Cholesterol Homeostasis and the Developing Brain: Implications for Autism Spectrum Disorders

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Introduction

Cholesterol is an essential molecule for cell structure and function that is supplied to the body through endogenous synthesis and exogenous supply. Precise levels of cholesterol are important to cells, as cholesterol mediates fluidity and permeability in the plasma membrane that surrounds them, and regulates several trans-membrane proteins.[1] Neuron cells in the nervous system have additional roles for cholesterol in establishing connections and conducting signals. Cholesterol is a major component of the myelin sheath, a fatty layer that surrounds axons of neurons, and is also abundant in thickened areas of the plasma membrane called lipid rafts, which are important for neuron growth and connectivity[2]. Therefore, problems in the brain and nervous system may occur when the body's homeostasis is disrupted by endogenous or exogenous cholesterol supply, as is thought to occur in Alzheimer's disease[3]. If such disruption occurs during development, such as occurs in the disorder Smith-Lemli-Opitz Syndrome (SLOS), abnormalities in body and brain functioning are common. Various symptoms of SLOS that implicate changes to the nervous system are hyperactivity, irritability, and ritualistic behavior[4].

Autism spectrum disorders (ASD) have also commonly been found to be associated with abnormalities in cholesterol metabolism. This neurodevelopmental disorder does not only commonly accompany cholesterol metabolism disorders such as SLOS, but often ASD individuals themselves display perturbations in cholesterol metabolism. Some current hypotheses on the onset of ASD look to the apparent correlation between ASD and cholesterol disruption, and suggests that differential connection in the brain's neural networks give rise to ASD symptoms[5]. In this review paper, I will reflect on our current understanding of cholesterol metabolism, especially in regards to the brain and fetal developmental, the role it plays in neurodevelopment, and how it is suspected to relate to the onset of ASD.

Cholesterol Metabolism

Due to the immeasurable importance of cholesterol throughout the entire life of cells, almost all are able to produce their own supply through *de novo* synthesis. Briefly, the cholesterol synthesis pathway converts acetyl coenzyme A (CoA) to cholesterol in approximately twenty steps. The rate-limiting enzymes of this pathway are hydroxymethylglutaryl-CoA reductase

[1] Brown AJ, Sharpe LJ. 2016. Cholesterol Synthesis. Sixth Edit. Elsevier.

[2] Hussain G, Wang J, Rasul A, Anwar H, Imran A, Qasim M, Zafar S, Kamran SKS, Razzaq A, Aziz N, et al. 2019. Role of cholesterol and sphingolipids in brain development and neurological diseases. Lipids Health Dis. 18(1):1–12. doi:10.1186/s12944-019-0965-z.

[3] Petrov AM, Kasimov MR, Zefirov AL. 2017. Cholesterol in the pathogenesis of alzheimer's, parkinson's diseases and autism: Link to synaptic dysfunction. Acta Naturae. 9(1):26–37. doi:10.32607/20758251-2017-9-1-26-37.

[4] Allen LB, Genaro-Mattos TC, Porter NA, Mirnics K, Korade Z. 2019. Desmosterolosis and desmosterol homeostasis in the developing mouse brain. J Inherit Metab Dis. 42(5):934–943. doi:10.1002/jimd.12088.

[5] Wang H. 2014. Lipid rafts: A signaling platform linking cholesterol metabolism to synaptic deficits in autism spectrum disorders. Front Behav Neurosci. 8:1–6. doi:10.3389/fnbeh.2014.00104.

(HMGCR) and squalene monooxygenase (SQLE)[6]. Both enzymes are highly regulated by negative feedback mechanisms that prevent too much cholesterol from accumulating within the cell. Other processes, including covalent modification of HMGCR, are also known to affect cholesterol synthesis.

One of the most extensively studied feedback mechanisms uses sterol regulatory element binding protein 2 (SREBP2) and SREBP-cleavage activating protein (SCAP), both found in the membrane of the endoplasmic reticulum (ER). SREBP2 is transported from the ER membrane to the Golgi apparatus through vesicular transport, where serine proteases (S1P and S2P) release nuclear SREBP2 from its N-terminus (Fig. 1). Nuclear SREBP2 then diffuses to the nucleus, where it binds sterol regulatory element (SRE) sequences in DNA, promoting transcription of HMGCR and SQLE. SCAP mediates this process by binding SREBP2 and COP II, the coating protein responsible for vesicle formation. There is a sensitive sterol-sensing domain (SSD) in SCAP that responds to cholesterol in the ER membrane. In response to cholesterol, the sterol-sensing domain will interact with insulin-induced gene (INSIG) proteins, which will block COP II from binding and inhibit vesicle formation. SREBP2 transport to the Golgi apparatus is greatly inhibited when cholesterol exceeds a very sharp threshold of 5% of the lipid composition of the membrane[7]. When SREBP2 cannot enter the Golgi apparatus, it does not interact with serine proteases, and transcription of HMGCR and SQLE stops.

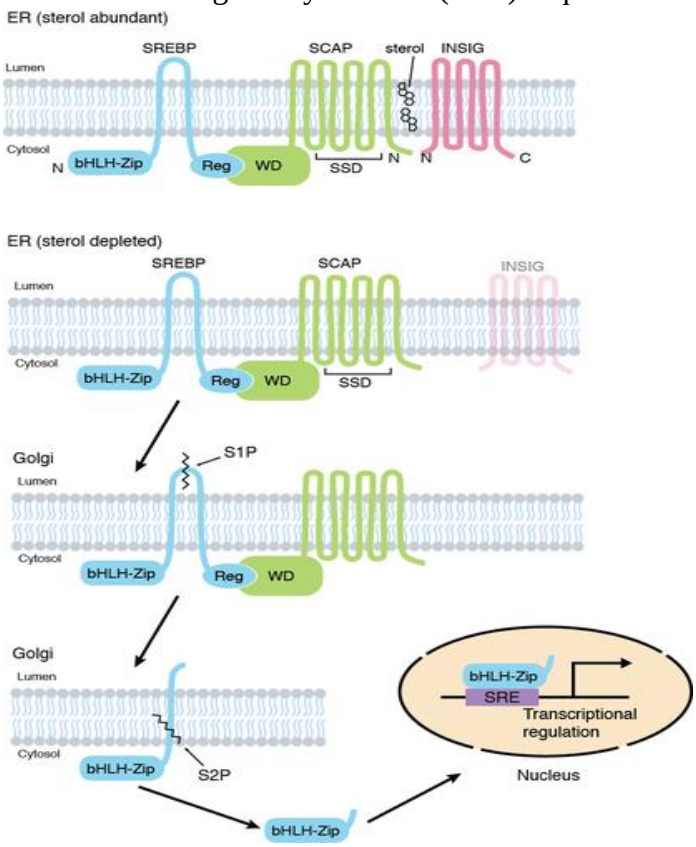

Fig. 1. "SREBP activation by proteolytic cleavage" by Artemister used under CC BY-SA 4.0. The SREBP-SCAP mechanism mediates the synthesis of cholesterol synthesis enzymes in response to cholesterol levels. Here, nuclear SREBP is referred to as bHLH-Zip, and the Reg-WD complex shown is the site of interaction between the two C-termini of SREBP and SCAP.

Another important mechanism that regulates cholesterol synthesis is the presence of oxysterols, which are oxidized forms of cholesterol that indicate a build-up of cholesterol in the cell. Oxysterols impact the SREBP-SCAP mechanism by binding INSIGs and promoting their binding to SCAP. Oxysterols also induce HMGCR degradation, and promotes the activity of the liver X receptor (LXR), which will be described shortly. This brief list of regulatory processes for the

[6] Luo J, Yang H, Song BL. 2020. Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol. 21(4):225–245. doi:10.1038/s41580-019-0190-7.

[7] Brown MS, Radhakrishnan A, Goldstein JL. 2018. Retrospective on cholesterol homeostasis: The central role of SCAP. Annu Rev Biochem. 87:783–807. doi:10.1146/annurev-biochem-062917-011852.

cholesterol synthesis pathway is by no means exhaustive, as most mechanisms are beyond the purposes of this paper and have been reviewed in detail elsewhere[6].

Cholesterol can also be delivered by the blood stream to tissues from the liver and intestines, as occurs after consuming cholesterol-rich foods. The cholesterol is carried by small molecules called lipoproteins, which are composed primarily of triacylglycerols that surround cholesteryl esters. Lipoproteins vary in size and composition as they gain and release cargo, and the resulting changes in surface lipids and proteins determine their intermolecular interactions[8]. Very low-density lipoproteins (VLDL) deliver mainly triacylglycerol, and in the process become low-density lipoproteins (LDL). LDL has a higher concentration of cholesterol than VLDL, and thus becomes the main vehicle of cholesterol delivery to body cells. LDL carries apolipoprotein B (apoB) at its surface, which is able to bind LDL receptor (LDLR) on cell membranes, inducing receptor-mediated endocytosis. An endosome forms from the engulfing of the LDL molecule, and the acidic pH inside causes LDL to release from its receptor. LDLR is moved back to the membrane for recycling as the LDL undergoes degradation. Cholesterol becomes available to the cell once the endosome fuses with a lysosome, and lysosomal acid lipase hydrolyzes the cholesteryl esters in the LDL. Once in the cytosol, cholesterol inhibits *de novo* synthesis, as described previously.

Maintaining the proper balance of cholesterol within the cell also entails removing cholesterol from the cell and excreting it from the body, a process accomplished through reverse cholesterol transport. This form of cholesterol transport is mostly accomplished by yet another class of lipoprotein called high-density lipoprotein (HDL). HDL is created by the liver rather than as remnants of larger lipoproteins, and functions to return cholesterol to the liver from peripheral cells and macrophages. While travelling through the body, HDL matures and remodels as it exchanges cargo with other lipoproteins using cholesteryl ester transport protein (CETP), which shuttles cholesteryl esters and exchanges them for triacylglycerols[9]. Active LXRs increase the expression of ATP-binding cassette (ABC) proteins, which encourages efflux of cholesterol from the cell onto HDL[10]. HDL molecules are eventually internalized by hepatic cells for biliary excretion of cholesterol as the final step of reverse cholesterol transport[8].

Brain Cholesterol Metabolism

The central nervous system faces a unique problem with cholesterol homeostasis due to the blood-brain barrier (BBB), which prevents neural tissue from accessing lipoproteins in the blood. The BBB is composed of astrocytes that surround capillaries projecting into brain tissue and create a seal of tight junctions[11] (Fig. 2). Small, lipid-based molecules can diffuse through the lipophilic membranes and enter brain tissue while other substances must be transported using proteins associated with the BBB. As a result, LDL cannot pass from the blood into the brain, and thus the brain must synthesize cholesterol locally.

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[8] Zanoni P, Velagapudi S, Yalcinkaya M, Rohrer L, von Eckardstein A. 2018. Endocytosis of lipoproteins. Atherosclerosis. 275:273–295. doi:10.1016/j.atherosclerosis.2018.06.881.

[9] Malajczuk CJ, Gandhi NS, Mancera RL. 2021. Structure and intermolecular interactions in spheroidal high-density lipoprotein subpopulations. J Struct Biol X. 5. doi:10.1016/j.yjsbx.2020.100042.

[10] Courtney R, Landreth GE. 2016. LXR regulation of brain cholesterol: From development to disease. Trends Endocrinol Metab. 27(6):404–414. doi:10.1016/j.tem.2016.03.018.

[11] Zhang J, Liu Q. 2015. Cholesterol metabolism and homeostasis in the brain. Protein Cell. 6(4):254–264. doi:10.1007/s13238-014-0131-3.

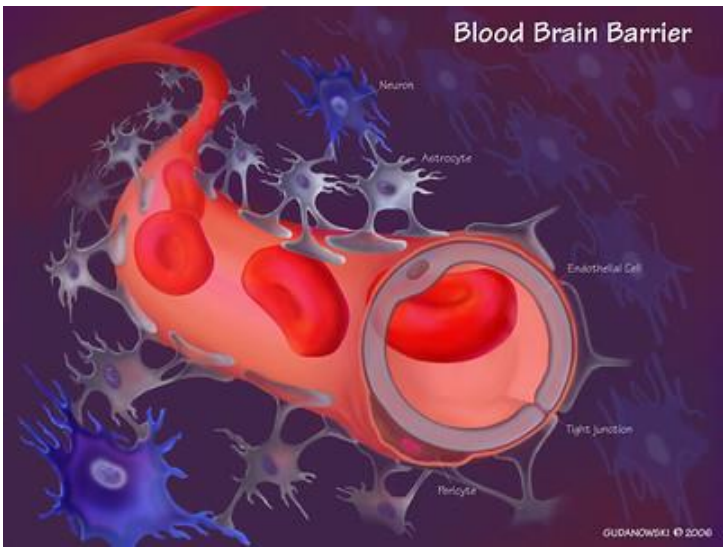

Fig. 2. "Blood brain barrier" by Chrejsa. Used under CC BY-NC-SA 2.0.

The majority of cholesterol synthesis occurs in astrocytes, which then transport cholesterol to the neurons[12]. Neurons carry out some degree of cholesterol synthesis themselves, but are very inefficient, and therefore require an external cholesterol source[11]. Astrocytes produce a surplus of cholesterol to supply themselves as well as surrounding neurons. Cholesterol is released from the astrocytes and incorporated onto apoE4 proteins by ABC proteins[13]. The resulting apoE4-containing lipoproteins are taken up by neurons using receptors LDLR and LDL receptor related protein 1 (LRP1). LDLR is more highly expressed in glial cells and LRP1 in neuron cells, so LRP1 is the main receptor for neuronal uptake of apoE lipoproteins in the brain[11].

As in any other cell of the body, cholesterol homeostasis is essential for the proper functioning of the neuron. Therefore, surplus cholesterol must be removed from neurons by secretion into the blood[11]. The secretion of cholesterol from the brain to the blood can be accomplished through two different enzyme processes. The first is specific to cells in the cortex and cerebellum, and uses the enzyme cholesterol 24-hydroxylase to convert cholesterol to the oxysterol 24-hydroxycholesterol (24-OHC)[14]. Cell membranes are permeable to oxysterols due to excess hydrophilic groups on the molecule that change the properties of cholesterol and allow it to pry phospholipids further apart, allowing the oxysterol through[15]. Therefore, once converted to 24-OHC, cholesterol can pass through cell membranes and passively flow through the BBB. The second process by which cholesterol can be returned to the blood uses ABC transporters, especially ABCA1, which are expressed in all neurons in the brain to release cholesterol from cellular membranes onto apoA-containing lipoproteins similar to HDL[11]. Brain capillary endothelial cells at the BBB have LRP1 or scavenger receptor class B1 (SR B1) receptors that take up these lipoproteins, removing them from the brain. From here, ABCA1 is used once again to transfer cholesterol to apolipoproteins and HDL in the blood[13].

Fetal Cholesterol Metabolism

[12] van Deijk ALF, Camargo N, Timmerman J, Heistek T, Brouwers JF, Mogavero F, Mansvelder HD, Smit AB, Verheijen MHG. 2017. Astrocyte lipid metabolism is critical for synapse development and function in vivo. Glia. 65(4):670–682. doi:10.1002/glia.23120.

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[15] Olkkonen VM, Hynynen R. 2009. Interactions of oxysterols with membranes and proteins. Mol Aspects Med. 30(3):123–133. doi:10.1016/j.mam.2009.02.004.

The cholesterol needs of a developing fetus are greater than those of an adult due to rapid formation of new cells and organ systems, particularly in the nervous system. In the first eight weeks of development, the fetus receives its cholesterol supply from the secondary yolk sac[16]. Once the cardiovascular system develops in the fetus, it is able to obtain cholesterol and other essential molecules from the maternal blood using an area of nutrient exchange in the chorionic membrane called the placenta (Fig. 3). In the placenta, maternal arteries and veins open into the intervillous space, where the maternal blood pools[17]. Fetal arteries and veins project into intervillous spaces as chorionic villi, enclosed in the placental barrier composed of three cell layers. Cholesterol travelling to the fetus must traverse the placental barrier from the intervillous space. LDLR is used to take up cholesterol from LDL. While transport of cholesterol from the outer cell layer to fetal blood is still being investigated, it is known that apoB is secreted from the basal membrane of the placental barrier, which suggests that cholesterol can be released while complexed to apoB[17].

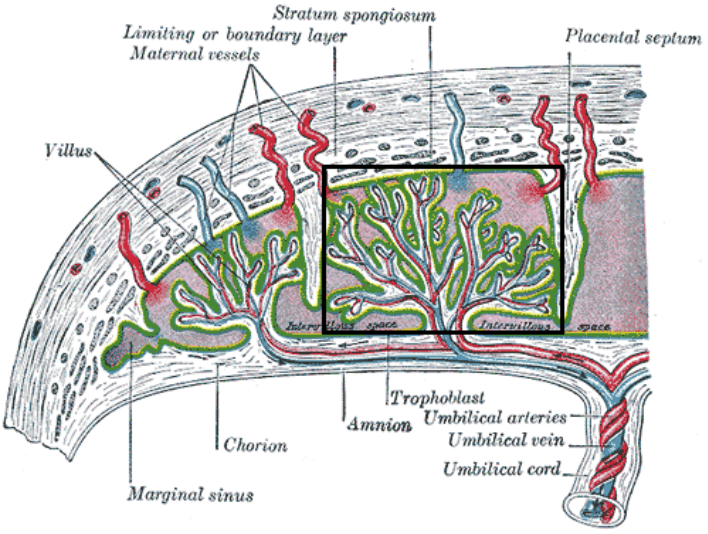

Fig. 3. "Structure of the placenta, with a placental cotyledon marked in rectangle" by Mikael Häggström. A cotyledon refers to a segment of intervillous space that contains a stem of chorionic villi. Public domain image.

Approximately during weeks 12-20, the fetus becomes capable of producing lipids from maternal substrates, and at around week 19 cholesterol precursors begin to accumulate in amniotic fluid, indicating synthesis by the fetus[18,19]. From this time onward, the fetus becomes increasingly independent from maternal cholesterol and lipid supply, requiring only carbohydrates and fatty acids for lipid synthesis in the fetal liver.

Causes of Cholesterol Deficiency During Fetal Development

Maternal Diet

Throughout the course of pregnancy, maternal serum lipid profiles naturally change to accommodate the needs of a developing fetus[19]. Likely caused by an increased level of estrogen produced by the placenta, the liver increases production of VLDL and decreases lipase activity, and throughout the body CETP activity increases, resulting in higher concentrations of LDL and

[16] Kallol S, Albrecht C. 2020. Materno-fetal cholesterol transport during pregnancy. Biochem Soc Trans. 48(3):775–786. doi:10.1042/BST20190129.

[17] Chatuphonprasert W, Jarukamjorn K, Ellinger I. 2018. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. Front Pharmacol. 9:1–29. doi:10.3389/fphar.2018.01027.

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HDL in maternal blood. However, abnormally high cholesterol levels, as can occur in obesity and gestational diabetes mellitus (GDM), can impede the fetus's ability to obtain cholesterol from the maternal body[16]. This condition, termed supraphysiological hypercholesterolemia, is associated with endothelial dysfunction in the placental vasculature, and in obesity shows evidence of increasing accumulation of oxidized LDL in the placenta, causing oxidative stress[20]. Additional to placental damage, obesity appears to increase LDLR activity in the liver, increasing LDL uptake and removing it from serum. It appears that in cases of maternal hypercholesterolemia, the fetal liver accumulates triacylglycerol and displays depressed lipogenesis, including cholesterol synthesis, possibly as a protective mechanism against triacylglycerol[21]. Both obesity and GDM are associated with increased risk of ASD developing in the child.

Genetic Metabolism Disorders

As the fetus must eventually produce its own cholesterol, developmental problems can also occur in genetic disorders that interrupt the production or uptake of cholesterol, such as SLOS, Rett syndrome, and phenylketonuria. In the case of SLOS, mentioned previously, the final step of cholesterol synthesis is disrupted, causing the cholesterol precursor 7-dehydrocholesterol to accumulate[4]. Rett syndrome is generally caused by mutations in a protein involved in gene silencing, and is connected to cholesterol metabolism by both reducing SR B1, the lipoprotein receptor protein involved in LDL and HDL uptake, and dysregulating expression of the rate-limiting enzymes involved in cholesterol synthesis[22]. Finally, phenylketonuria (PKU) is caused by a mutation in phenylalanine hydroxylase, which increases blood concentration of phenylalanine[23]. This increase in phenylalanine apparently causes a decrease in HMGCR activity and LDL-cholesteryl levels, likely by behaving as a competitive inhibitor for HMGCR similar to a statin. These disorders all have neurological symptoms, and are known to often occur with ASD and/or ASD symptoms.

ASD and Cholesterol

As mentioned, the cholesterol homeostasis disruptions listed above are associated with increased risk of the development of autism spectrum disorders (ASD). Furthermore, individuals with ASD tend to show signs of disrupted cholesterol metabolism. In one study, ASD individuals were found to have higher levels of 24-OHC present in their brains, indicating greater cholesterol turnover compared to neurotypical controls[24]. Whether there is a common underlying cause to this observation is still unknown, but the presence of 24-OHC implies less cholesterol is being retained in neurons, and may cause additional problems such as cytotoxicity and oxidative stress in the

[20] Park BY, Yao R, Tierney E, Brucato M, Hong X, Wang G, Ji Y, Pearson C, Fallin MD, Wang X, et al. 2021. The association between maternal lipid profile after birth and offspring risk of autism spectrum disorder. Ann Epidemiol. 53:50-55.e1. doi:10.1016/j.annepidem.2020.08.009.

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brain. ASD children are also known to have fewer myelinated neurons in their brain, which account for much of its cholesterol density[25]. Finally, a study that examined the effects of a known ASD risk factor called valproate (VPA) found that VPA impacted the phosphorylation of HMGCR in certain brain areas, seemingly increasing its activity in the nucleus accumbens and decreasing its activity in the cerebellum and dorsal striatum in adolescent mice[26]. The hippocampus also displayed decreased cholesterol and myelination after VPA exposure. How cholesterol may connect to ASD is currently an area of popular research. The answer may lie in a greater understanding of the role cholesterol plays in the structure and function of neurons.

The Role of Cholesterol in Forming Neural Networks

Much of the cholesterol in neurons occurs with sphingolipids in concentrated areas of the plasma membrane called lipid rafts. Strong associations between cholesterol and sphingolipids separate them from phospholipids in the membrane, and the resulting lipid rafts are major anchoring sites for proteins[27]. Lipid rafts form clusters of neurotransmitter receptors and ion channels at postsynaptic membranes, and contribute to the fluidity of membranes and therefore exocytosis of neurotransmitter at the presynaptic membrane[5]. Lipid rafts also carry glycosylphosphatidylinositol (GPI)-anchored proteins, many of which contribute to axonal and dendritic growth, guidance, differentiation, and myelination, and the formation of synapses[28]. If cholesterol is not abundant in the cell, the precise localization of such proteins would be affected and synapses may form abnormally and have impacted fluidity. The importance of these proteins in the emergence of ASD is evidenced by many of the genetic differences that accompany the disorder, as many are mutations of synaptic proteins such as the N-methyl-D-aspartate receptor (NMDAR)[29].

Previous studies have found that reducing cholesterol produced by astrocytes leads to the production of more immature synapses and fewer, less mature vesicles[12]. In this study, the main protein complex responsible appeared to be SNAP-25, a SNARE complex in pre-synaptic neurons responsible for vesicle docking, which is associated with lipid rafts. Dispersion of SNAP-25 and other proteins (such as synaptophysin, which affects the curvature of the membrane) lead to an altered synaptic vesicle cycle and possible impairments in synaptic transmission and plasticity. Another cholesterol depletion study also observed reduced evoked vesicle release, but found a more likely explanation was an interruption in signal propagation preceding vesicle release[30]. In fact, when observing spontaneous vesicle release, cholesterol deprivation appeared to increase the amount of exocytosis. The study did not look at why interruption in signal propagation occurred,

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[25] Dimond D, Schuetze M, Smith RE, Dhollander T, Cho I, Vinette S, Ten Eycke K, Lebel C, McCrimmon A, Dewey D, et al. 2019. Reduced white matter fiber density in autism spectrum disorder. Cereb Cortex. 29(4):1–11. doi:10.1093/cercor/bhy348.

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[27] Tracey TJ, Steyn FJ, Wolvetang EJ, Ngo ST. 2018. Neuronal lipid metabolism: Multiple pathways driving functional outcomes in health and disease. Front Mol Neurosci. 11:1–25. doi:10.3389/fnmol.2018.00010.

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[29] Qiu S, Aldinger KA, Levitt P. 2012. Modeling of autism genetic variations in mice: Focusing on synaptic and microcircuit dysfunctions. Dev Neurosci. 34(2–3):88–100. doi:10.1159/000336644.

[30] Korinek M, Gonzalez-Gonzalez IM, Smejkalova T, Hajdukovic D, Skrenkova K, Krusek J, Horak M, Vyklicky L. 2020. Cholesterol modulates presynaptic and postsynaptic properties of excitatory synaptic transmission. Sci Rep. 10(1):1–18. doi:10.1038/s41598-020-69454-5.

but noted that an increase in membrane fluidity by increasing temperature had a similar effect, and suggested disruption in the axon causing "leakiness" or disturbed function of ion channels in the axon as possible causes. Another finding of this study was that cholesterol increased the open probability of NMDAR.

Lipid rafts have also been implicated in the growth and polarization of newly developing neurons, likely by concentrating signalling molecules such as netrin, semaphorins, and ephrins in the growth cone to determine directional growth[31]. Growth cones are structures located at the end of a growing axon or dendrite that produces tubulin, a component of microtubules, to provide directional axonal growth in response to extracellular signals (Fig. 4). These signal molecules induce a cascade of second messengers such as calcium ions and cAMP through several types of transmembrane proteins, which stimulate or inhibit growth of microtubules and fusion of vesicles towards the signal. SNARE complex proteins are involved in the process of vesicle endocytosis, as another important protein associated with lipid rafts[32].

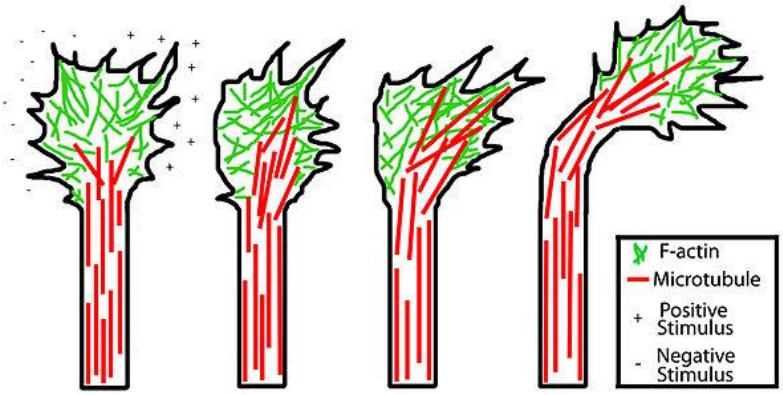

Fig. 4. "Cartoon of Growth Cone mediated axon guidance" by Chris1387 used under CC BY-SA 3.0. Neurons grow in response to external stimuli, mediated by the growth cone.

Current Theories on ASD

Current theories into the underlying causes of ASD suggest that pathfinding and/or synapse formation are altered in ASD individuals and provide explanation to core autistic behaviours. This theory provides a reasonable link between the known functions of cholesterol in the nervous system and the frequency at which ASD occurs with disruptions to cholesterol metabolism. Furthermore, this theory explains why ASD can also arise when genetic mutations disrupt proteins involved in neuron growth and/or synaptogenesis[29]. Synaptogenesis in the developing brain occurs in two major phases: prenatally, neurons grow and establish synapses within and between brain structures, and postnatally, connections are adjusted based on experience and sensory information[32]. During the prenatal period, at approximately 24 weeks of gestation, synapses begin to form in the cortical plate and subplate of the developing cerebral cortex. After most of these short-range circuits are in place, innervation from thalamic nuclei into the cortex begins and establishes the core of what will become long-range connections. Following the 24-week mark and continuing until the end of gestation, activity increases in the cortical plate relative to the subplate, and long-range connections between sensory regions of the cortex and between the cortex and limbic system are established. Different structures of the limbic system, namely the hippocampus and anterior cingulate gyrus, form circuits with the frontal cortex at different times and at different rates, making this system particularly sensitive to disruption. If the timing of

[31] Igarashi M. 2019. Molecular basis of the functions of the mammalian neuronal growth cone revealed using new methods. Proc Japan Acad Ser B Phys Biol Sci. 95(7):358–377. doi:10.2183/pjab.95.026.

[32] Carroll L, Braeutigam S, Dawes JM, Krsnik Z, Kostovic I, Coutinho E, Dewing JM, Horton CA, Gomez-Nicola D, Menassa DA. 2021. Autism spectrum disorders: Multiple routes to, and multiple consequences of, abnormal synaptic function and connectivity. Neuroscientist. 27(1):10–29. doi:10.1177/1073858420921378.

synaptogenesis is disrupted in one structure relative to the other, later connections formed in the limbic system will be altered. After birth, the structures of the synapses change based on their use and disuse by the child and their experiences with their environment, but the number of established synapses remains the same.

Post-mortem studies of young children with ASD reveal differences in cortical architecture, providing evidence that the underlying circuitry from prenatal development may be a major cause of these disorders[33]. In these studies, disorganized regions appeared in the frontal and temporal cortices, but not the occipital cortex. Various neuroimaging studies also show patterns of hypoconnectivity between cortical brain regions in ASD individuals, and hyperconnectivity between the thalamus and cortical brain regions[32]. In general, long-range connections appear mostly impaired, while shorter-range connections are more likely to be varied in whether they are impaired, enhanced, or unaffected.

Onset of ASD is thought to occur as two "hits"; the first occurring in prenatal development and the second in postnatal development. In this model, abnormalities that occur during the establishment of initial synapses sets up an underlying neural circuitry that is "built to fail"[34]. During infancy and adolescence, as the synapses change to provoke appropriate responses to the surrounding world, symptoms of ASD emerge if the synapses form incorrectly due to differences in neural circuitry. In regularly developing children, the synapses being formed at this time establish the balance between excitatory signals, such as those produced by glutamate, and inhibitory signals, such as those produced by gamma-Aminobutyric acid transmission (GABA). This balance fine-tunes the brain's ability to respond appropriately to stimuli without overwhelming the individual[3]. It thus logically follows that alterations in synaptic development could cause an imbalance in these transmissions, leading to the symptoms and behaviours of ASD including sensory-seeking and sensory-avoidant behaviours.

Future Directions

Since ASD is observed to occur in many cases where cholesterol metabolism is disturbed and is thought to result from failure of the prenatal circuitry development, it is reasonable to look to disruptions in the function of lipid rafts and their resulting effects on growth cones to further our understanding of ASD. A few such studies have already been performed. One study disrupted lipid rafts in *Xenopus* neuron cultures and found that responses to extracellular signalling molecules BDNF, netrin-1, and Sema3A were hindered[35]. The experimental neurons were subjected to methyl-β-cyclodextrin (MCD), a water-soluble cyclic oligomer that extracts cholesterol from the plasma membrane, and then were exposed to signal molecule gradients. While intact neurons responded to the guidance molecules by growing towards them, experimental neurons continued to grow straight, revealing that cholesterol-deprived neurons in a nervous system could potentially follow altered paths during development.

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Experiments such as this one provide evidence that abnormalities in the behaviour of growth cones can occur in cases when cholesterol supply is insufficient. The previously referenced study that examined cholesterol depletion in the synapse also indicated a loss or reduction of functioning in such conditions[30]. What remains to be answered is the underlying cause of the patterns seen in ASD, where certain synapses are underdeveloped, some overdeveloped, and some unaffected. If particular brain areas are implicated in the abnormalities seen in ASD, and if cholesterol depletion is at least one way for these abnormalities to appear, do these brain areas exhibit a different response to cholesterol depletion? There are some differences in cholesterol metabolism that have been demonstrated between certain brain areas; for example, only the cerebrum and cerebellum eliminate cholesterol via 24-OHC, and HMGCR responds differently to VPA depending on the brain region it is in[11,26]. Therefore, vulnerability to cholesterol depletion and/or response to factors that can cause cholesterol depletion may be regionally-specific, though the literature pursuing this hypothesis is still limited and warrants further investigation to increase our understanding of the mechanisms that lead to ASD.

Conclusion

ASD is a neurodevelopmental disorder that is not well understood in terms of how it affects the brain and nervous system, but cholesterol homeostasis is likely connected to its onset and pathology. By increasing our knowledge on the behaviour of cholesterol-deprived neurons, we may begin to understand the exact causes of symptoms seen in ASD. By pursuing such research, we will not only increase our knowledge of neurodevelopment in ASD children, but may be able to connect cholesterol metabolism to more neurodevelopmental and neurodegenerative disorders. We may also eventually be able to create a model for ASD that shows how it progresses in early childhood, which will increase our understanding of the symptoms and struggles of ASD children in cases where these symptoms cannot be easily communicated. Once we understand exactly where the issue occurs and what has caused it, we can more successfully address symptoms to provide ASD children more effective and empathetic treatments and coping strategies.