resemble Parkinsonism in symptomatic expression (Perry et al., 1978, Purdy et al., 1979).

Carnosine is a metabolite found solely in the tissues of animals, with the highest concentrations being found in the brain and muscle. Histidine and β-alanine form the dipeptide known as Carnosine. Its pertinence and usefulness lies in its reported potential. Carnosine has the potential to inhibit many pathological changes such as protein oxidation, glycation, cross-linking, and several others (Hipkiss, 2006). Changes in doses of the compound have provided different responses in patients diagnosed as autistics. Taken as a dietary supplement has reported an improvement in the conditions attributed to the disorder, but in high doses "hyperactivity" has been noted and believed to be caused by the increase in cortisone levels (McGinnis, 2005, Rossignol, 2009). The antioxidant features of the compound are believed to act on a mechanism involving chelating against metal ions, superoxide dismutase (SOD)-like activity, ROS and free radicals scavenging ability (Guney et al., 2006). In the PFC group a significantly higher level of the compound is present, and in this group one of the visible characteristics of the animals is hyperactivity, potentially being explained by the same factors reported in higher doses in human patients of elevated cortisone levels (several peaks in the IC are suggestive of cortisone, but as previously mentioned further experimental conditions are required for positive absolute confirmation). The LRR group showed the lowest levels and it appears that the levels plateau to somewhere below the PFC levels. This observation is possibly due to the immediate depletion of the compound in the brain in this area at the immediate LRR but as TD irreversibly progresses

the remaining tissue, whether brain or possibly muscle sends quantities of the compound to reach pre-TD levels.

4.2 Neural Stem-Progenitor Cell

An identical metabolic analysis (Meinhardt et al., 2015) was performed on rat brains extracted from PTD rats reversed and replenished with thiamine and tail-vein transplanted with NSPC labelled with CFSE. Several unidentifiable compounds from the previous analysis groups produced significance with regards to statistical correlation, summarized in Table 3. This suggested that NSPC can offer an indication of different metabolites that do play roles in TD not possibly identified previously. One of the more notable metabolites detected was arachidonic acid, a polyunsaturated, essential fatty acid. The fatty acid and its usual conversion compounds into the various eicosanoids, are readily found in the liver and brain (along with DHA are the most abundant in this area) and play a crucial role in the regulation of inflammation, although there remains a difference in the literature concerning whether increased levels in certain target areas cause decreases in some inflammatory factors or if the opposite is the case (Selim et al., 2014, Barakat et al., 2015, Fritsche, 2015, Wurtz et al., 2015). It is the substrate in cell membrane phospholipids for the biosynthesis of other compounds that include prostaglandins, thromboxanes, and leukotrienes (Pompeia et al., 2002, Pompeia et al., 2003). These compounds can act as mediators and regulators in processes, such as clotting, platelet aggregation, inflammatory cytokine production, and immune function. Arachidonic acid offers a protection to the brain (shown in the hippocampus specifically) against oxidative stress through activation of peroxisome proliferator-activated receptor gamma (Wang et al., 2006). A very crucial function of arachidonic acid is its involvement in the repair and growth of neurons via the activation of syntaxin-3 (Darios and Davletov, 2006). It is important to note that this compound is of statistical significance only in the thalamus but no noticeable correlation is noted in the IC. Conversely, the transcript arachidonate 5-LOX activator shows a significant expression increase in the IC (Vemuganti et al., 2006) but not in the thalamus. This transcript uses arachidonic acid for activation and also functions as an inflammation regulator (specifically in cardiovascular diseases and thought to be involved in the vascular pathology of AD) (Manev and Manev, 2006, Lucas et al., 2014). The over expression of the transcript has been shown to trigger c-Myc (shown to be one of the 4 necessary factors for pluripotent reprogramming of somatic cells) and

its up regulation (Sarveswaran et al., 2015). The transcription factor has demonstrated an ability to promote neuronal differentiation (Zinin et al., 2014). A global overview of the relevance of arachidonic acid being present in high doses in the thalamus in the PFC group (possibly to mediate the inflammatory responses in that area of the brain) and an increased expression of arachidonate 5-LOX activator exclusively in the IC in the TD group infers a more dramatic neuronal loss in the IC that the brain regulatory mechanisms want to mediate as best as possible. This metabolite once again provides an indication that NSPC signaling and differentiation is possibly at play and find functional importance during key time points of the TD development. It is noteworthy to mention that a change in the relative metabolite concentrations of arachidonic acid and several others was found following NSPC transplantations in both the PFC group and the TD group.

4.3 Statistical Analysis Integrating and Identifying Gene-Metabolism pathways mainly involved

As a final method of correlation, an integrated pathway analysis was performed (via MetaboAnalyst 3.0) to search for a significance between the metabolomics data obtained and the previously highlighted data from the Vemuganti et al. (2006) transcript identification. Only the data with the same experimental conditions were used, i.e. excluding the NSPC transplanted and the LRR groups. Figure 20 highlights the pathways of greatest significance. The 2 most important pathways based on the correlation are the VEGF signalling pathway and the Jak-STAT signalling pathway. The former is a pathway involved in the stimulation of vasculogenesis and angiogenesis, providing functional restoration for the oxygen supply in poor or depleted blood supplied tissue. Of greater pertinence to this study is the involvement of the Jak-STAT pathway. It has been a recent point of emphasis by our group to highlight astrocytes as a major target in TD and in WE showing the downregulation of various cell specific proteins including EAAT1, EAAT2, glial fibrillary acidic protein, and glutamine synthetase (Hazell et al., 2010).

The JAK–STAT pathway has shown pleiotropic effects on various processes involved in cell fate regulation (Artavanis-Tsakonas et al., 1999, Gaiano and Fishell, 2002, Skjesol et al., 2014), including stimulation of cell proliferation and survival. In NSPC, this pathway demonstrated

direct implication in the promotion of differentiation in astrocytes and up-regulation of the astrocytic marker glial fibrillary acidic protein (GFAP) (Rajan and McKay, 1998, Chambers et al., 2001, Kamakura et al., 2004).

Recently STAT-3 has been acknowledged as a key contributor to the regulation of astrogenesis. For proper progression of the neurogenesis phase during the formation of required neurons the Inhibition of astrogenesis is a requirement. This requires the silencing of the JAK/STAT pathway. The method by which this is accomplished is through the activation of the JAK receptors being phosphorylated and acetylated, activating the STAT3 transcription factor. STAT3 dimerizes followed by translocation to the nucleus to stimulation gene transcription. Due to this importance recent research has focused on the inhibition of STAT3 and the mechanisms involved in accomplishing this. Reports have indicated that during neurogenesis, p300/CBP co-activator complex is sequestered from STAT3 resulting in inhibition of STAT3-dependent transcription in NSPCs. p300/CBP has been suggested to reduce acetylation at the STAT3 binding site of the GFAP promoter (Benekli et al., 2003). JAK/STAT signaling is also down regulated by the methylation of the STAT3 to the promoters of astrocytic genes. This in turn deters binding of STAT3 at the promoter of the astrocytic genes GFAP and S100B (Rajan and McKay, 1998). It has been demonstrated that conditional deletion of DNMT1 in NSPC brings about a cellular production switch from neurogenesis to astrogenesis (Nakashima et al., 1999).

Another noted phenomenon for deactivating STAT3 is through de-acetylation which has an added function of preventing astrogenesis but also induces neurogenesis through the up-regulation of proneural gene expression. Some extrinsic factors may also trigger inhibition of the JAK/STAT pathway, and they include neurotrophins. Brain-derived neurotrophic factor (BDNF) (noted in the 2006 transcript study) can activate the neurogenic SHP2-Ras-MEK-ERK pathway (a significant correlation has been noted above indicating the involvement of the MAPK pathway also referred to as Ras-MEK-ERK pathway), which can promote neurogenesis (Zhang et al., 2009). The phosphatase SHP2 is a key modulator of the MEK-ERK pathway (Abranches et al., 2009), and helps regulate the neuron-to-astrocyte switch. A knockdown study of SHPs in NSPC initiated the differentiation of astroglial cells during the more advanced stages of neurogenesis (Satow et al., 2001). SHP2 has also been shown to prompt pro-neural MEK-ERK signaling while inhibiting JAK/STAT pathway. The dephosphorylation of STAT3 is also accomplished by SHP2 in non-neuronal cells (Hanke et al., 1996). The MAPK signaling pathway could effectively

inhibit STAT3 transcription, in turn down regulating astrogenesis, and although very few candidates for such inhibition have been identified, but BDNF can fill this role as it is expressed prior to the production stage of astrocytes (Lobie et al., 1996, Lefrancois-Martinez et al., 2011) and remains a key regulator of NSPC differentiation (Ram and Waxman, 1997, Qu and Shi, 2009, Imayoshi et al., 2010).

CONCLUSION

Through a non-targeted metabolomics comparative study key metabolites were identified in rats with TD. The study confirmed that through NSPC transplantation of TD rats, unique metabolites could be detected in key vulnerable areas, specifically the thalamus and IC. The findings also suggested an involvement of endogenous NSPC in focal areas of the thalamus and IC. Labelled NSPC were transplanted and in conjunction with the metabolomics study provided evidence for NSPC involvement in trying to ameliorate brain irregularities, suggesting that these cells could offer a therapeutic alternative to alleviate some of symptomatic degeneration. Further studies to focus on quantitative metabolomics of certain known and unknown metabolites found in this study could offer potential confirmation and elucidation of the mechanisms. The implications of the findings provided potential targets for quick detection of early onset stages of TD, and possibly other neurodegenerative disorders, with similar biochemical manifestations/dysfunction, prospectively through blood and/or urine samples due to the traceability of these metabolites.

REFERENCES

Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V, Black PM, Breakefield XO, Snyder EY (2000) Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proceedings of the National Academy of Sciences of the United States of America 97:12846-12851.

Abranches E, Silva M, Pradier L, Schulz H, Hummel O, Henrique D, Bekman E (2009) Neural differentiation of embryonic stem cells in vitro: a road map to neurogenesis in the embryo. PloS one 4:e6286.

Aikawa H, Watanabe IS, Furuse T, Iwasaki Y, Satoyoshi E, Sumi T, Moroji T (1984) Low energy levels in thiamine-deficient encephalopathy. Journal of neuropathology and experimental neurology 43:276-287.

Akin E, Clower B, Tibbs R, Tang J, Zhang J (2002) Bilirubin produces apoptosis in cultured bovine brain endothelial cells. Brain research 931:168-175.

Allen NJ, Karadottir R, Attwell D (2004) Reversal or reduction of glutamate and GABA transport in CNS pathology and therapy. Pflugers Archiv : European journal of physiology 449:132-142.

Amaral JD, Viana RJ, Ramalho RM, Steer CJ, Rodrigues CM (2009) Bile acids: regulation of apoptosis by ursodeoxycholic acid. Journal of lipid research 50:1721-1734.

Arriza JL, Eliasof S, Kavanaugh MP, Amara SG (1997) Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proceedings of the National Academy of Sciences of the United States of America 94:4155-4160.

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284:770-776.

Barakat R, Abou El-Ela NE, Sharaf S, El Sagheer O, Selim S, Tallima H, Bruins MJ, Hadley KB, El Ridi R (2015) Efficacy and safety of arachidonic acid for treatment of school-age children in Schistosoma mansoni high-endemicity regions. The American journal of tropical medicine and hygiene 92:797-804.

Barateiro A, Domingues HS, Fernandes A, Relvas JB, Brites D (2014) Rat cerebellar slice cultures exposed to bilirubin evidence reactive gliosis, excitotoxicity and impaired myelinogenesis that is prevented by AMPA and TNF-alpha inhibitors. Molecular neurobiology 49:424-439.

Benavides J, Fage D, Carter C, Scatton B (1987) Peripheral type benzodiazepine binding sites are a sensitive indirect index of neuronal damage. Brain research 421:167-172.

Benekli M, Baer MR, Baumann H, Wetzler M (2003) Signal transducer and activator of transcription proteins in leukemias. Blood 101:2940-2954.

Berger UV, Hediger MA (1998) Comparative analysis of glutamate transporter expression in rat brain using differential double in situ hybridization. Anatomy and embryology 198:13-30.

Bijlsma S, Bobeldijk I, Verheij ER, Ramaker R, Kochhar S, Macdonald IA, van Ommen B, Smilde AK (2006) Large-scale human metabolomics studies: a strategy for data (pre-) processing and validation. Analytical chemistry 78:567-574.

Bilic G, Zeisberger SM, Mallik AS, Zimmermann R, Zisch AH (2008) Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. Cell transplantation 17:955-968.

Blakaj A, Lin H (2008) Piecing together the mosaic of early mammalian development through microRNAs. The Journal of biological chemistry 283:9505-9508.

Blanco Ayala T, Lugo Huitron R, Carmona Aparicio L, Ramirez Ortega D, Gonzalez Esquivel D, Pedraza Chaverri J, Perez de la Cruz G, Rios C, Schwarcz R, Perez de la Cruz V (2015) Alternative kynurenic acid synthesis routes studied in the rat cerebellum. Frontiers in cellular neuroscience 9:178.

Bogdanov M, Matson WR, Wang L, Matson T, Saunders-Pullman R, Bressman SS, Flint Beal M (2008) Metabolomic profiling to develop blood biomarkers for Parkinson's disease. Brain : a journal of neurology 131:389-396.

Botham KM, Boyd GS (1983) The metabolism of chenodeoxycholic acid to beta-muricholic acid in rat liver. European journal of biochemistry / FEBS 134:191-196.

Brites D, Rodrigues CM, van-Zeller H, Brito A, Silva R (1998) Relevance of serum bile acid profile in the diagnosis of intrahepatic cholestasis of pregnancy in an high incidence area: Portugal. European journal of obstetrics, gynecology, and reproductive biology 80:31-38.

Brito MA, Lima S, Fernandes A, Falcao AS, Silva RF, Butterfield DA, Brites D (2008) Bilirubin injury to neurons: contribution of oxidative stress and rescue by glycoursodeoxycholic acid. Neurotoxicology 29:259-269.

Bruder SP, Jaiswal N, Haynesworth SE (1997) Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. Journal of cellular biochemistry 64:278-294.

Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S (1998) Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 16:155-162.

Butterworth RF (1986) Cerebral thiamine-dependent enzyme changes in experimental Wernicke's encephalopathy. Metabolic brain disease 1:165-175.

Butterworth RF (1989) Effects of thiamine deficiency on brain metabolism: implications for the pathogenesis of the Wernicke-Korsakoff syndrome. Alcohol Alcohol 24:271-279.

Butterworth RF, Giguere JF, Besnard AM (1985) Activities of thiamine-dependent enzymes in two experimental models of thiamine-deficiency encephalopathy: 1. The pyruvate dehydrogenase complex. Neurochemical research 10:1417-1428.

Butterworth RF, Kril JJ, Harper CG (1993) Thiamine-dependent enzyme changes in the brains of alcoholics: relationship to the Wernicke-Korsakoff syndrome. Alcoholism, clinical and experimental research 17:1084-1088.

Byers B, Cord B, Nguyen HN, Schule B, Fenno L, Lee PC, Deisseroth K, Langston JW, Pera RR, Palmer TD (2011) SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate alpha-synuclein and are susceptible to oxidative stress. PloS one 6:e26159.

Cagnin A, Myers R, Gunn RN, Lawrence AD, Stevens T, Kreutzberg GW, Jones T, Banati RB (2001) In vivo visualization of activated glia by [11C] (R)-PK11195-PET following herpes encephalitis reveals projected neuronal damage beyond the primary focal lesion. Brain : a journal of neurology 124:2014-2027.

Calingasan NY, Gandy SE, Baker H, Sheu KF, Kim KS, Wisniewski HM, Gibson GE (1995) Accumulation of amyloid precursor protein-like immunoreactivity in rat brain in response to thiamine deficiency. Brain research 677:50-60.

Cardoso FL, Kittel A, Veszelka S, Palmela I, Toth A, Brites D, Deli MA, Brito MA (2012) Exposure to lipopolysaccharide and/or unconjugated bilirubin impair the integrity and function of brain microvascular endothelial cells. PloS one 7:e35919.

Carvey PM, Hendey B, Monahan AJ (2009) The blood-brain barrier in neurodegenerative disease: a rhetorical perspective. Journal of neurochemistry 111:291-314.

Chambers CB, Peng Y, Nguyen H, Gaiano N, Fishell G, Nye JS (2001) Spatiotemporal selectivity of response to Notch1 signals in mammalian forebrain precursors. Development 128:689-702.

Cho JG, Lee JH, Hong SH, Lee HN, Kim CM, Kim SY, Yoon KJ, Oh BJ, Kim JH, Jung SY, Asahara T, Kwon SM, Park SG (2015) Tauroursodeoxycholic acid, a bile acid, promotes blood vessel repair by recruiting vasculogenic progenitor cells. Stem Cells 33:792-805.

Cohen RS, Wong RJ, Stevenson DK (2010) Understanding neonatal jaundice: a perspective on causation. Pediatrics and neonatology 51:143-148.

Cortez LM, Campeau J, Norman G, Kalayil M, Van der Merwe J, McKenzie D, Sim VL (2015) Bile acids reduce prion conversion, reduce neuronal loss, and prolong male survival in models of prion disease. Journal of virology.

Coskun PE, Busciglio J (2012) Oxidative Stress and Mitochondrial Dysfunction in Down's Syndrome: Relevance to Aging and Dementia. Current gerontology and geriatrics research 2012:383170.

D'Ambrosio R, Gordon DS, Winn HR (2002) Differential role of KIR channel and Na(+)/K(+)-pump in the regulation of extracellular K(+) in rat hippocampus. Journal of neurophysiology 87:87-102.

Danbolt NC (2001) Glutamate uptake. Progress in neurobiology 65:1-105.

Danbolt NC, Storm-Mathisen J, Kanner BI (1992) An [Na+ + K+]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience 51:295-310.

Darios F, Davletov B (2006) Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 440:813-817.

Dehnes Y, Chaudhry FA, Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC (1998) The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:3606-3619.

Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI (1999) A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 14:700-709.

Dettmer K, Aronov PA, Hammock BD (2007) Mass spectrometry-based metabolomics. Mass spectrometry reviews 26:51-78.

Devine MJ, Ryten M, Vodicka P, Thomson AJ, Burdon T, Houlden H, Cavaleri F, Nagano M, Drummond NJ, Taanman JW, Schapira AH, Gwinn K, Hardy J, Lewis PA, Kunath T (2011) Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus. Nature communications 2:440.

Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321:1218-1221.

Dionisio PA, Amaral JD, Ribeiro MF, Lo AC, D'Hooge R, Rodrigues CM (2015) Amyloid-beta pathology is attenuated by tauroursodeoxycholic acid treatment in APP/PS1 mice after disease onset. Neurobiology of aging 36:228-240.

Diorio D, Welner SA, Butterworth RF, Meaney MJ, Suranyi-Cadotte BE (1991) Peripheral benzodiazepine binding sites in Alzheimer's disease frontal and temporal cortex. Neurobiology of aging 12:255-258.

Dolmetsch R, Geschwind DH (2011) The human brain in a dish: the promise of iPSC-derived neurons. Cell 145:831-834.
Donnino MW, Vega J, Miller J, Walsh M (2007) Myths and misconceptions of Wernicke's encephalopathy: what every emergency physician should know. Annals of emergency medicine 50:715-721.
Ebert AD, Yu J, Rose FF, Jr., Mattis VB, Lorson CL, Thomson JA, Svendsen CN (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457:277-280.
Eriksson S, Lundquist A, Gustafson Y, Lundin-Olsson L (2009) Comparison of three statistical methods for analysis of fall predictors in people with dementia: negative binomial regression (NBR), regression tree (RT), and partial least squares regression (PLSR). Archives of gerontology and geriatrics 49:383-389.
Falcao AS, Fernandes A, Brito MA, Silva RF, Brites D (2006) Bilirubin-induced immunostimulant effects and toxicity vary with neural cell type and maturation state. Acta neuropathologica 112:95-105.
Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E (2012) Mitochondria, oxidative stress and neurodegeneration. Journal of the neurological sciences 322:254-262.
Feng Y, Wang J, Ling S, Li Z, Li M, Li Q, Ma Z, Yu S (2014) Differentiation of mesenchymal stem cells into neuronal cells on fetal bovine acellular dermal matrix as a tissue engineered nerve scaffold. Neural regeneration research 9:1968-1978.
Fernandes A, Falcao AS, Silva RF, Gordo AC, Gama MJ, Brito MA, Brites D (2006) Inflammatory signalling pathways involved in astroglial activation by unconjugated bilirubin. Journal of neurochemistry 96:1667-1679.
Fernandes A, Vaz AR, Falcao AS, Silva RF, Brito MA, Brites D (2007) Glycoursodeoxycholic acid and interleukin-10 modulate the reactivity of rat cortical astrocytes to unconjugated bilirubin. Journal of neuropathology and experimental neurology 66:789-798.
Fritsche KL (2015) The science of fatty acids and inflammation. Adv Nutr 6:293S-301S.
Fuhrer T, Heer D, Begemann B, Zamboni N (2011) High-throughput, accurate mass metabolome profiling of cellular extracts by flow injection-time-of-flight mass spectrometry. Analytical chemistry 83:7074-7080.
Gaiano N, Fishell G (2002) The role of notch in promoting glial and neural stem cell fates. Annual review of neuroscience 25:471-490.
Gaitonde MK, Fayein NA, Johnson AL (1975) Decreased metabolism in vivo of glucose into amino acids of the brain of thiamine-deficient rats after treatment with pyrithiamine. Journal of neurochemistry 24:1215-1223.
Gardner-Medwin AR, Coles JA, Tsacopoulos M (1981) Clearance of extracellular potassium: evidence for spatial buffering by glial cells in the retina of the drone. Brain research 209:452-457.
Gerhard A, Banati RB, Goerres GB, Cagnin A, Myers R, Gunn RN, Turkheimer F, Good CD, Mathias CJ, Quinn N, Schwarz J, Brooks DJ (2003) [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. Neurology 61:686-689.
Gibson GE, Ksiezak-Reding H, Sheu KF, Mykytyn V, Blass JP (1984) Correlation of enzymatic, metabolic, and behavioral deficits in thiamin deficiency and its reversal. Neurochemical research 9:803-814.
Gika HG, Ji C, Theodoridis GA, Michopoulos F, Kaplowitz N, Wilson ID (2012) Investigation of chronic alcohol consumption in rodents via ultra-high-performance liquid chromatography-mass spectrometry based metabolite profiling. Journal of chromatography A 1259:128-137.

Gould E, Reeves AJ, Graziano MS, Gross CG (1999) Neurogenesis in the neocortex of adult primates. Science 286:548-552.
Gu B, Desjardins P, Butterworth RF (2008) Selective increase of neuronal cyclooxygenase-2 (COX-2) expression in vulnerable brain regions of rats with experimental Wernicke's encephalopathy: effect of nimesulide. Metabolic brain disease 23:175-187.
Gubler CJ, Adams BL, Hammond B, Yuan EC, Guo SM, Bennion M (1974) Effect of thiamine deprivation and thiamine antagonists on the level of gamma-aminobutyric acid and on 2-oxoglutarate metabolism in rat brain. Journal of neurochemistry 22:831-836.
Guney Y, Turkcu UO, Hicsonmez A, Andrieu MN, Guney HZ, Bilgihan A, Kurtman C (2006) Carnosine may reduce lung injury caused by radiation therapy. Medical hypotheses 66:957-959.
Hakim AM (1984) The induction and reversibility of cerebral acidosis in thiamine deficiency. Annals of neurology 16:673-679.
Hakim AM, Pappius HM (1983) Sequence of metabolic, clinical, and histological events in experimental thiamine deficiency. Annals of neurology 13:365-375.
Ham D, Karska-Wysocki B (2005) Energy and glucose pathways in thiamine deficient primary rat brain microvascular endothelial cells. General physiology and biophysics 24:467-474.
Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, Weringer EJ, Pollok BA, Connelly PA (1996) Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. The Journal of biological chemistry 271:695-701.
Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, Yow A, Soldner F, Hockemeyer D, Hallett PJ, Osborn T, Jaenisch R, Isacson O (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proceedings of the National Academy of Sciences of the United States of America 107:15921-15926.
Haugeto O, Ullensvang K, Levy LM, Chaudhry FA, Honore T, Nielsen M, Lehre KP, Danbolt NC (1996) Brain glutamate transporter proteins form homomultimers. The Journal of biological chemistry 271:27715-27722.
Hazell AS (2009a) Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. Neurochemistry international 55:129-135.
Hazell AS (2009b) Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. Neurochemistry international 55:129-135.
Hazell AS, Faim S, Wertheimer G, Silva VR, Marques CS (2013) The impact of oxidative stress in thiamine deficiency: a multifactorial targeting issue. Neurochemistry international 62:796-802.
Hazell AS, Rao KV, Danbolt NC, Pow DV, Butterworth RF (2001) Selective down-regulation of the astrocyte glutamate transporters GLT-1 and GLAST within the medial thalamus in experimental Wernicke's encephalopathy. Journal of neurochemistry 78:560-568.
Hazell AS, Sheedy D, Oanea R, Aghourian M, Sun S, Jung JY, Wang D, Wang C (2010) Loss of astrocytic glutamate transporters in Wernicke encephalopathy. Glia 58:148-156.
Hazell AS, Wang C (2005) Downregulation of complexin I and complexin II in the medial thalamus is blocked by N-acetylcysteine in experimental Wernicke's encephalopathy. Journal of neuroscience research 79:200-207.
Hazell AS, Wang D, Oanea R, Sun S, Aghourian M, Yong JJ (2014) Pyrithiamine-induced thiamine deficiency alters proliferation and neurogenesis in both neurogenic and vulnerable areas of the rat brain. Metabolic brain disease 29:145-152.

Hernandez-Benitez R, Vangipuram SD, Ramos-Mandujano G, Lyman WD, Pasantes-Morales H (2013) Taurine enhances the growth of neural precursors derived from fetal human brain and promotes neuronal specification. Developmental neuroscience 35:40-49.

Heroux M, Butterworth RF (1988) Reversible alterations of cerebral gamma-aminobutyric acid in pyrithiamine-treated rats: implications for the pathogenesis of Wernicke's encephalopathy. J Neurochem 51:1221-1226.

Heroux M, Butterworth RF (1995) Regional alterations of thiamine phosphate esters and of thiamine diphosphate-dependent enzymes in relation to function in experimental Wernicke's encephalopathy. Neurochemical research 20:87-93.

Hertz L, Dringen R, Schousboe A, Robinson SR (1999) Astrocytes: glutamate producers for neurons. Journal of neuroscience research 57:417-428.

Hipkiss AR (2006) Would carnosine or a carnivorous diet help suppress aging and associated pathologies? Annals of the New York Academy of Sciences 1067:369-374.

Holt IJ, Harding AE, Morgan-Hughes JA (1988) Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 331:717-719.

Hunt DP, Irvine KA, Webber DJ, Compston DA, Blakemore WF, Chandran S (2008) Effects of direct transplantation of multipotent mesenchymal stromal/stem cells into the demyelinated spinal cord. Cell transplantation 17:865-873.

Imayoshi I, Sakamoto M, Yamaguchi M, Mori K, Kageyama R (2010) Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:3489-3498.

Inoue K, Furukawa T, Kumada T, Yamada J, Wang T, Inoue R, Fukuda A (2012) Taurine inhibits K+-Cl- cotransporter KCC2 to regulate embryonic Cl- homeostasis via with-no-lysine (WNK) protein kinase signaling pathway. The Journal of biological chemistry 287:20839-20850.

Johansson CB, Svensson M, Wallstedt L, Janson AM, Frisen J (1999) Neural stem cells in the adult human brain. Experimental cell research 253:733-736.

Kaddurah-Daouk R, Krishnan KR (2009) Metabolomics: a global biochemical approach to the study of central nervous system diseases. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:173-186.

Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y (2004) Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling. Nature cell biology 6:547-554.

Kaur C, Ling EA (2008) Blood brain barrier in hypoxic-ischemic conditions. Current neurovascular research 5:71-81.

Kazumori H, Ishihara S, Takahashi Y, Amano Y, Kinoshita Y (2011) Roles of Kruppel-like factor 4 in oesophageal epithelial cells in Barrett's epithelium development. Gut 60:608-617.

Keene CD, Rodrigues CM, Eich T, Chhabra MS, Steer CJ, Low WC (2002) Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease. Proceedings of the National Academy of Sciences of the United States of America 99:10671-10676.

Kepplinger B, Baran H, Kainz A, Ferraz-Leite H, Newcombe J, Kalina P (2005) Age-related increase of kynurenic acid in human cerebrospinal fluid - IgG and beta2-microglobulin changes. Neuro-Signals 14:126-135.

Kim DS, Kim JY, Kang M, Cho MS, Kim DW (2007) Derivation of functional dopamine neurons from embryonic stem cells. Cell transplantation 16:117-123.

Kornblum HI (2007) Introduction to neural stem cells. Stroke; a journal of cerebral circulation 38:810-816.

Kugler P, Schmitt A (1999) Glutamate transporter EAAC1 is expressed in neurons and glial cells in the rat nervous system. Glia 27:129-142.
Kunkanjanawan T, Noisa P, Parnpai R (2011) Modeling neurological disorders by human induced pluripotent stem cells. Journal of biomedicine & biotechnology 2011:350131.
Lakshmipathy U, Hart RP (2008) Concise review: MicroRNA expression in multipotent mesenchymal stromal cells. Stem Cells 26:356-363.
Lazaridis KN, Gores GJ, Lindor KD (2001) Ursodeoxycholic acid 'mechanisms of action and clinical use in hepatobiliary disorders'. Journal of hepatology 35:134-146.
Le O, Heroux M, Butterworth RF (1991) Pyrithiamine-induced thiamine deficiency results in decreased Ca(2+)-dependent release of glutamate from rat hippocampal slices. Metabolic brain disease 6:125-132.
Lee G, Papapetrou EP, Kim H, Chambers SM, Tomishima MJ, Fasano CA, Ganat YM, Menon J, Shimizu F, Viale A, Tabar V, Sadelain M, Studer L (2009) Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461:402-406.
Lefrancois-Martinez AM, Blondet-Trichard A, Binart N, Val P, Chambon C, Sahut-Barnola I, Pointud JC, Martinez A (2011) Transcriptional control of adrenal steroidogenesis: novel connection between Janus kinase (JAK) 2 protein and protein kinase A (PKA) through stabilization of cAMP response element-binding protein (CREB) transcription factor. The Journal of biological chemistry 286:32976-32985.
Lehre KP, Levy LM, Ottersen OP, Storm-Mathisen J, Danbolt NC (1995) Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:1835-1853.
Leong DK, Le O, Oliva L, Butterworth RF (1994) Increased densities of binding sites for the "peripheral-type" benzodiazepine receptor ligand [3H]PK11195 in vulnerable regions of the rat brain in thiamine deficiency encephalopathy. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 14:100-105.
Liu C, Huang Y, Pang M, Yang Y, Li S, Liu L, Shu T, Zhou W, Wang X, Rong L, Liu B (2015) Tissue-engineered regeneration of completely transected spinal cord using induced neural stem cells and gelatin-electrospun poly (lactide-co-glycolide)/polyethylene glycol scaffolds. PloS one 10:e0117709.
Liu J, Wu JQ, Yang JJ, Wei JY, Gao WN, Guo CJ (2010) Metabolomic study on vitamins B(1), B(2), and PP supplementation to improve serum metabolic profiles in mice under acute hypoxia based on (1)H NMR analysis. Biomedical and environmental sciences : BES 23:312-318.
Liu SP, Fu RH, Huang SJ, Huang YC, Chen SY, Chang CH, Liu CH, Tsai CH, Shyu WC, Lin SZ (2013) Stem cell applications in regenerative medicine for neurological disorders. Cell transplantation 22:631-637.
Liu SP, Fu RH, Yu HH, Li KW, Tsai CH, Shyu WC, Lin SZ (2009) MicroRNAs regulation modulated self-renewal and lineage differentiation of stem cells. Cell transplantation 18:1039-1045.
Lobie PE, Ronsin B, Silvennoinen O, Haldosen LA, Norstedt G, Morel G (1996) Constitutive nuclear localization of Janus kinases 1 and 2. Endocrinology 137:4037-4045.
Lucas CM, Harris RJ, Giannoudis A, McDonald E, Clark RE (2014) Low leukotriene B4 receptor 1 leads to ALOX5 downregulation at diagnosis of chronic myeloid leukemia. Haematologica 99:1710-1715.
Mackenzie AE, Milligan G (2015) The emerging pharmacology and function of GPR35 in the nervous system. Neuropharmacology.

Malcolm RA, Richard P, Nicholas AW (2002) Cardiac stem cells. The Journal of pathology 197:468-478.
Manev H, Manev R (2006) 5-Lipoxygenase (ALOX5) and FLAP (ALOX5AP) gene polymorphisms as factors in vascular pathology and Alzheimer's disease. Medical hypotheses 66:501-503.
Maragakis NJ, Rothstein JD (2004) Glutamate transporters: animal models to neurologic disease. Neurobiology of disease 15:461-473.
Marcheselli VL, Bazan NG (1996) Sustained induction of prostaglandin endoperoxide synthase-2 by seizures in hippocampus. Inhibition by a platelet-activating factor antagonist. The Journal of biological chemistry 271:24794-24799.
Matsushima K, MacManus JP, Hakim AM (1997) Apoptosis is restricted to the thalamus in thiamine-deficient rats. Neuroreport 8:867-870.
McCandless DW (1982) Energy metabolism in the lateral vestibular nucleus in pyrithiamin-induced thiamin deficiency. Annals of the New York Academy of Sciences 378:355-364.
McGinnis WR (2005) Oxidative stress in autism. Alternative therapies in health and medicine 11:19.
McLaren FH, Svendsen CN, Van der Meide P, Joly E (2001) Analysis of neural stem cells by flow cytometry: cellular differentiation modifies patterns of MHC expression. Journal of neuroimmunology 112:35-46.
Meinhardt MW, Sevin DC, Klee ML, Dieter S, Sauer U, Sommer WH (2015) The neurometabolic fingerprint of excessive alcohol drinking. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 40:1259-1268.
Melo A, Monteiro L, Lima RM, Oliveira DM, Cerqueira MD, El-Bacha RS (2011) Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. Oxidative medicine and cellular longevity 2011:467180.
Meyers DE, Basha HI, Koenig MK (2013) Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. Texas Heart Institute journal / from the Texas Heart Institute of St Luke's Episcopal Hospital, Texas Children's Hospital 40:385-394.
Morris GS, Simmonds HA, Davies PM (1986) Use of biological fluids for the rapid diagnosis of potentially lethal inherited disorders of human purine and pyrimidine metabolism. Biomedical chromatography : BMC 1:109-118.
Muller N, Schwarz M (2006) Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission. Neurotoxicity research 10:131-148.
Munujos P, Vendrell M, Ferrer I (1993) Proto-oncogene c-fos induction in thiamine-deficient encephalopathy. Protective effects of nicardipine on pyrithiamine-induced lesions. Journal of the neurological sciences 118:175-180.
Murayama A, Matsuzaki Y, Kawaguchi A, Shimazaki T, Okano H (2002) Flow cytometric analysis of neural stem cells in the developing and adult mouse brain. Journal of neuroscience research 69:837-847.
Myers R, Manjil LG, Cullen BM, Price GW, Frackowiak RS, Cremer JE (1991) Macrophage and astrocyte populations in relation to [3H]PK 11195 binding in rat cerebral cortex following a local ischaemic lesion. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 11:314-322.
N M, K V, V R, Gn P, V K, At N, S A, Km C (2007) Use of Bone Marrow derived Stem Cells in Patients with Cardiovascular Disorders. Journal of stem cells & regenerative medicine 3:28-29.

Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T (1999) Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 284:479-482.

Namihira M, Kohyama J, Abematsu M, Nakashima K (2008) Epigenetic mechanisms regulating fate specification of neural stem cells. Philosophical transactions of the Royal Society of London Series B, Biological sciences 363:2099-2109.

Navarro D, Zwingmann C, Hazell AS, Butterworth RF (2005) Brain lactate synthesis in thiamine deficiency: a re-evaluation using 1H-13C nuclear magnetic resonance spectroscopy. Journal of neuroscience research 79:33-41.

Neri M, Cantatore S, Pomara C, Riezzo I, Bello S, Turillazzi E, Fineschi V (2011) Immunohistochemical expression of proinflammatory cytokines IL-1beta, IL-6, TNF-alpha and involvement of COX-2, quantitatively confirmed by Western blot analysis, in Wernicke's encephalopathy. Pathology, research and practice 207:652-658.

Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schule B, Dolmetsch RE, Langston W, Palmer TD, Pera RR (2011) LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell stem cell 8:267-280.

Ouchi Y, Yagi S, Yokokura M, Sakamoto M (2009) Neuroinflammation in the living brain of Parkinson's disease. Parkinsonism & related disorders 15 Suppl 3:S200-204.

Owen F, Poulter M, Waddington JL, Mashal RD, Crow TJ (1983) [3H]R05-4864 and [3H]flunitrazepam binding in kainate-lesioned rat striatum and in temporal cortex of brains from patients with senile dementia of the Alzheimer type. Brain research 278:373-375.

Palmela I, Cardoso FL, Bernas M, Correia L, Vaz AR, Silva RF, Fernandes A, Kim KS, Brites D, Brito MA (2011) Elevated levels of bilirubin and long-term exposure impair human brain microvascular endothelial cell integrity. Current neurovascular research 8:153-169.

Palmela I, Correia L, Silva RF, Sasaki H, Kim KS, Brites D, Brito MA (2015) Hydrophilic bile acids protect human blood-brain barrier endothelial cells from disruption by unconjugated bilirubin: an in vitro study. Frontiers in neuroscience 9:80.

Palmela I, Sasaki H, Cardoso FL, Moutinho M, Kim KS, Brites D, Brito MA (2012) Time-dependent dual effects of high levels of unconjugated bilirubin on the human blood-brain barrier lining. Frontiers in cellular neuroscience 6:22.

Papadopoulos V (1993) Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. Endocrine reviews 14:222-240.

Paulsen Bda S, da Silveira MS, Galina A, Rehen SK (2013) Pluripotent stem cells as a model to study oxygen metabolism in neurogenesis and neurodevelopmental disorders. Archives of biochemistry and biophysics 534:3-10.

Perry TL, Hansen S, Currier RD, Berry K (1978) Abnormalities in neurotransmitter amino acids in dominantly inherited cerebellar disorders. Advances in neurology 21:303-314.

Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U, Amadio S, Bergami A, Furlan R, Comi G, Vescovi AL, Martino G (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422:688-694.

Plum J, Nohr MK, Hansen SH, Holm R, Nielsen CU (2014) The anti-epileptic drug substance vigabatrin inhibits taurine transport in intestinal and renal cell culture models. International journal of pharmaceutics 473:395-397.

Pompeia C, Freitas JJ, Kim JS, Zyngier SB, Curi R (2002) Arachidonic acid cytotoxicity in leukocytes: implications of oxidative stress and eicosanoid synthesis. Biology of the cell / under the auspices of the European Cell Biology Organization 94:251-265.

Pompeia C, Lima T, Curi R (2003) Arachidonic acid cytotoxicity: can arachidonic acid be a physiological mediator of cell death? Cell biochemistry and function 21:97-104.
Poupon RE, Poupon R, Balkau B (1994) Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. The New England journal of medicine 330:1342-1347.
Purdy A, Hahn A, Barnett HJ, Bratty P, Ahmad D, Lloyd KG, McGeer EG, Perry TL (1979) Familial fatal Parkinsonism with alveolar hypoventilation and mental depression. Annals of neurology 6:523-531.
Qin W, Li X, Peng Y, Harlow LM, Ren Y, Wu Y, Li J, Qin Y, Sun J, Zheng S, Brown T, Feng JQ, Ke HZ, Bauman WA, Cardozo CC (2015) Sclerostin antibody preserves the morphology and structure of osteocytes and blocks the severe skeletal deterioration after motor-complete spinal cord injury in rats. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.
Qu Q, Shi Y (2009) Neural stem cells in the developing and adult brains. Journal of cellular physiology 221:5-9.
Rajan P, McKay RD (1998) Multiple routes to astrocytic differentiation in the CNS. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:3620-3629.
Ram PA, Waxman DJ (1997) Interaction of growth hormone-activated STATs with SH2-containing phosphotyrosine phosphatase SHP-1 and nuclear JAK2 tyrosine kinase. The Journal of biological chemistry 272:17694-17702.
Rao VL, Bowen KK, Dempsey RJ (2001) Transient focal cerebral ischemia down-regulates glutamate transporters GLT-1 and EAAC1 expression in rat brain. Neurochemical research 26:497-502.
Reye P, Sullivan R, Scott H, Pow DV (2002) Distribution of two splice variants of the glutamate transporter GLT-1 in rat brain and pituitary. Glia 38:246-255.
Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255:1707-1710.
Rodrigues CM, Sola S, Brito MA, Brites D, Moura JJ (2002) Bilirubin directly disrupts membrane lipid polarity and fluidity, protein order, and redox status in rat mitochondria. Journal of hepatology 36:335-341.
Rodrigues CM, Sola S, Brito MA, Brondino CD, Brites D, Moura JJ (2001) Amyloid beta-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. Biochem Biophys Res Commun 281:468-474.
Rodrigues CM, Sola S, Nan Z, Castro RE, Ribeiro PS, Low WC, Steer CJ (2003) Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. Proceedings of the National Academy of Sciences of the United States of America 100:6087-6092.
Rodrigues CM, Sola S, Silva R, Brites D (2000) Bilirubin and amyloid-beta peptide induce cytochrome c release through mitochondrial membrane permeabilization. Mol Med 6:936-946.
Rossignol DA (2009) Novel and emerging treatments for autism spectrum disorders: a systematic review. Annals of clinical psychiatry : official journal of the American Academy of Clinical Psychiatrists 21:213-236.
Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675-686.
Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncl RW (1994) Localization of neuronal and glial glutamate transporters. Neuron 13:713-725.

Rudolph G, Kloeters-Plachky P, Sauer P, Stiehl A (2002) Intestinal absorption and biliary secretion of ursodeoxycholic acid and its taurine conjugate. European journal of clinical investigation 32:575-580.
Russell DW (2003) The enzymes, regulation, and genetics of bile acid synthesis. Annual review of biochemistry 72:137-174.
Safranow K (2000) [Identification and quantitation of purine derivatives in urinary calculi as markers of abnormal purine metabolism by using high-performance liquid chromatography (HPLC)]. Annales Academiae Medicae Stetinensis 46:35-49.
Safranow K, Machoy Z, Ciechanowski K (2000) Analysis of purines in urinary calculi by high-performance liquid chromatography. Analytical biochemistry 286:224-230.
Sarveswaran S, Chakraborty D, Chitale D, Sears R, Ghosh J (2015) Inhibition of 5-lipoxygenase selectively triggers disruption of c-Myc signaling in prostate cancer cells. The Journal of biological chemistry 290:4994-5006.
Satow T, Bae SK, Inoue T, Inoue C, Miyoshi G, Tomita K, Bessho Y, Hashimoto N, Kageyama R (2001) The basic helix-loop-helix gene hesr2 promotes gliogenesis in mouse retina. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:1265-1273.
Schaap FG, Trauner M, Jansen PL (2014) Bile acid receptors as targets for drug development. Nature reviews Gastroenterology & hepatology 11:55-67.
Schober AL, Mongin AA (2015) Intracellular levels of glutamate in swollen astrocytes are preserved via neurotransmitter reuptake and de novo synthesis: implications for hyponatremia. Journal of neurochemistry.
Schoemaker H, Morelli M, Deshmukh P, Yamamura HI (1982) [3H]Ro5-4864 benzodiazepine binding in the kainate lesioned striatum and Huntington's diseased basal ganglia. Brain research 248:396-401.
Scholer HR (2007) The Potential of Stem Cells: An Inventory. In: Humanbiotechnology as Social Challenge(Knoepffler, N. et al., eds), p 28: Ashgate Publishing.
Schousboe A (1981) Transport and metabolism of glutamate and GABA in neurons are glial cells. International review of neurobiology 22:1-45.
Sechi G, Serra A (2007) Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management. Lancet neurology 6:442-455.
Selim S, El Sagheer O, El Amir A, Barakat R, Hadley K, Bruins MJ, El Ridi R (2014) Efficacy and safety of arachidonic acid for treatment of Schistosoma mansoni-infected children in Menoufiya, Egypt. The American journal of tropical medicine and hygiene 91:973-981.
Shihabuddin LS, Ray J, Gage FH (1997) FGF-2 is sufficient to isolate progenitors found in the adult mammalian spinal cord. Experimental neurology 148:577-586.
Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell 61:931-937.
Silva RF, Rodrigues CM, Brites D (2001) Bilirubin-induced apoptosis in cultured rat neural cells is aggravated by chenodeoxycholic acid but prevented by ursodeoxycholic acid. Journal of hepatology 34:402-408.
Silva RF, Rodrigues CM, Brites D (2002) Rat cultured neuronal and glial cells respond differently to toxicity of unconjugated bilirubin. Pediatric research 51:535-541.
Silva SL, Vaz AR, Barateiro A, Falcao AS, Fernandes A, Brito MA, Silva RF, Brites D (2010) Features of bilirubin-induced reactive microglia: from phagocytosis to inflammation. Neurobiology of disease 40:663-675.
Silva SL, Vaz AR, Diogenes MJ, van Rooijen N, Sebastiao AM, Fernandes A, Silva RF, Brites D (2012) Neuritic growth impairment and cell death by unconjugated bilirubin is

mediated by NO and glutamate, modulated by microglia, and prevented by glycoursodeoxycholic acid and interleukin-10. Neuropharmacology 62:2398-2408.
Şimşek G, Şimşek E (2012) Stem Cells, Challenging Era Beyond Debate. Niche 1:46-48.
Skjesol A, Liebe T, Iliev DB, Thomassen EI, Tollersrud LG, Sobhkhez M, Lindenskov Joensen L, Secombes CJ, Jorgensen JB (2014) Functional conservation of suppressors of cytokine signaling proteins between teleosts and mammals: Atlantic salmon SOCS1 binds to JAK/STAT family members and suppresses type I and II IFN signaling. Developmental and comparative immunology 45:177-189.
Sola S, Castro RE, Laires PA, Steer CJ, Rodrigues CM (2003) Tauroursodeoxycholic acid prevents amyloid-beta peptide-induced neuronal death via a phosphatidylinositol 3-kinase-dependent signaling pathway. Mol Med 9:226-234.
Sorrentino A, Ferracin M, Castelli G, Biffoni M, Tomaselli G, Baiocchi M, Fatica A, Negrini M, Peschle C, Valtieri M (2008) Isolation and characterization of CD146+ multipotent mesenchymal stromal cells. Experimental hematology 36:1035-1046.
Strauer BE, Schannwell CM, Brehm M (2009) Therapeutic potentials of stem cells in cardiac diseases. Minerva cardioangiologica 57:249-267.
Suh H, Jung EJ, Kim TH, Lee HY, Park YH, Kim KW (1997) Anti-angiogenic activity of ursodeoxycholic acid and its derivatives. Cancer letters 113:117-122.
Sun H, Lu G, Ren S, Chen J, Wang Y (2011) Catabolism of branched-chain amino acids in heart failure: insights from genetic models. Pediatric cardiology 32:305-310.
Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861-872.
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.
Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276:1699-1702.
Temple S (1999) CNS development: The obscure origins of adult stem cells. Current biology : CB 9:R397-399.
Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K (2008) Targeting bile-acid signalling for metabolic diseases. Nature reviews Drug discovery 7:678-693.
Toledo-Sherman LM, Prime ME, Mrzljak L, Beconi MG, Beresford A, Brookfield FA, Brown CJ, Cardaun I, Courtney SM, Dijkman U, Hamelin-Flegg E, Johnson PD, Kempf V, Lyons K, Matthews K, Mitchell WL, O'Connell C, Pena P, Powell K, Rassoulpour A, Reed L, Reindl W, Selvaratnam S, Friley WW, Weddell DA, Went NE, Wheelan P, Winkler C, Winkler D, Wityak J, Yarnold CJ, Yates D, Munoz-Sanjuan I, Dominguez C (2015) Development of a series of aryl pyrimidine kynurenine monooxygenase inhibitors as potential therapeutic agents for the treatment of Huntington's disease. Journal of medicinal chemistry 58:1159-1183.
Troncoso JC, Johnston MV, Hess KM, Griffin JW, Price DL (1981) Model of Wernicke's encephalopathy. Archives of neurology 38:350-354.
Trujillo CA, Schwindt TT, Martins AH, Alves JM, Mello LE, Ulrich H (2009) Novel perspectives of neural stem cell differentiation: from neurotransmitters to therapeutics. Cytometry Part A : the journal of the International Society for Analytical Cytology 75:38-53.
Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL (2000) Direct isolation of human central nervous system stem cells.

Proceedings of the National Academy of Sciences of the United States of America 97:14720-14725.
Urbach A, Bar-Nur O, Daley GQ, Benvenisty N (2010) Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells. Cell stem cell 6:407-411.
Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P (2005) Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proceedings of the National Academy of Sciences of the United States of America 102:8692-8697.
Vaz AR, Delgado-Esteban M, Brito MA, Bolanos JP, Brites D, Almeida A (2010) Bilirubin selectively inhibits cytochrome c oxidase activity and induces apoptosis in immature cortical neurons: assessment of the protective effects of glycoursodeoxycholic acid. Journal of neurochemistry 112:56-65.
Vemuganti R, Kalluri H, Yi JH, Bowen KK, Hazell AS (2006) Gene expression changes in thalamus and inferior colliculus associated with inflammation, cellular stress, metabolism and structural damage in thiamine deficiency. The European journal of neuroscience 23:1172-1188.
Viana RJ, Nunes AF, Castro RE, Ramalho RM, Meyerson J, Fossati S, Ghiso J, Rostagno A, Rodrigues CM (2009) Tauroursodeoxycholic acid prevents E22Q Alzheimer's Abeta toxicity in human cerebral endothelial cells. Cellular and molecular life sciences : CMLS 66:1094-1104.
Victor M, Adams, R.D., Collins, G.H. (1989) The Wernicke–Korsakoff Syndrome and Related Neurologic Disorders due to Alcoholism and Malnutrition. (FA Davies, P., ed).
Vladykovskaya E, Sithu SD, Haberzettl P, Wickramasinghe NS, Merchant ML, Hill BG, McCracken J, Agarwal A, Dougherty S, Gordon SA, Schuschke DA, Barski OA, O'Toole T, D'Souza SE, Bhatnagar A, Srivastava S (2012) Lipid peroxidation product 4-hydroxy-trans-2-nonenal causes endothelial activation by inducing endoplasmic reticulum stress. The Journal of biological chemistry 287:11398-11409.
Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annual review of genetics 39:359-407.
Wallace DC (2008) Mitochondria as chi. Genetics 179:727-735.
Wallace DC, Fan W, Procaccio V (2010) Mitochondrial energetics and therapeutics. Annual review of pathology 5:297-348.
Wallace DC, Lott MT, Procaccio V (2007) Mitochondrial genes in degenerative diseases, cancer and aging. In: Emery and Rimoin's Principles and Practice of Medical Genetics, vol. 1 (Rimoin, D. L. et al., eds), pp 194–298 Philadelphia: Churchill Livingstone Elsevier.
Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ, 2nd, Nikoskelainen EK (1988a) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427-1430.
Wallace DC, Zheng XX, Lott MT, Shoffner JM, Hodge JA, Kelley RI, Epstein CM, Hopkins LC (1988b) Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. Cell 55:601-610.
Walz W (1987) Swelling and potassium uptake in cultured astrocytes. Canadian journal of physiology and pharmacology 65:1051-1057.
Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O,

Clish CB, Gerszten RE (2011) Metabolite profiles and the risk of developing diabetes. Nature medicine 17:448-453.

Wang ZJ, Liang CL, Li GM, Yu CY, Yin M (2006) Neuroprotective effects of arachidonic acid against oxidative stress on rat hippocampal slices. Chemico-biological interactions 163:207-217.

Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA (1996) Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:7599-7609.

Wendel U, Claussen U, Diekmann E (1983) Prenatal diagnosis for methylenetetrahydrofolate reductase deficiency. The Journal of pediatrics 102:938-940.

Wennstrom M, Nielsen HM, Orhan F, Londos E, Minthon L, Erhardt S (2014) Kynurenic Acid levels in cerebrospinal fluid from patients with Alzheimer's disease or dementia with lewy bodies. International journal of tryptophan research : IJTR 7:1-7.

Witowski N, Lusczek E, Determan C, Jr., Lexcen D, Mulier K, Ostrowski B, Beilman G (2015) A four-compartment metabolomics analysis of the liver, muscle, serum, and urine response to polytrauma with hemorrhagic shock following carbohydrate prefeed. PloS one 10:e0124467.

Woo SJ, Kim JH, Yu HG (2010) Ursodeoxycholic acid and tauroursodeoxycholic acid suppress choroidal neovascularization in a laser-treated rat model. Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics 26:223-229.

Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkila V, Jula A, Kahonen M, Lehtimaki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasan RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M, Salomaa V (2015) Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation 131:774-785.

Wurtz P, Soininen P, Kangas AJ, Ronnemaa T, Lehtimaki T, Kahonen M, Viikari JS, Raitakari OT, Ala-Korpela M (2013) Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes care 36:648-655.

Xia J, Psychogios N, Young N, Wishart DS (2009) MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic acids research 37:W652-660.

Xia J, Sinelnikov IV, Han B, Wishart DS (2015) MetaboAnalyst 3.0-making metabolomics more meaningful. Nucleic acids research 43:W251-257.

Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, Yamanaka S, Okano H, Suzuki N (2011) Modeling familial Alzheimer's disease with induced pluripotent stem cells. Human molecular genetics 20:4530-4539.

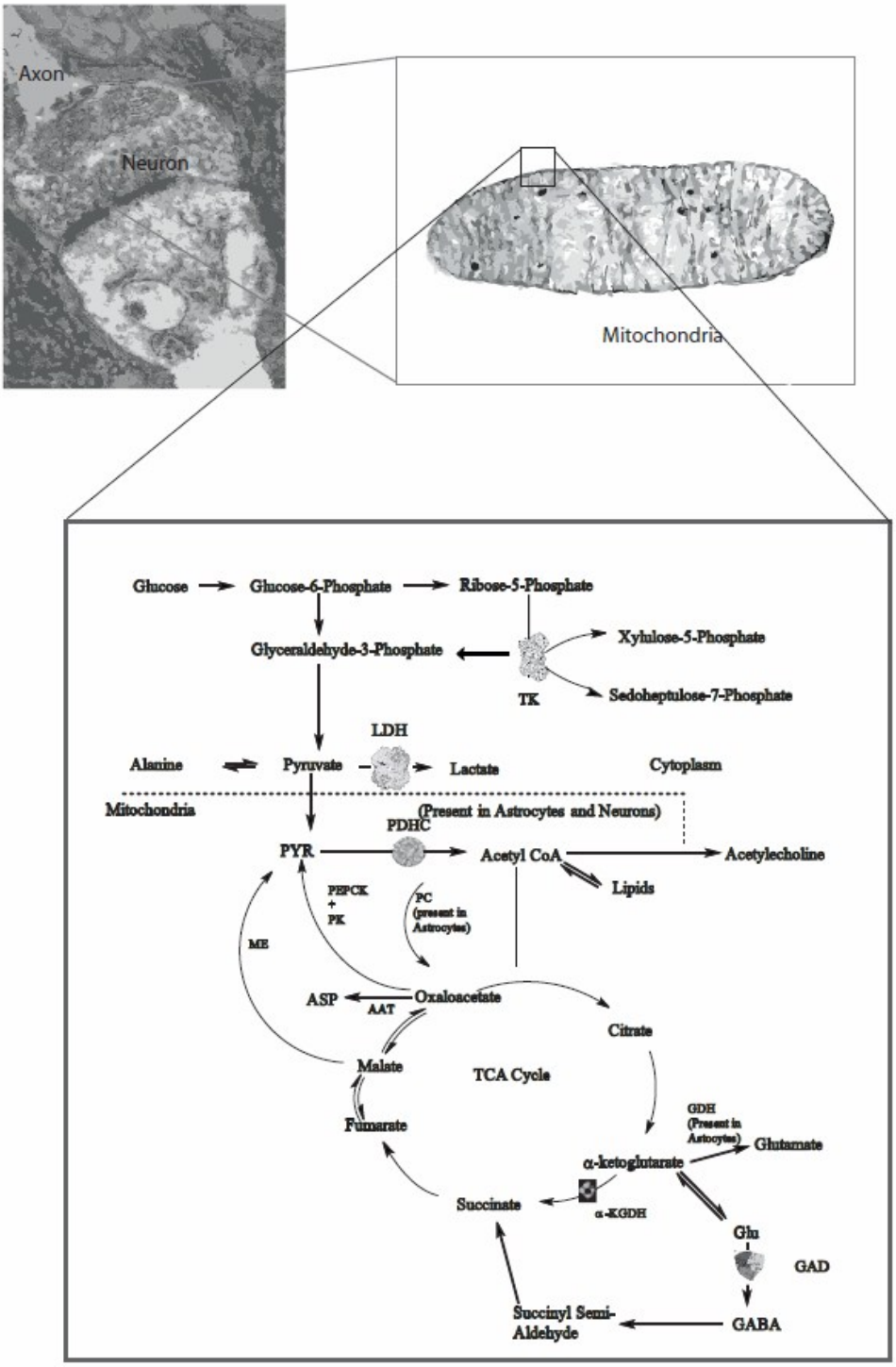
Yahata N, Asai M, Kitaoka S, Takahashi K, Asaka I, Hioki H, Kaneko T, Maruyama K, Saido TC, Nakahata T, Asada T, Yamanaka S, Iwata N, Inoue H (2011) Anti-Abeta drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. PloS one 6:e25788.

Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE (2011) Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 471:230-234.

Yenkoyan K, Safaryan K, Chavushyan V, Meliksetyan I, Navasardyan G, Sarkissian J, Galoyan A, Aghajanov M (2011) Neuroprotective action of proline-rich polypeptide-1 in beta-amyloid induced neurodegeneration in rats. Brain research bulletin 86:262-271.

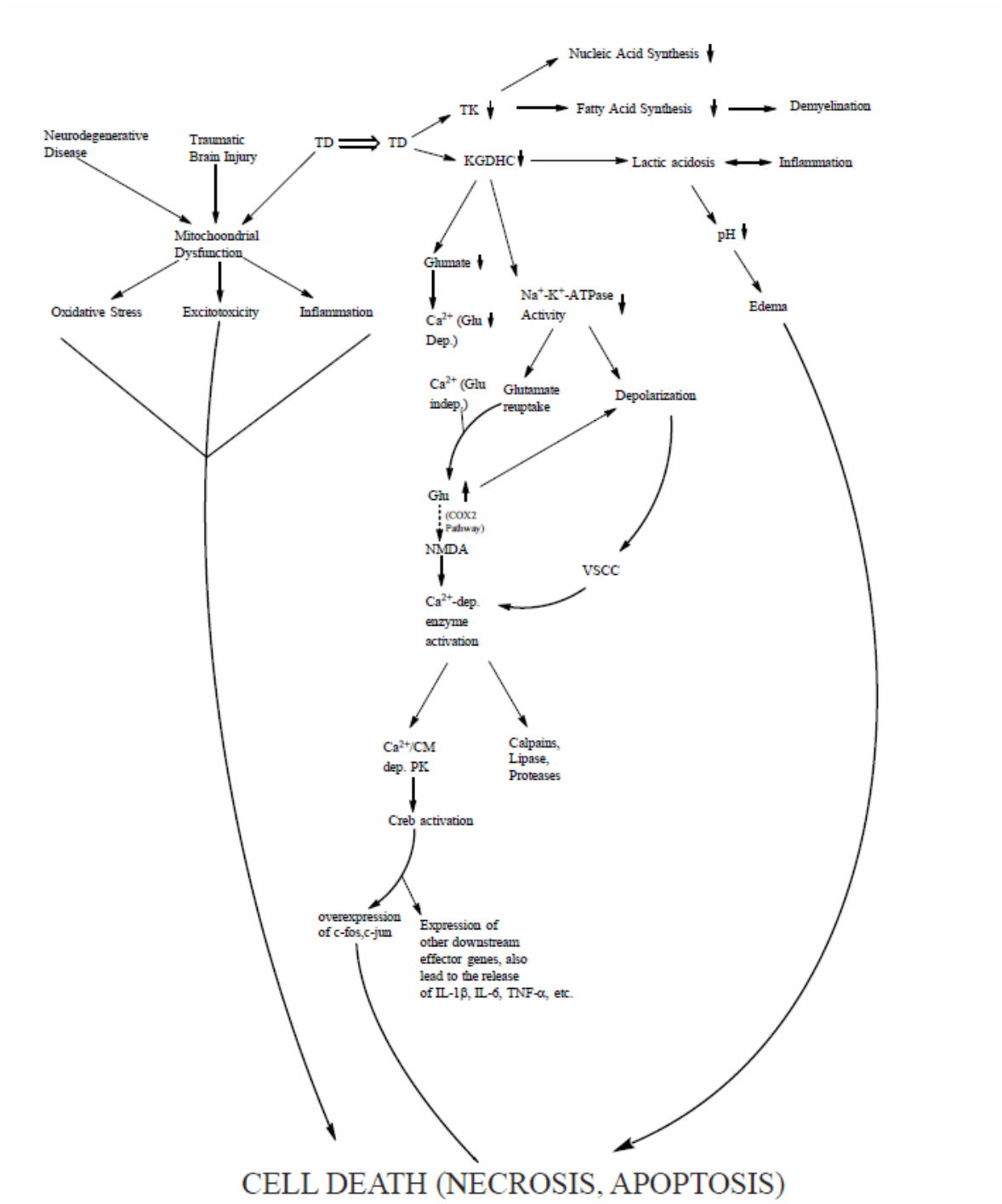
Yi JH, Hazell AS (2006) Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochemistry international 48:394-403.
Zhang Q, Yang G, Li W, Fan Z, Sun A, Luo J, Ke ZJ (2011) Thiamine deficiency increases beta-secretase activity and accumulation of beta-amyloid peptides. Neurobiology of aging 32:42-53.
Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Current opinion in neurobiology 20:588-594.
Zhang Y, Lian JB, Stein JL, van Wijnen AJ, Stein GS (2009) The Notch-responsive transcription factor Hes-1 attenuates osteocalcin promoter activity in osteoblastic cells. Journal of cellular biochemistry 108:651-659.
Zhao J, Sun X, Yu Z, Pan X, Gu F, Chen J, Dong W, Zhao L, Zhong C (2011) Exposure to pyrithiamine increases beta-amyloid accumulation, Tau hyperphosphorylation, and glycogen synthase kinase-3 activity in the brain. Neurotoxicity research 19:575-583.
Ziegler L, Grigoryan S, Yang IH, Thakor NV, Goldstein RS (2011) Efficient generation of schwann cells from human embryonic stem cell-derived neurospheres. Stem cell reviews 7:394-403.
Zinin N, Adameyko I, Wilhelm M, Fritz N, Uhlen P, Ernfors P, Henriksson MA (2014) MYC proteins promote neuronal differentiation by controlling the mode of progenitor cell division. EMBO reports 15:383-391.

FIGURE 1



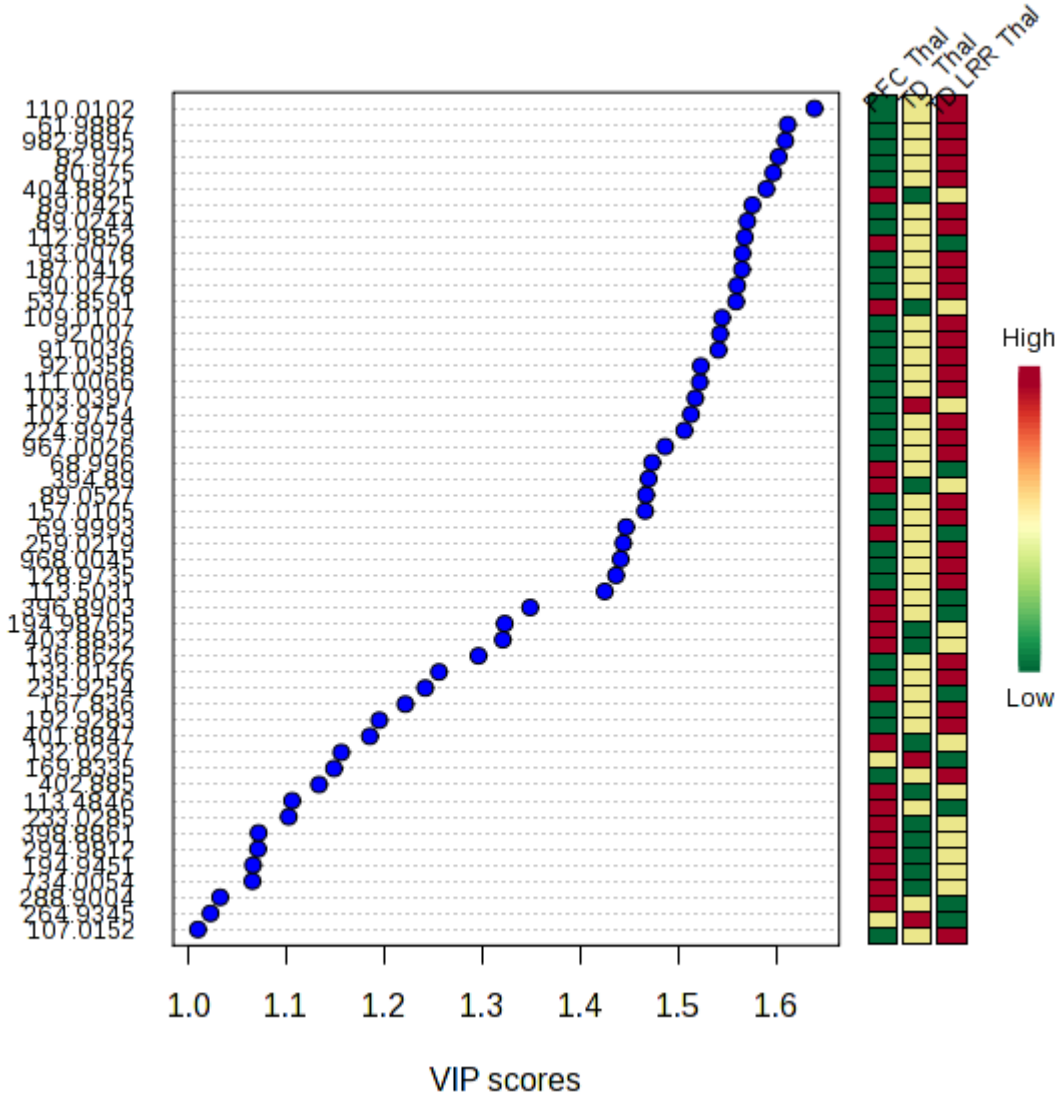
Biochemical overview of the metabolism associated with thiamine. Boxes show transmission electron microscope images (modified using Adobe Illustrator) of a mitochondrion and relevant biochemical pathways associated with TD

FIGURE 2



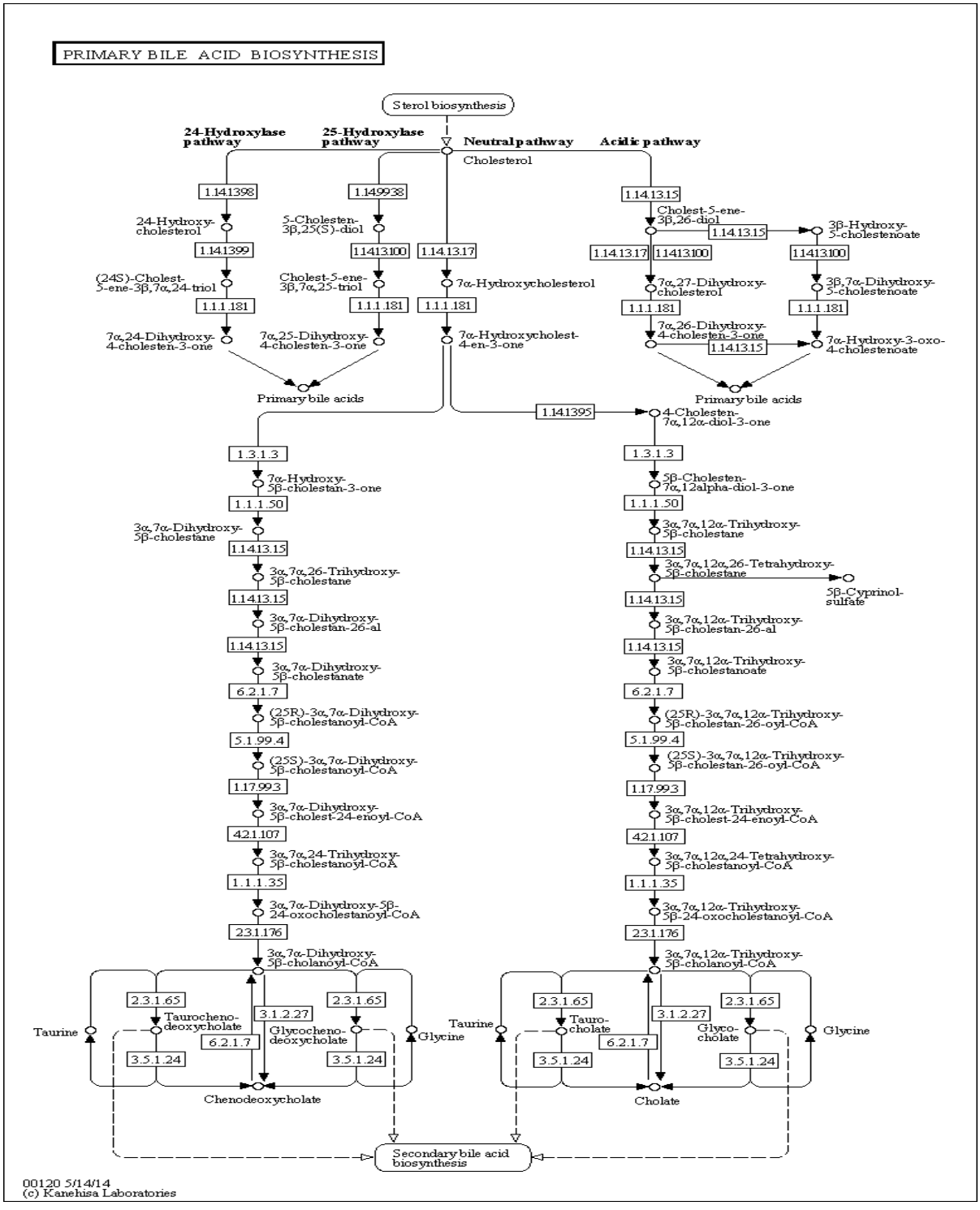
Cell death indicators, identifying some key contributors: cytokines (IL-6, TNFα, etc) involved in inflammatory responses, transcription factors (c-fos, more specifically involved in the post-translational phosphorylation of kinases including MAPK and plays a crucial role in cell proliferation and differentiation)

FIGURE 3



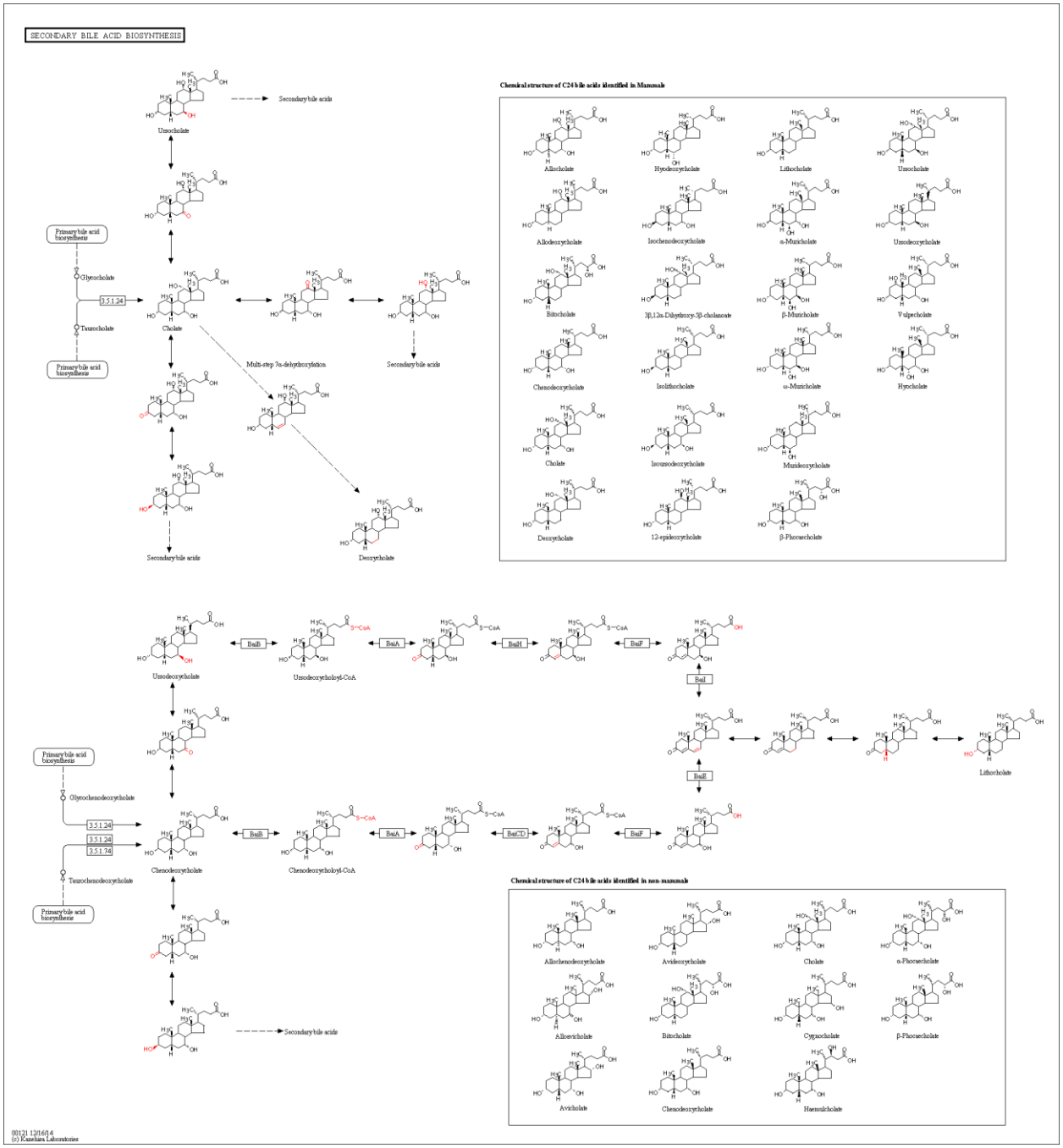
List of detected peaks associated with metabolites in the thalamus showing a significant correlation. Key metabolites of interest included kynurenic acid (187.0142), and the various bile acids. Included are the peaks observed at 398.8861 and 396.8903 which are very suggestive if Urso.

FIGURE 4



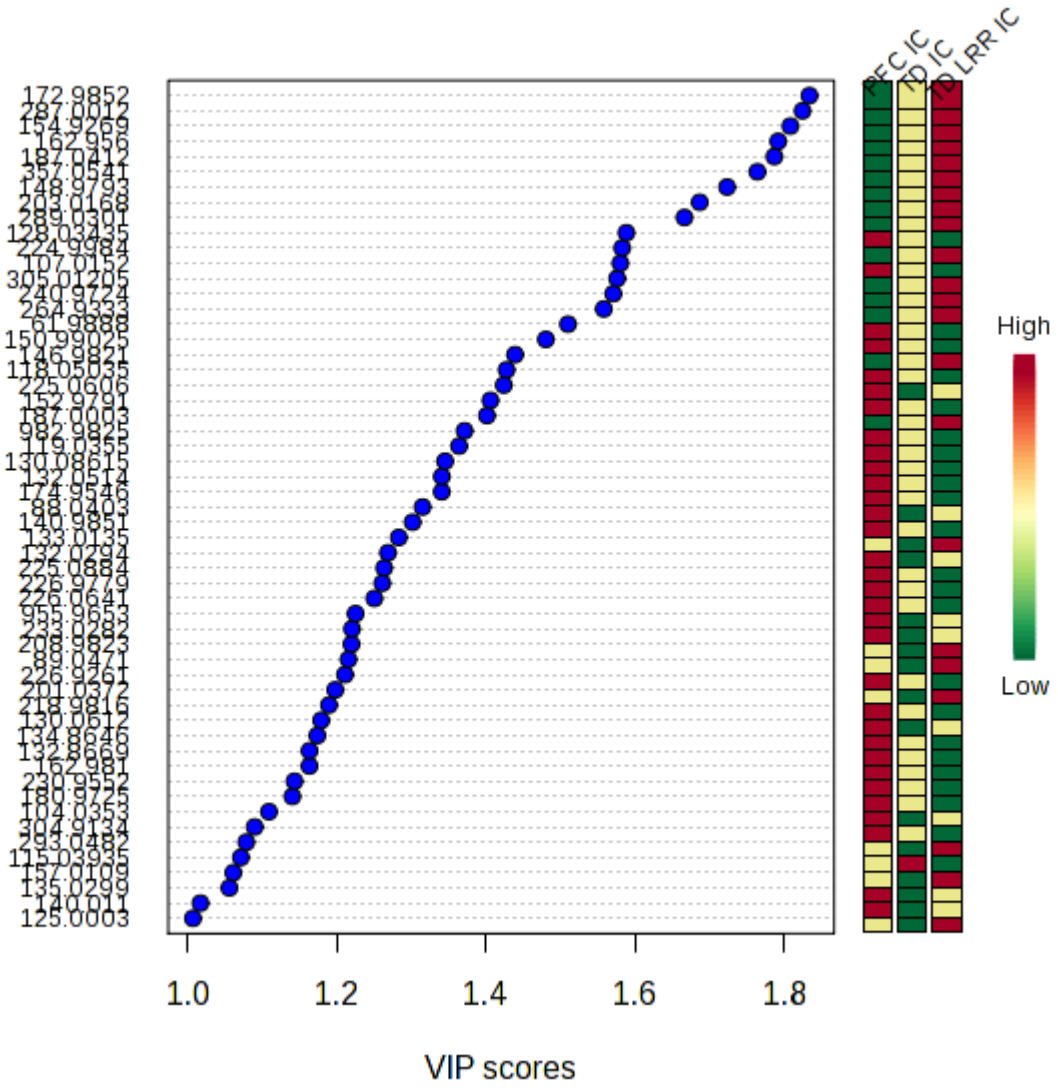
Primary bile metabolites (KEGG PATHWAYS). Many of the key identified bile acids detected in the metabolomics analysis are those outlined in between the metabolism of the primary bile acids to the secondary bile acids.

FIGURE 5



Secondary bile metabolites (KEGG PATHWAYS).

FIGURE 6



List of detected peaks associated with metabolites in the IC showing a significant correlation (any VIP values over 1 were considered to be of significance). Several amino acids (e.g. arginine, glutamic acid, tryptophan, serine, leucine, tyrosine, and aspartic acid are some of the clearly distinguishable) peaks were identified along with nucleic acid peaks. Highlighted metabolites for discussion were taurine, 125.0003 and l-carnosine, 225.0884.

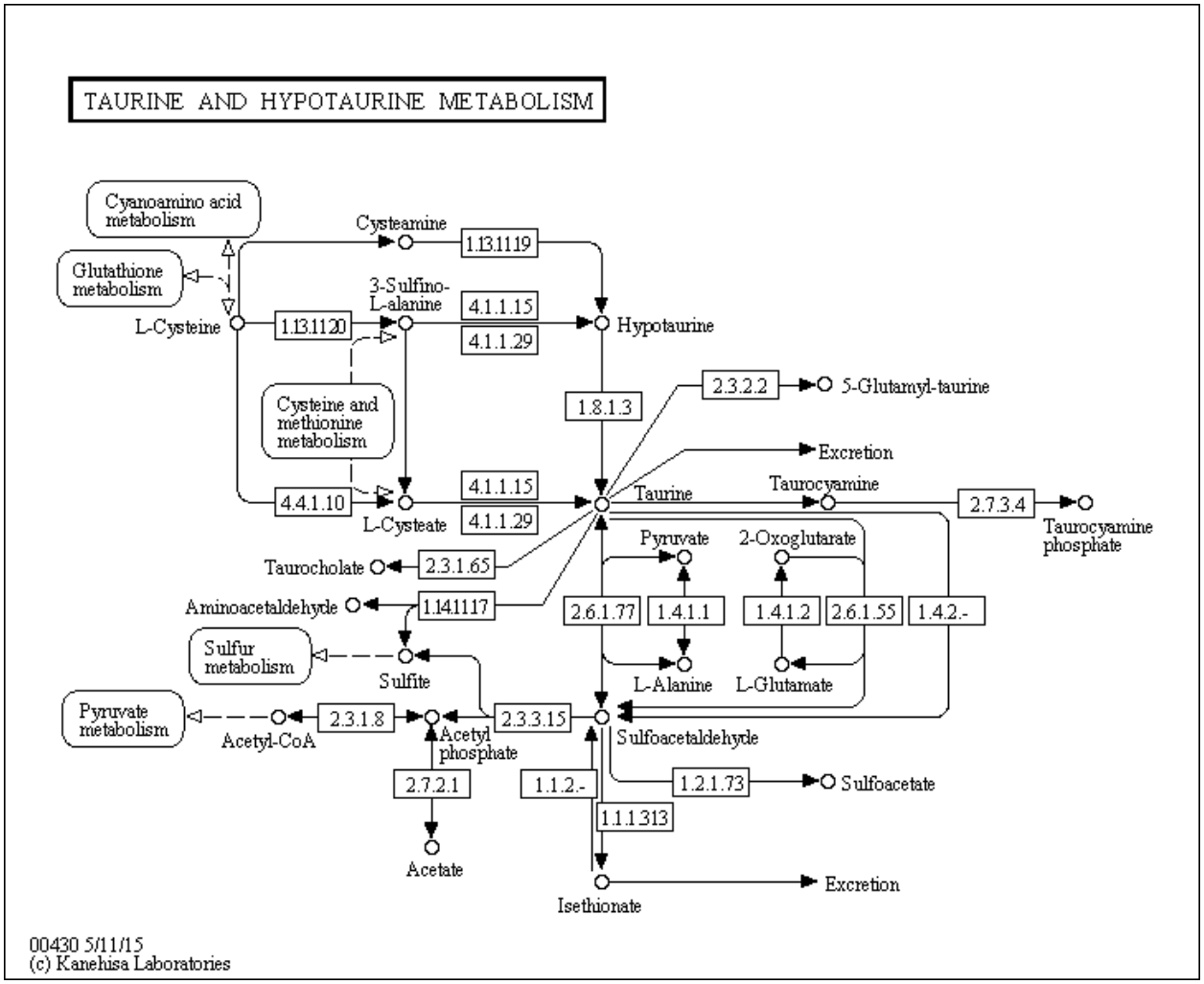
FIGURE 7

Result from Pathway Analysis

	Total	Expected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Aminoacyl-tRNA biosynthesis	67	1.15	6	6.48E-04	7.34E+00	5.25E-02	5.25E-02	0.15
Histidine metabolism	15	0.26	2	2.57E-02	3.66E+00	1.00E+00	8.35E-01	0.31
Arginine and proline metabolism	44	0.75	3	3.68E-02	3.30E+00	1.00E+00	8.35E-01	0.20
Ubiquinone and other terpenoid-quinone biosynthesis	3	0.05	1	5.05E-02	2.99E+00	1.00E+00	8.35E-01	0.50
Alanine, aspartate and glutamate metabolism	24	0.41	2	6.16E-02	2.79E+00	1.00E+00	8.35E-01	0.04
Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.07	1	6.68E-02	2.71E+00	1.00E+00	8.35E-01	0.25
Cysteine and methionine metabolism	28	0.48	2	8.10E-02	2.51E+00	1.00E+00	8.35E-01	0.11
Cyanoamino acid metabolism	6	0.10	1	9.86E-02	2.32E+00	1.00E+00	8.35E-01	0.00
Glycine, serine and threonine metabolism	32	0.55	2	1.02E-01	2.28E+00	1.00E+00	8.35E-01	0.19
Purine metabolism	68	1.16	3	1.07E-01	2.24E+00	1.00E+00	8.35E-01	0.04
Taurine and hypotaurine metabolism	8	0.14	1	1.29E-01	2.05E+00	1.00E+00	8.35E-01	0.17
Phenylalanine metabolism	9	0.15	1	1.44E-01	1.94E+00	1.00E+00	8.35E-01	0.00
Methane metabolism	9	0.15	1	1.44E-01	1.94E+00	1.00E+00	8.35E-01	0.00
Nitrogen metabolism	9	0.15	1	1.44E-01	1.94E+00	1.00E+00	8.35E-01	0.11
Valine, leucine and isoleucine biosynthesis	11	0.19	1	1.74E-01	1.75E+00	1.00E+00	9.37E-01	0.29
Pentose phosphate pathway	19	0.33	1	2.81E-01	1.27E+00	1.00E+00	1.00E+00	0.08
Butanoate metabolism	20	0.34	1	2.94E-01	1.23E+00	1.00E+00	1.00E+00	0.00
Propanoate metabolism	20	0.34	1	2.94E-01	1.23E+00	1.00E+00	1.00E+00	0.06
Citrate cycle (TCA cycle)	20	0.34	1	2.94E-01	1.23E+00	1.00E+00	1.00E+00	0.07
Sphingolipid metabolism	21	0.36	1	3.06E-01	1.18E+00	1.00E+00	1.00E+00	0.03
Glutathione metabolism	26	0.45	1	3.64E-01	1.01E+00	1.00E+00	1.00E+00	0.03
Valine, leucine and isoleucine degradation	38	0.65	1	4.86E-01	7.22E-01	1.00E+00	1.00E+00	0.02
Pyrimidine metabolism	41	0.70	1	5.12E-01	6.69E-01	1.00E+00	1.00E+00	0.03
Tryptophan metabolism	41	0.70	1	5.12E-01	6.69E-01	1.00E+00	1.00E+00	0.08
Tyrosine metabolism	42	0.72	1	5.21E-01	6.52E-01	1.00E+00	1.00E+00	0.12

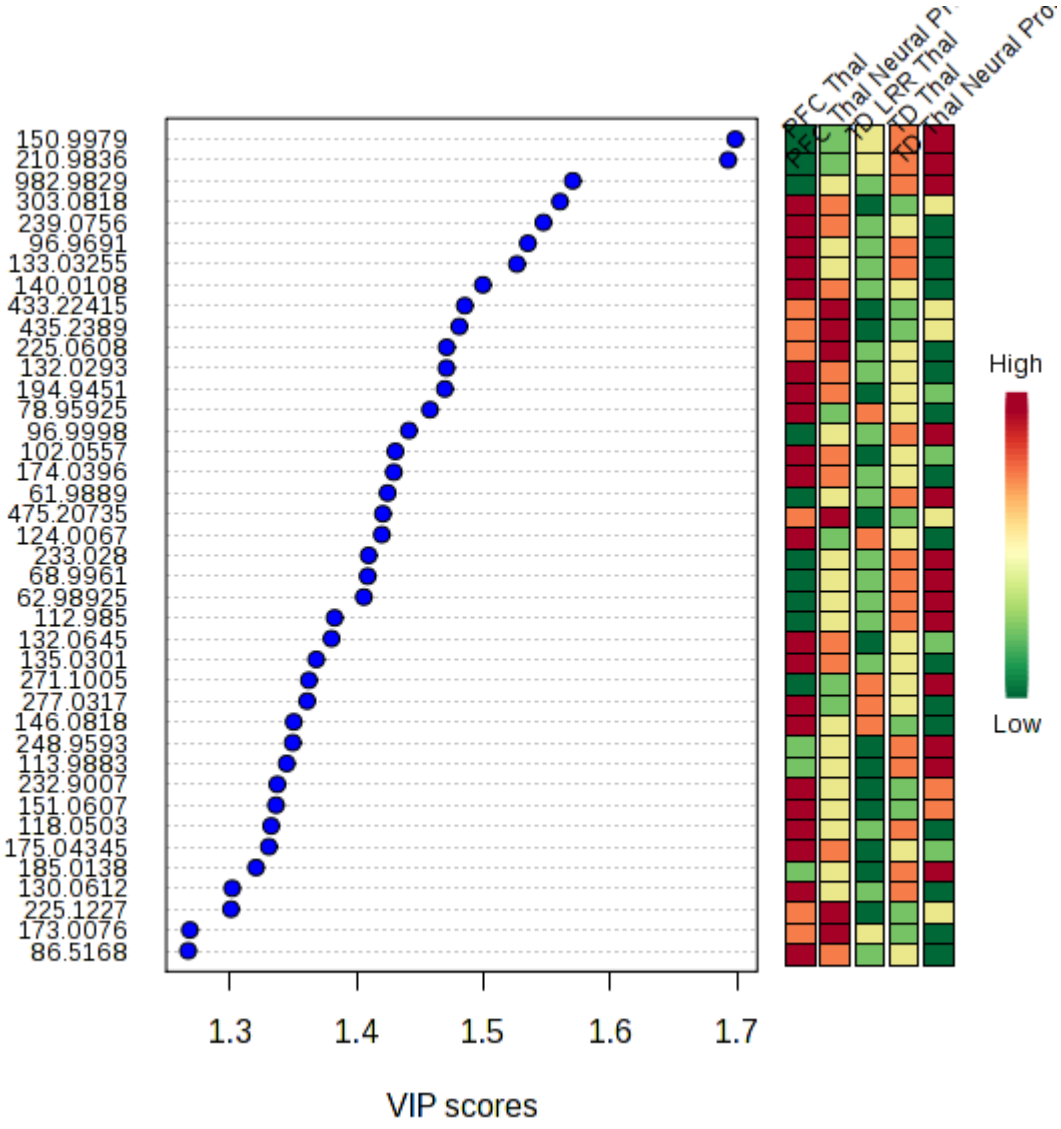
Results from a Metabolism Pathway Enrichment (Metaboanalyst 3.0) analysis showing a number of metabolic pathways involved and their statistical significance in regards to their involvement. The impact of each pathway is calculated using algorithms that take into account the compounds detected and their occurrence, participation, and involvement in various pathways. Key pathways based on the impact value (seen in the far right column) include Ubiquinone and other Terpenoid-quinone biosynthesis; Histidine Metabolism; Phenylalanine, Tyrosine and Tryptophan biosynthesis; and Valine, Leucine and Isoleucine biosynthesis.

FIGURE 8



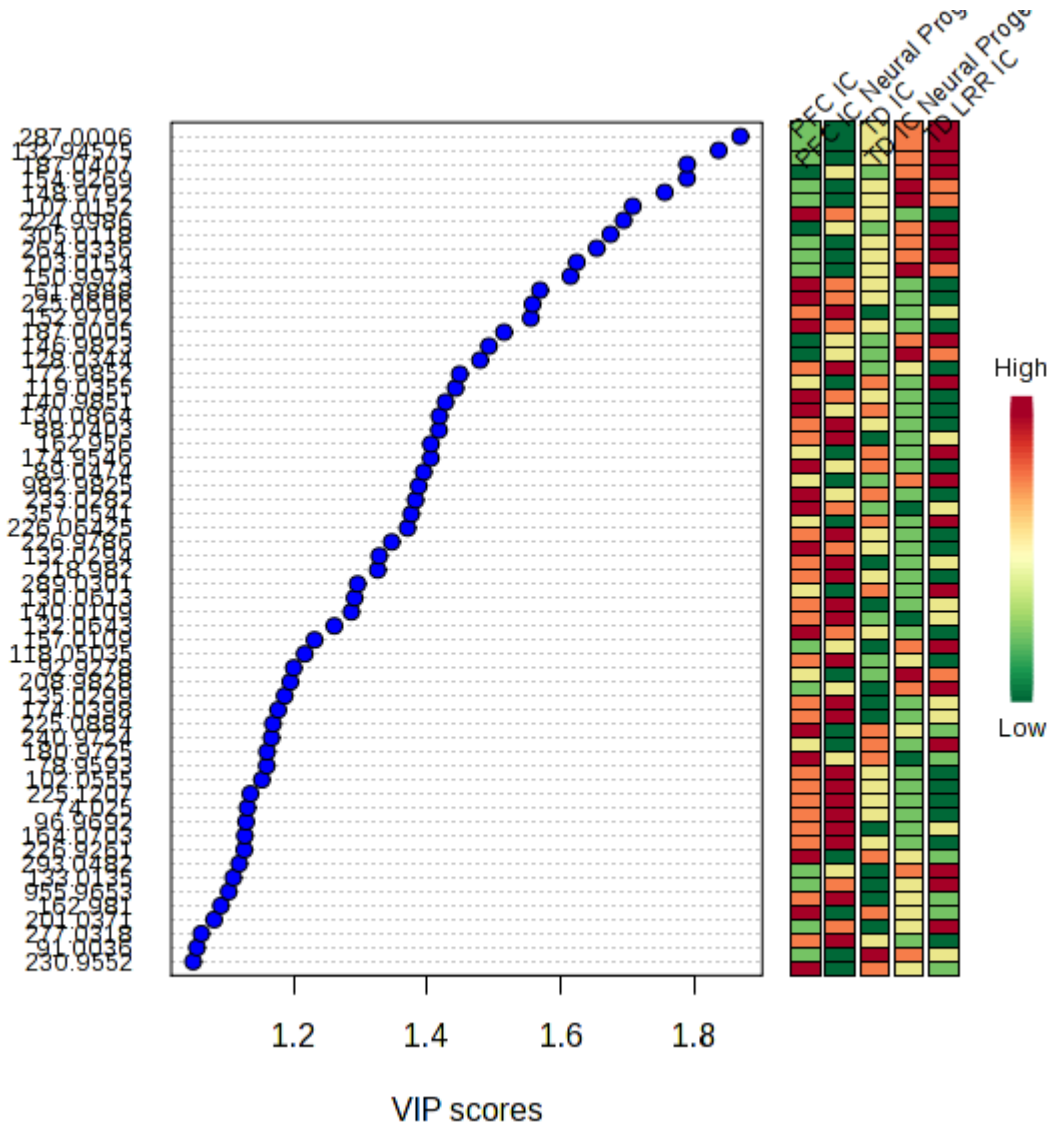
Taurine metabolism (KEGG PATHWAYS). Taurine is an important amino acid involved in the metabolism, excretion and processing of other various compounds, including some of the amino acids detected in the IC.

FIGURE 9



Listing of detected peaks associated with metabolites in the thalamus showing a significant correlation with the inclusion of the samples that were transplanted with NSPC. Many of the peaks that were not of statistical significance are now relevant and offer a potential window into the possible other metabolites of importance specifically with regards to NSPC.

FIGURE 10



Listing of detected peaks associated with metabolites in the IC demonstrating a significant correlation with the inclusion of the samples that were transplanted with NSPC.

FIGURE 11

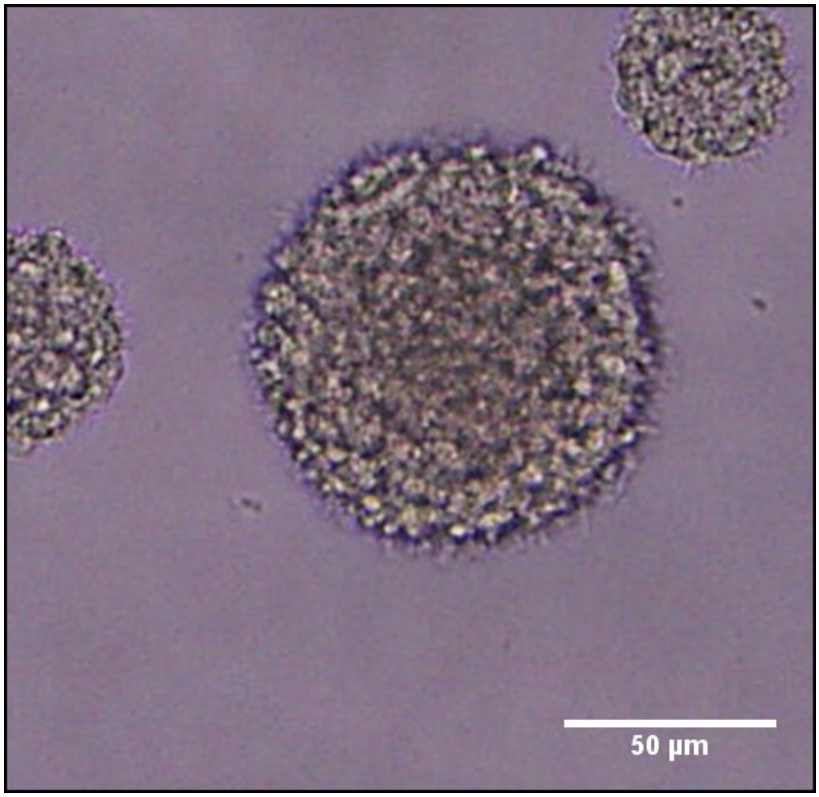
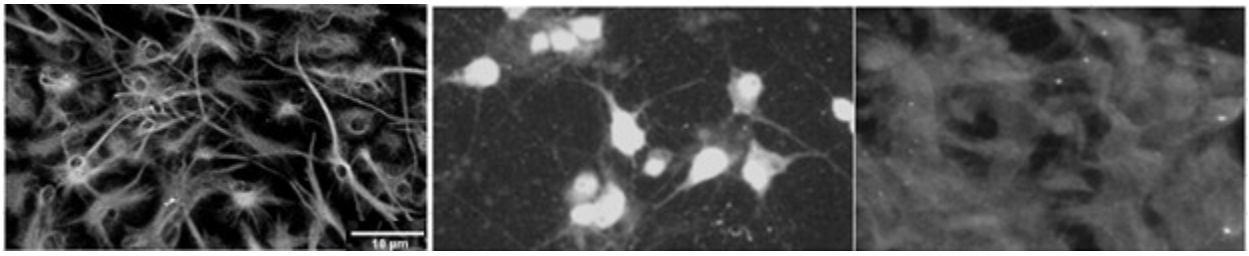


Image of the formation of neurospheres following a second passage (P2) at day 2 of the subculture. These are the same neurospheres that contained the NSPC that were titerated and tail-vein transplanted as single cells. Passages are performed every 4 days when flasks are usually at a maximal cell density of 500,000 cells/mL. Scale bar : 50µm.

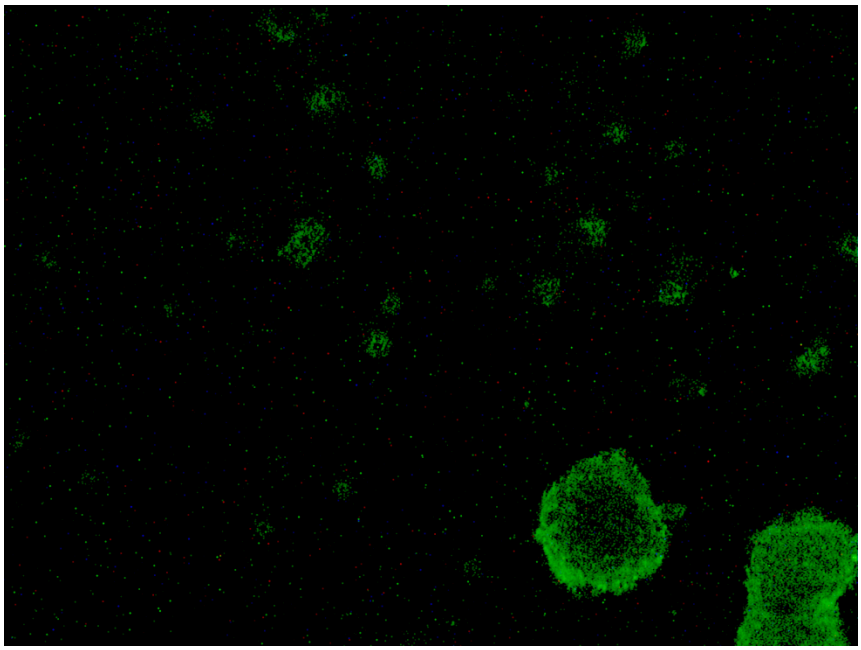
FIGURE 12

A **B** **C**



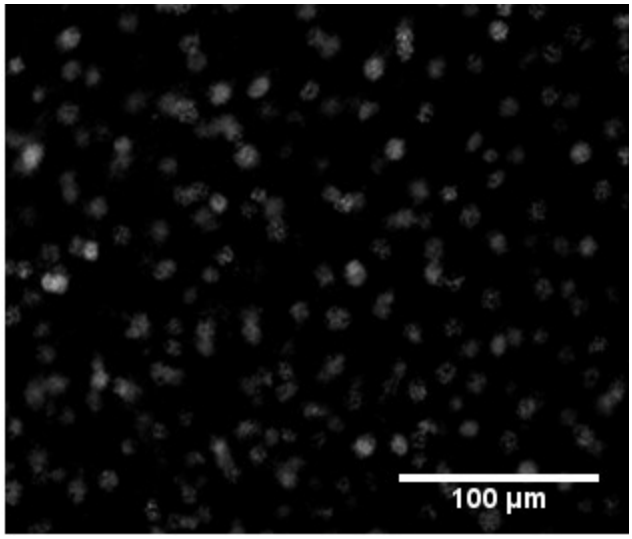
Images of differentiated cell types from the neurosphere (to confirm the differentiability of the stem cells). A: astrocytes, GFAP confirmation; B: neurons, NeuN confirmation; C:oligodentrocytes β-tubulin, Tuj-1. Scale Bar for all images is 10 µm

FIGURE 13



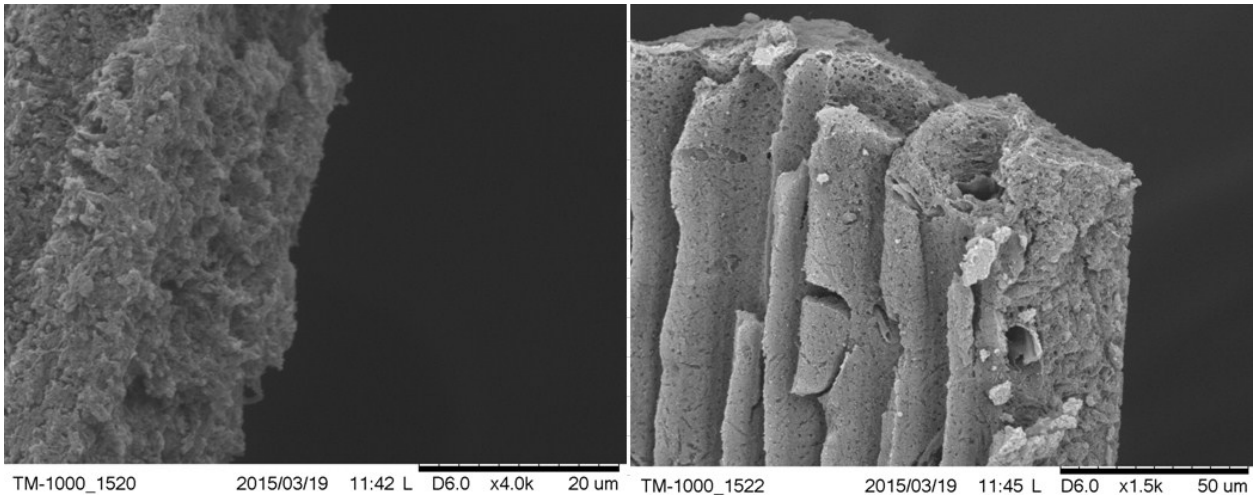
Labelled neurospheres (containing NSPC) with CFSE. Confirmation performed using FITC filter. Scale Bar identical to Figure 11.

FIGURE 14



NSPC labelled with CFSE localized in the inferior colliculus. Scale Bar 100µm

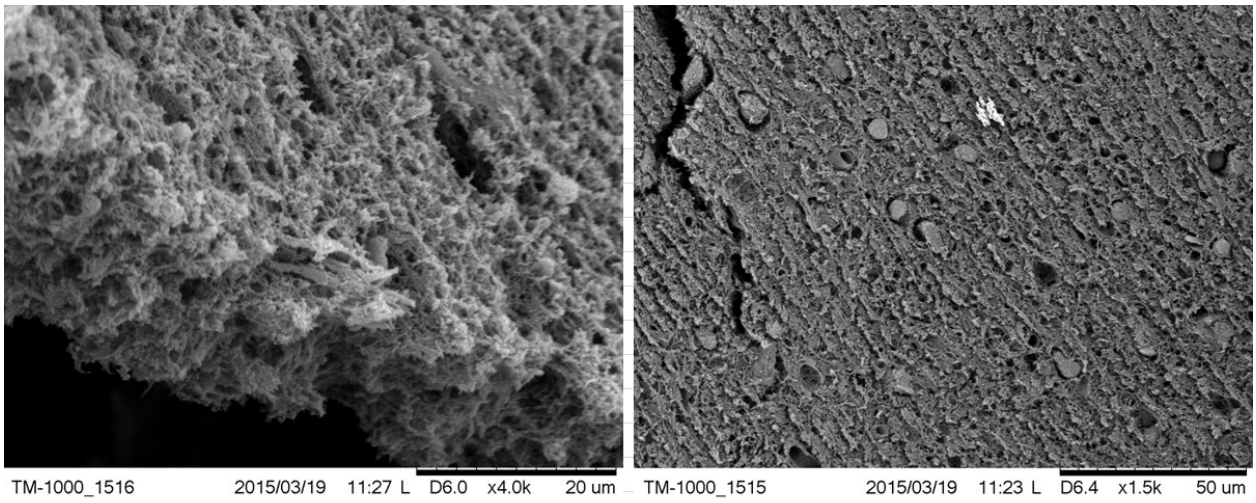
FIGURE 15



A B

PFC thalamus section, A: side view at 4K magnification, B 1.5K magnification. Scale bars are 20 µm and 50 µm respectively for A and B.

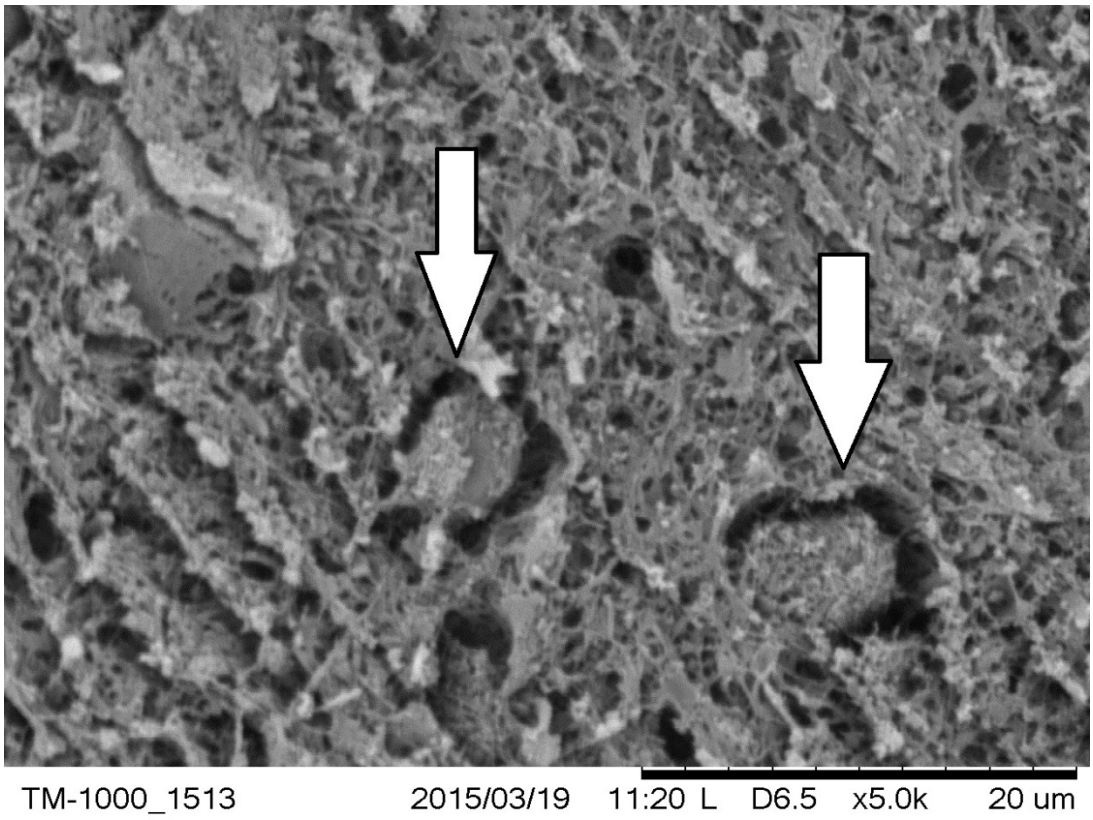
FIGURE 16



A B

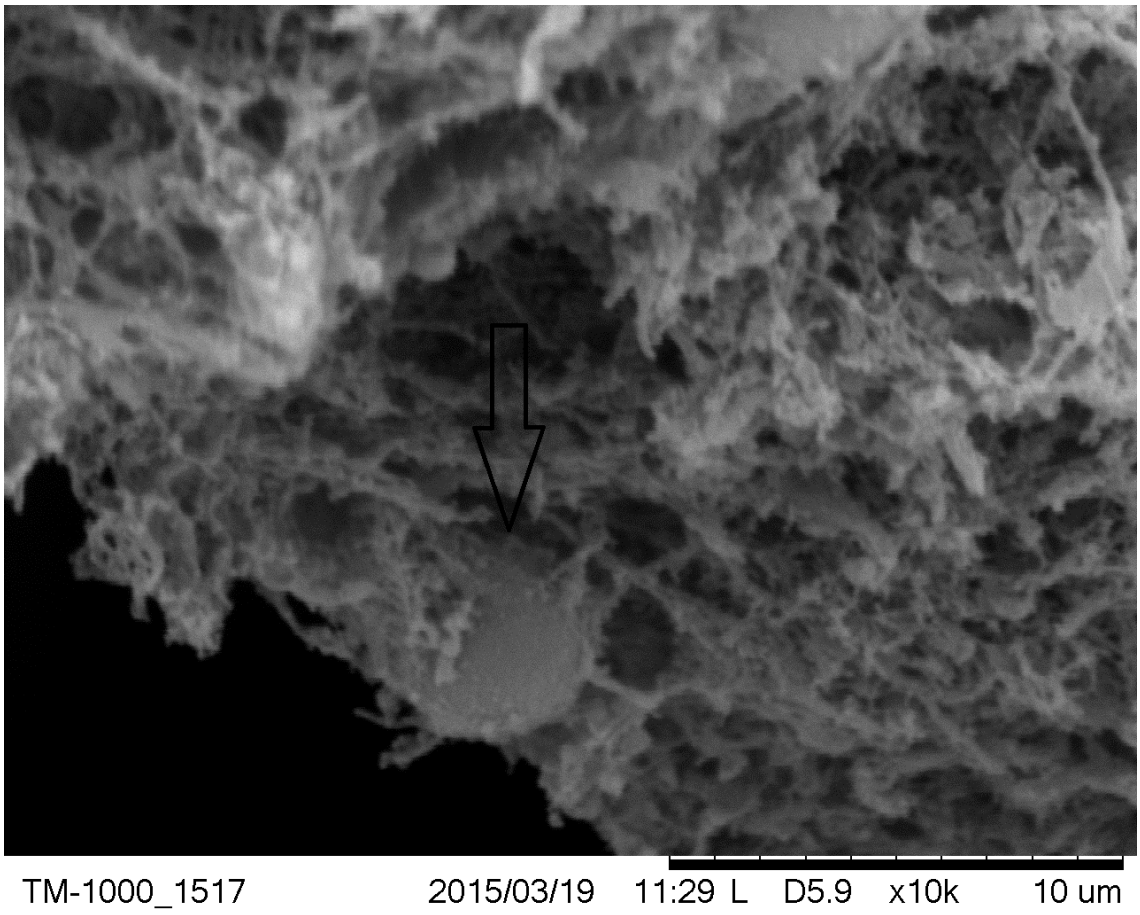
TD (non-transplanted) sections of the thalamus, A: side view at 4K maginification, B: overhead view perspective at 1.5K magnification. The tissue sample connectivity appears to be less consistent compared to the PFC tissue sample shown in Figure 15. Scale bars are 20 µm and 50 µm respectively for A and B.

FIGURE 17



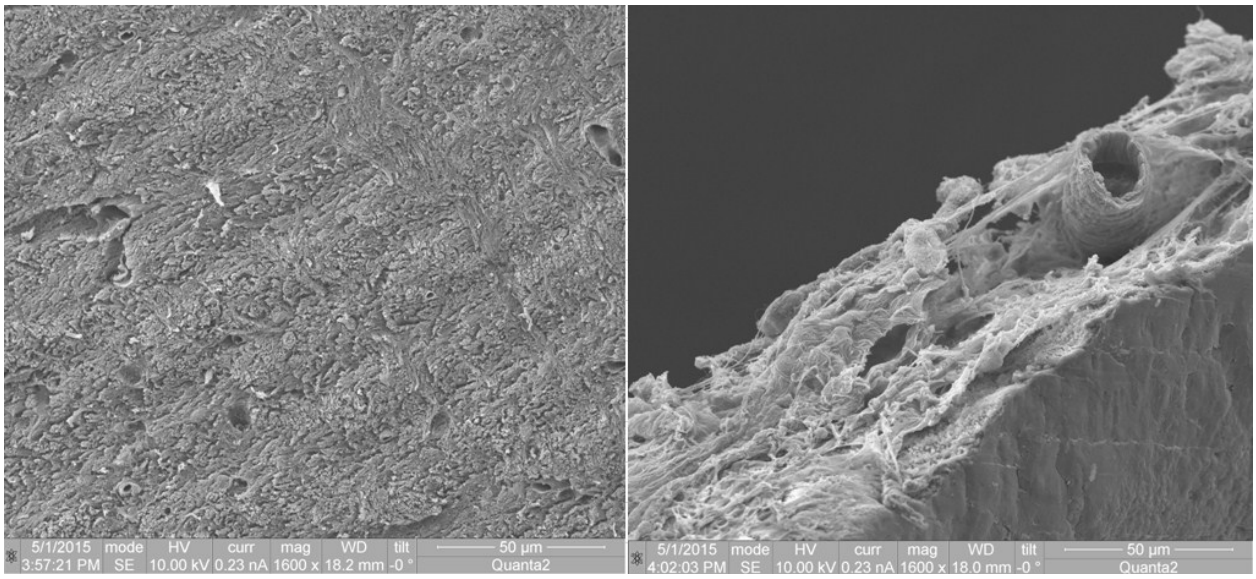
SEM image at 5K magnification of TD (non-transplanted) thalamus brain section (from a overhead view perspective) showing morphological similarities to NSPC. Refer to images from Figures 21-23. Scale bar is 20 μm.

FIGURE 18



SEM image (side view) at 10 K magnification of TD (non-transplanted) IC brain section showing morphological similarities to NSPC. Scale bar is μm.

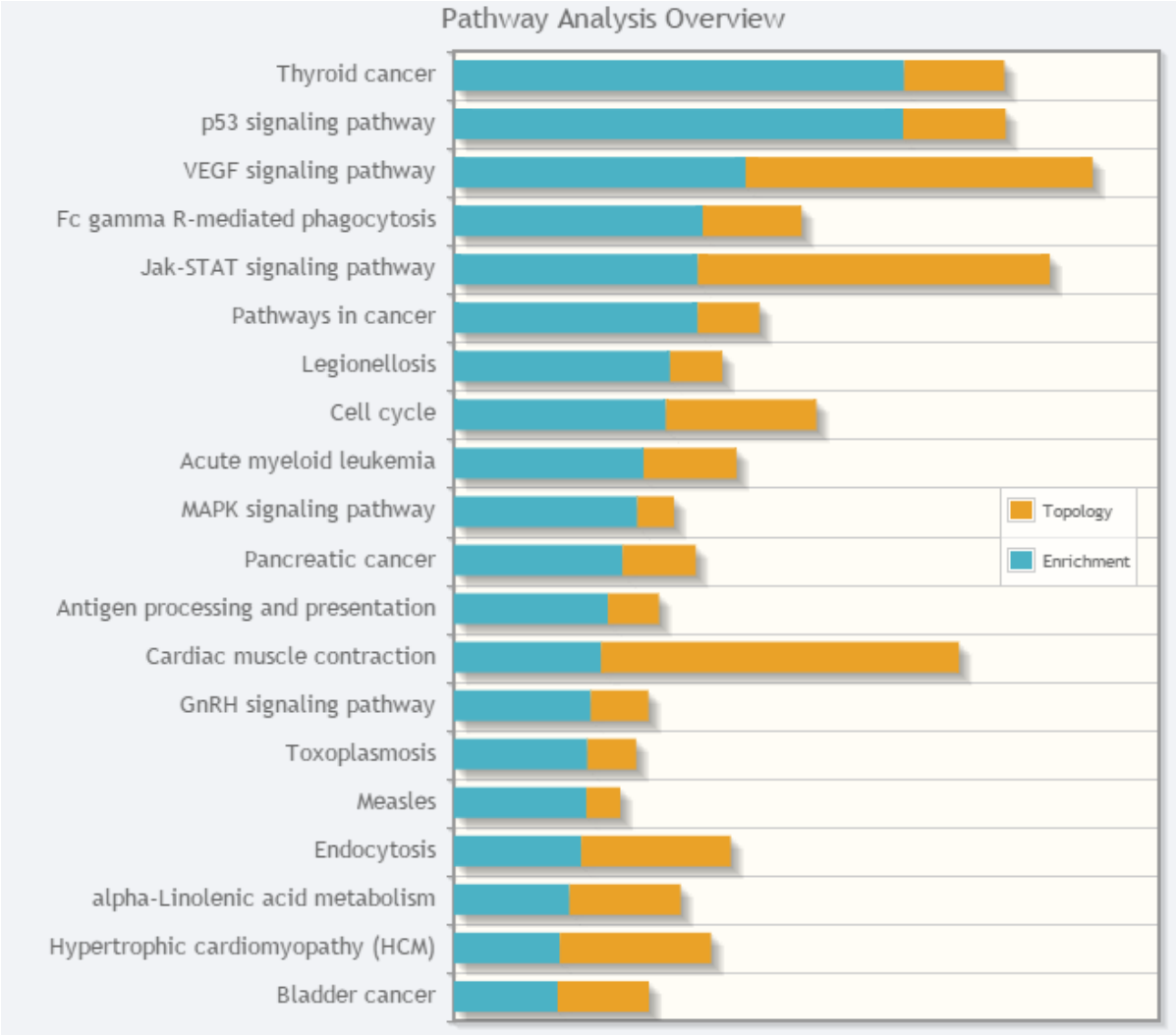
FIGURE 19



A B

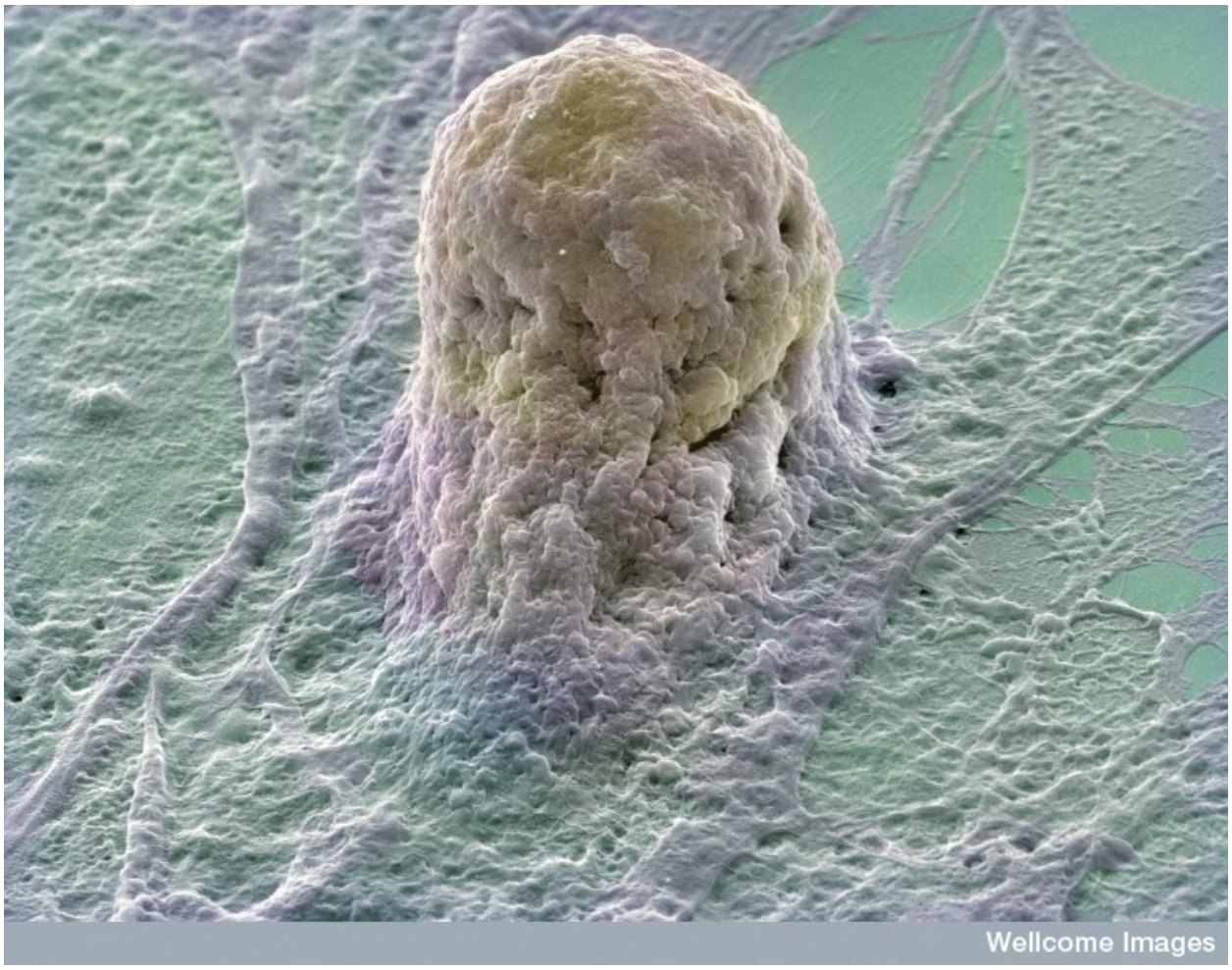
SEM images (overhead view and side view of section) of the thalamus of TD animals 48 hours post NSPC transplantation (post perfusion fixation). Tissue appears to be in better condition (connectivity of tissue more consistent) compared with the TD (non-tranplanted) sections, seen in Figures 16-18. Scale bars are 50 µm for both A and B.

FIGURE 20



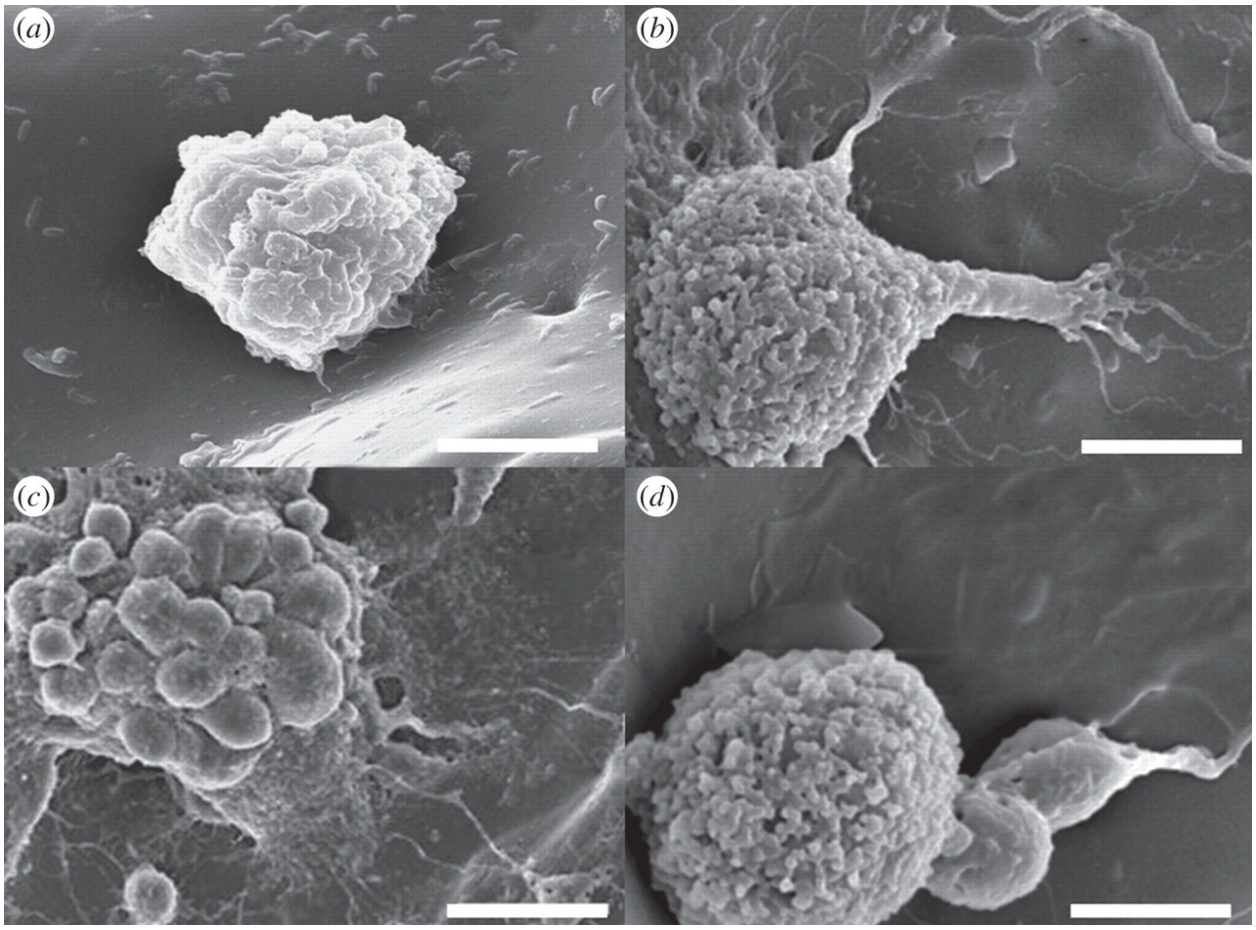
Integrative statistical analysis of Gene-Metabolite pathways involved in TD (Metaboanalyst 3.0). Key Pathways are observed known to have direct consequence on neurogenesis/astrogenesis and angiogenesis, which are respectively the JAK-STAT and VEGF pathways. Again, suggesting the involvement of a possible regenerative and protective mechanism.

FIGURE 21



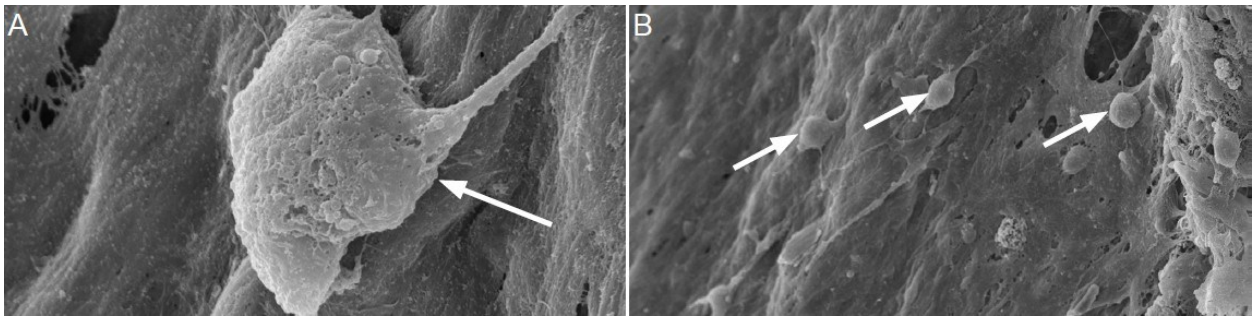
Scanning electron microscopic image of an embryonic stem cell growing on fibroblast. Image B0006219 from Wellcome Images credited to Annie Cavanagh. Image provided as a comparative sample to show morphological similarities with stem cells identified in the SEM images of the TD brain tissue samples previously illustrated.

FIGURE 22



Images of neural stem cells (Wang et al. 2012, ***Hyaluronic acid-based scaffold for central neural tissue***) adhered to hydrogels. Scale bars are 5μm and 10 μm for (a) and (b)-(d) respectively. Images serve the same functional purpose as the previous image illustrated in Figure 21.

FIGURE 23



Images of neural stem cells (Yuan et al. 2014, ***Neural stem cell transplantation in a double-layer collagen membrane with unequal pore sizes for spinal cord injury repair***) attached to the double-layer collagen membrane. The arrows in (A) neural stem cell with a neurite at 2500 k and (B) neural stem cells at 500 k. Scale bars would be 50μm and 100 μm respectively.